'SOME ASPECTS OF IMAGING SQUAMOUS CELL CARCINOMA USING TECHNETIUM-99m (V) DIMERCAPTOSUCCINIC ACID (PENTAVALENT DMSA)'

By

Senior Registrar in Otolaryngology,
Guy's Hospital,
London.

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AD MAJOREM DEI GLORIAM
AND, TO ESMÉ
ABSTRACT

The purpose of this study was to establish an animal tumour model with squamous carcinoma and use it to evaluate the pharmacokinetics, biodistribution and optimal imaging characteristics of the new head and neck tumour imaging agent Technetium-99m (v) Dimercaptosuccinic Acid (Tc $^{99m}$ (v) DMSA). These parameters were then compared with similar studies in humans with squamous carcinoma and the combined data used to calculate the effective dose equivalent in man.

Seventy one patients with malignant tumours and 10 with benign lesions were studied.

Eighty nine rabbits were studied (27 non-tumour, 62 tumour). Tc $^{99m}$ (v) DMSA had a bi-exponential blood clearance and cumulative urine excretion in rabbits and humans.

The major organ biodistribution of Tc $^{99m}$ (v) DMSA in rabbits was in bone, kidneys and the blood pool. There was no significant difference in biodistribution between non-tumour and tumour groups and the main route of excretion was in the urine. There was no evidence of active tumour accumulation
or specific intracellular localisation of Tc $^{99m}$ (v) DMSA in rabbits or humans.

The optimal imaging time for rabbits was four hours and for humans between two and four hours. In man, the normal biodistribution of Tc $^{99m}$ (v) DMSA was in the lacrimal glands, nasal mucosa, blood pool, kidneys and bladder.

In rabbits, palpation was more efficient than Tc $^{99m}$ (v) DMSA planar scintigraphy in detecting superficially transplanted tumours. In humans, clinical examination was superior to Tc $^{99m}$ (v) DMSA scintigraphy (planar and SPECT) in detecting squamous carcinoma.

Computerised tomography was as accurate as clinical examination (but more accurate than Tc $^{99m}$ (v) DMSA planar imaging) in detecting patients with squamous carcinoma.

Tc $^{99m}$ (v) DMSA is a cheap safe radiopharmaceutical with a low radiation dose (5.1 uSv/MBq) which is stable to two hours in-vitro. However, Tc $^{99m}$ (v) DMSA imaging has no role to play in the management of patients with head and neck squamous carcinoma.
DECLARATION

The work in this Thesis is entirely original and was carried out by the author in the Departments of Surgery, Nuclear Medicine and Biochemistry at Guy's Hospital. In one instance, work has been included from a previous study (Watkinson, 1987) and this has been made clear in the text.

Technetium-99m (v) Dimercaptosuccinic Acid was prepared by Dr. Colin Lazarus, Principal Radiopharmacist in the Department of Nuclear Medicine at Guy's. The rabbit scintigraphic studies were performed with the help of Peter Liepins, Senior Technician in the Radiological Sciences Research Laboratory at Guy's. The human scintigraphic studies were performed by the technical staff in the Department of Nuclear Medicine at Guy's and both animal and human scans were reported by Dr. Susan Clarke, Consultant Physician in Nuclear Medicine at Guy's. The computerised axial tomographic, magnetic resonance and ultrasound scans were all performed in the Department of Diagnostic Radiology at Guy's, and the computerised axial tomographic scans were reported jointly by Dr. Sheila Rankin (Consultant Radiologist) and Dr. Colin Todd (Senior Registrar in Radiology). The magnetic resonance images were reported by Dr. John Bingham, Consultant Radiologist at Guy's, and the ultrasound scan was reported by Dr. Colin Todd.
The animal pathology specimens were analysed by Dr. Iain Lindsay, Senior Lecturer and Consultant Histopathologist at Charing Cross Hospital, London. The human pathological specimens were all analysed in the Department of Pathology at Guy's, and the African flesh eating beetles came from the Rayne Institute, St. Thomas' Hospital, London.
"Because the newer methods of treatment are good, it does not follow that the old ones were bad: for if our honourable and worshipful ancestors had not recovered from their ailments, you and I would not be here today"

Confucius 551-478 B.C.

"Diagnosis precedes treatment"

Russell John Howard 1875-1942

"There is a tremendous literature on cancer, but what we know for sure about it can be printed on a calling card"

August Bier 1861-1949

"We're saving thousands of lives today that weren't saved years ago".

The Director of the US National Cancer Institute, 1985.
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INTRODUCTION

Head and neck carcinoma dates back to prehistoric times. The oldest known instance of a human tumour is thought to be the "kanam mandible", a fossilised mandibular fragment found in East Africa by Leakey, and which was deformed by an irregular lesion that was 'fairly extensive, extending onto the lingual and labial surfaces of the jaw in the region of the symphysis'. This fossil probably dates back to the middle Pleistocene (more than 500,000 years ago), and has been interpreted by some as an osteogenic sarcoma and by others as a reaction to trauma (McCarty and Million, 1984, p 1).

The oldest medical documents, written on Egyptian papyri (1600 B.C.) mention diseases such as "eating ulcers of the gums" and "illnesses of the tongue" but concentrated on medical management (McCarty and Million, 1984, p 3). The ancient Greek physicians recognised head and neck cancer and Hippocrates (400 B.C.) referred to what was probably tongue cancer in his Prorheticon (Martin, 1940). The Greco-Roman physician Asklepiades is credited with the first use of tracheotomy around 50 B.C., and it was he who rejected the Hippocratic doctrine of the four humors and founded the Methodist School of Medicine in Rome (McCarty and Million, 1984, p 3). In A.D. 30, Celsus compiled an encyclopedia of
current Roman medical knowledge and in it described an operation for lip cancer with local flap repair (McCarty and Million, 1984, p 3).

By the time of Galen (A.D. 129-199), the humoralists were ascendant and Galen's great synthesis and forceful development of the humoral theory made it the basis for medical practice for some 1500 years to come. Galen described many head and neck operations and was the first to demonstrate the function of the recurrent laryngeal nerve and describe accurately the anatomy of the pharynx and larynx (McCarty and Million, 1984, p 4).

All of these very early medical writings, although fragmentary and often obscure, formed the basis of Western medicine until sometime after 1600 A.D. Probably the greatest Byzantine medical writer was Paul of Aegina (700 A.D.). Although his work was principally a compilation of earlier writings, he was the first to discuss neck dissection, albeit for scrofulous (and not carcinomatous) neck nodes (McCarty and Million, 1984, p 4). Both Arabic and Byzantine medical texts were available to physicians at Salerno (in Southern Italy) which, by the late eleventh century A.D. had become the centre of practical surgical knowledge. In his surgery (1170 A.D.), Master Roger of Salerno taught that cancer of the head and neck should be completely excised by
knife or cautery, unless it was located close to major nerves or arteries when it should be left alone. Scrofulous (tuberculous) neck nodes were excised if not very enlarged but cancerous nodes were left alone (McCarty and Million, 1984, p 5). However, up to the latter part of the nineteenth century, most cervical lymph node swellings were grouped together under the general term 'scrofula', whether or not they were benign or malignant. Under such conditions, obvious errors in diagnosis occurred (Martin, 1940).

In the fifteenth century, the work of modern anatomists began with Italy the centre of activity. Subsequently, many Italian and French surgeons described operations to resect head and neck cancers, most of which had arisen following an epidemic of syphilis. There were also some early attempts at reconstruction, not only following these head and neck resections but also for the mutilation which followed punishment for theft and for treatment of war injuries.

By the late eighteenth century, Paris was the centre of the medical world. The Parisian physicians discarded much of the humoralist system in favour of "localism", i.e. the idea that cancer might have a local rather than a systemic origin, and could therefore be treated in its early stages.
Such thoughts kindled a theoretical basis for the development of modern cancer surgery. Parisian medicine was mostly clinical in its orientation and resisted advances in microscopy, experimental physiology and medicinal chemistry. Others were not so conservative and Germany became the centre of the medical world in the nineteenth century.

In the mid-nineteenth century, anaesthesia and antisepsis were developed, both of which were crucial for the development of modern surgery. About this time indirect laryngoscopy was discovered by Manoel Garcia who used a warmed dental mirror to examine his own larynx (Stevenson and Guthrie, 1949, p 99). Following his discovery, indirect laryngoscopy was adopted into the chief medical centres of the world. Sir Morrell McKenzie was one of the first British laryngologists. He founded the Throat Hospital in Golden Square, London, wrote the classical book "Growth in the Larynx" (1870) and co-founded, in 1887, the Journal of Laryngology and Otology (Stevenson and Guthrie, 1949).

In Victorian days, the differential diagnosis between simple infection, syphilis, tuberculosis and malignant disease was always difficult, and sometimes impossible, even for an experienced laryngologist. Such was the problem faced by Sir Morrell McKenzie when dealing with the Crown Prince
Frederick who was subsequently found to have cancer of the larynx. Koch did not discover the tubercle bacillus until 1882, Röntgen discovered X-rays in 1895 and Wasserman introduced his test for syphilis in 1906, so that the Victorian laryngologist was obliged to rely solely on his clinical acumen and experience. By the late-nineteenth century, laryngo-fissure and laryngectomy were being performed for laryngeal cancer, as well as many other local resections for head and neck carcinoma by such distinguished surgeons as Billroth, Kocher, Glück and, in this country, Butlin and Whitehead. Billroth also recognised the importance of removing the regional lymph nodes in continuity when performing a resection such as laryngectomy (McCarty and Million, 1984, p 10).

However, the majority of these patients were not seen sufficiently early, so that at operation the growths were incompletely removed. In addition, poor anaesthetic techniques resulted in a high incidence of post-operative pneumonia. The more successful operations of today are a tribute, not only to an improvement in early diagnosis and surgical techniques, but also parallel advances in anaesthesia and post-operative care.
In the early part of the twentieth century, specialised instruments and surgical techniques for head and neck cancer operations were developed and operative mortality rates improved due to meticulous pre- and post-operative care. Further advances in the mid-twentieth century meant that the list of supportive measures available included antibiotics and blood transfusion together with further advances in anaesthetic, operative and rehabilitation skills.

Sir Henry Butlin, when discussing the treatment of pharyngeal tumours, recognised that the treatment of the neck was as important as the treatment of the primary growth (McCarty and Million, 1984, p 16). Early attempts to deal with neck lymph node metastases reached a culmination in 1906 when George Crile advocated en bloc resection of the "entire lymphatic-bearing tissue" in the neck, either on one or both sides, for patients with a wide variety of head and neck squamous carcinomas who had enlarged neck nodes (Crile, 1906).

Crile's work was subsequently popularised by Hayes Martin (Martin, 1941). The development of radiation therapy along with the birth of plastic surgery led to the concept of combined treatment of head and neck cancer, popularised first by Sir Stanford Cade in this country, and then by Hayes Martin in America.
Over the last four decades, there have been many advances in the management of head and neck cancer. Advances in diagnosis (TNM staging, microlaryngoscopy, monoclonal antibodies and Computerised Axial Tomography); reconstructive surgery (pedicled and free flaps); excisional surgery (partial laryngectomy and modified neck dissection) and multimodality treatment (surgery and radiotherapy, and/or chemotherapy). Recent reports suggest that despite such advances, cure rates have not improved dramatically although reconstruction and rehabilitation have improved patient morbidity (Stell and McCormack, 1985a).

Head and neck cancer continues to be a major disease with significant morbidity and mortality (Powell and Robin, 1983), and one of its greatest prognostic factors is the presence or absence, level and size of metastatic deposits in cervical lymph nodes. The detection of these deposits is difficult but crucial to correct management. There are errors in palpation, and current methods available to increase diagnostic sensitivity and specificity such as computerised axial tomography, magnetic resonance imaging, ultrasound and radioisotopes all have their limitations.

Current research in head and neck imaging using either fine resolution or three dimensional computerised axial
tomography, *in-vivo* and *in-vitro* magnetic resonance, or planar and tomographic radioscinography is directed towards demonstrating not only tumour anatomy, but also tumour physiology, in an attempt to detect occult primary and cervical metastatic disease together with residual and recurrent disease following surgery and/or radiation which cannot be identified using more conventional means.
CHAPTER 1

1.1. THE STAGING OF HEAD AND NECK SQUAMOUS CARCINOMA

1.1.1. Introduction

1.1.2. The Purpose of Classification

1.1.3. History

1.1.4. Current Status

1.1.5. Criticisms of the System
1.1.1. INTRODUCTION

A staging system for head and neck squamous carcinoma is important both for clinical and therapeutic research and as an acceptable and reproducible method of staging all sites within the region. It is mandatory to allow any meaningful comparison to be made between results from different centres, both nationally and internationally (Spiro et al., 1974a). The goals of any cancer staging system are therefore, by definition, far-reaching and multiple in nature. The system should act as a dictionary, allowing individual physicians and surgeons to compare and exchange information using language and vocabulary they can all understand. The staging data obtained must reflect prognosis and provide guidelines for treatment selection, allow analysis of isolated clinical factors such as pretreatment findings, age, complications and survival, and should be easy to both comprehend and update (Spiro et al., 1974a; Rapidis et al., 1977; Jacobs et al., 1985). In this section the history, current status and criticisms of the various staging systems applicable to head and neck squamous cell carcinoma are discussed.
1.1.2. THE PURPOSE OF CLASSIFICATION

The concept of the TNM (tumour, node, metastases) classification of cancer is really a logical one since it covers the life-span of any malignant disease process in the body (Beahrs et al, 1977). The surgeon, by using a numerical system, can state accurately where the tumour is in its natural development. The practice of dividing cancer cases into groups arose from the fact that recovery or survival rates were higher for cases where the disease was localised than for those where the tumour had extended beyond the site of origin.

Although the staging of cancer is steeped in tradition (Steinthal, 1905), its purpose should not merely be to satisfy the scientific urge to classify (Rubin, 1971; Harmer, 1977; Black and Gluckman, 1983). For the purpose of analysis of large groups of patients it is usual to employ a method of staging and the Union Internationale Contre Le Cancer (UICC) believes (UICC, 1987) that it is preferable to reach agreement on the recording of accurate information on the extent of disease for each site, because the precise clinical description and histopathological classification of malignant neoplasms may serve a number of important functions. These include helping the clinician in the planning of treatment,
the evaluation of prognosis and to assist in the analysis of treatment results. The information obtained should correlate with expected survival (Hoopes, 1969), be exchangeable between various centres both nationally and internationally, and should make an important contribution to the ongoing investigation of human cancer.

For clinical and therapeutic research it is mandatory to use a uniform and comprehensive classification of head and neck carcinoma. To facilitate the evaluation of different therapeutic approaches it is necessary to compare equivalent homogeneous groups of patients and to be able to summarise the salient clinical features of patients attending different treatment centres (Fries et al, 1973; Moss et al, 1973; Spiessel et al, 1973). This enables information interchange between such centres and for any classification to function, the definitions of the criteria used must be sufficiently exact so that each clinician will attach a similar meaning to each criterion. It is, therefore, important that data kept in hospital records should include sufficient detail to allow the subsequent classification of cases for both retrospective and prospective analysis (Hoopes, 1969; Binnie et al, 1972). To this end most head and neck units now utilise a data sheet (Von Haacke and Croft, 1984) which is kept separate from
traditional hospital records and whose information is often transferred onto a computer tumour registry (Black and Gluckman, 1983; Jacobs et al, 1985; Stell and McCormack, 1985 a-b; Fletcher and McManus, 1987).

The head and neck data sheet (Figure 1) is important, not only since it allows for retrospective and prospective analysis, but it also enforces the clinician to obtain all the relevant information at the primary consultation, and at each subsequent visit thereafter. This has not always been the case and, in one series (Batley, 1964), an extensive survey of hospital records showed that only 1% contained sufficient descriptive information to allow the application of a classification system.

Carr (1983) asked the question - Is staging of cancer of value? In his paper he stated there is ample evidence that localised cancers are more curable than their disseminated counterparts and, indeed, the AJC published a statement emphasising the value of staging in the evaluation of screening programmes in the reduction of morbidity and mortality due to cancer (AJCC, 1980). He concluded that staging of cancer is not only of value, but is indispensible in any practice of medicine that includes either the diagnosis of cancer, or any other aspect of the broad subject of oncology.
## Figure 1

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Head and neck data sheet (Guy's Hospital Head and Neck Oncology Clinic)
The one basic problem in the clinical staging of head and neck squamous carcinoma is the subjective interpretation of the clinical findings and this is dealt with in subsequent sections.
1.1.3. HISTORY

Although Steinthal (1905) proposed the first scientific classification for malignant breast tumours, Krishaber (1879) and Sebileau (1906) should be credited with the first attempts to classify head and neck malignancy. Krishaber divided laryngeal tumours into extrinsic and intrinsic and recognised, quite rightly, the former to be more malignant. Sebileau staged maxillary sinus cancer using a system which divided the upper jaw into a suprastructure, mesostructure and infrastructure by means of parallel lines. Steinthal's system, however, described three stages of disease of increased severity and thereby initiated the orderly, progressive, systematic reporting of clinical findings in the evaluation of malignant tumours. In 1929, The League of Nations Health Organisation began working on the clinical classification of cancer and nine years later published an atlas illustrating the division of cancer of the uterine cervix into four stages (Heyman, 1938). Since this work appeared, the idea of visual representation of the anatomical extent of malignant tumours at different stages of their development has become commonplace (UICC, 1985).

Following the conception of a TNM system (Denoix, 1946; Denoix, 1950 and Denoix, 1952) for classifying and staging malignant neoplasms, the International Union against Cancer appointed a committee on tumour nomenclature and statistics.
The Committee adopted, as a basis for its work on clinical stage classification, the general definitions of local tumor extension suggested by the World Health Organisation (WHO) Subcommittee on the registration of cancer cases as well as their statistical representation (WHO, 1952). Meeting with the Committee on Stage-Grouping in Cancer at the International Congress of Radiology in Copenhagen in 1953, the UICC reached agreement on a general technique for staging and, one year later, established a Special Committee on Clinical Stage Classification and Applied Statistics to extend the technique of classification to cover all sites. The Committee published its first recommendations for the clinical stage classification of cancers of the breast and larynx (UICC, 1958) and between 1960 and 1967 published a further nine brochures each describing the classification, and in several cases, clinical staging of 23 sites, which culminated in a separate paper outlining the roles of the TNM system. Of interest in this section, these brochures included the classification of malignant tumors of the oral cavity (including the lip), the pharynx and the larynx (UICC, 1963), malignant tumors of the lung (UICC, 1966a), oesophagus, stomach, colon and rectum (UICC, 1966b) and the skin (UICC, 1966c).

In 1966, the UICC reorganised its committee structure, and the classification committee was renamed the Committee on TNM Classification. National committees on TNM classification
were established in many countries, some of the most active being the American Joint Committee on Cancer (AJCC), The French TNM Group (FTNM), the European Organisation for Research on Treatment of Cancer (EORTC) and, in this country, the British Isles Joint TNM Classification Committee (BIJC).

In 1968, the UICC brochures were combined in a booklet, the Livre de Poche, (UICC, 1968), and one year later a complimentary booklet was published which contained recommendation for field trials, presentation of end results, and the determination and expression of cancer survival rates. The head and neck primary sites included the oral cavity and lip, pharynx and larynx, thyroid and external skin while the cervical trachea and oesophagus were included as sub-sites.

The regional lymph nodes were classified as in Table 1, a classification which had remained unchanged since the conception of the TNM system (UICC, 1958). In addition subcategories were employed (a) where lymph nodes were palpable but not expected to contain metastatic growth and (b) where nodes were considered to contain tumour (i.e. $N_{1a}$ or $N_{1b}$). The Livre de Poche was subsequently translated into eleven languages and subsequent 2nd and 3rd editions appeared in 1974 and 1978. No new primary head and neck sites were added and the staging of cervical lymphadenopathy remained the same until the third edition was revised in 1982 when the (a) and (b) subcategories were removed. The 4th edition, published
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<th><strong>UICC</strong></th>
<th><strong>AJC</strong></th>
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<tr>
<td><strong>N₀</strong></td>
<td>No evidence of lymph node metastasis</td>
<td>No evidence of lymph node metastasis</td>
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<td><strong>N₁</strong></td>
<td>Involvement of movable ipsilateral nodes</td>
<td>Single positive ipsilateral node 3 cm or less in diameter</td>
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<td><strong>N₂</strong></td>
<td>Involvement of movable contralateral or bilateral nodes</td>
<td>Single ipsilateral node more than 3 cm but not more than 6 cm (N₂a); or multiple ipsilateral nodes, none more than 6 cm (N₂b)</td>
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<td><strong>N₃</strong></td>
<td>Involvement of fixed regional lymph nodes</td>
<td>Ipsilateral nodes, one over 6 cm (N₃a); bilateral nodes (N₃b); or contralateral nodes (N₃c)</td>
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**UICC, 1982**

**AJCC, 1977**
in 1987, classifies and stages over 40 cancer sites of which nine are within the head and neck (including the external skin and cervical oesophagus as sub-sites) and, of these, the maxillary sinus and salivary glands are included for the first time. Major changes are also made in the classification of the regional lymph nodes, the system adopted (Table 2) being a modification of that suggested by the AJC in 1977 (Table 1).

Over 25 years ago, the AJC brought out its own classification for the larynx (Smith et al, 1961), and this was followed by similar classifications for the pharynx (Smith et al, 1963) and the oral cavity (Smith et al, 1967). The staging of regional lymphadenopathy at this time was the same as that which had been recommended by the UICC since its initial conception (UICC, 1958). The staging of supraglottic cancer was changed in 1972 and based on further recommendations (Chandler et al, 1976) changes were made in 1977, not only for the larynx but also for regional cervical lymphadenopathy, to coincide with the publication of the first AJC manual for the staging of cancer (AJCC, 1977). This listed similar head and neck sites to its equivalent UICC partner (UICC, 1974) except that the maxillary sinus and salivary glands were included.

The changes suggested by the AJC in 1977 when revising its 1972 staging system for supraglottic laryngeal cancer
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<tr>
<td>N₀</td>
<td>No regional lymph node metastasis</td>
</tr>
<tr>
<td>N₁</td>
<td>Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension</td>
</tr>
<tr>
<td>N₂</td>
<td>Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension, or in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension, or in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension.</td>
</tr>
<tr>
<td>N₂ᵃ</td>
<td>Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension</td>
</tr>
<tr>
<td>N₂ᵇ</td>
<td>Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension</td>
</tr>
<tr>
<td>N₂ᶜ</td>
<td>Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension</td>
</tr>
<tr>
<td>N₃</td>
<td>Metastasis in a lymph node more than 6 cm in greatest dimension.</td>
</tr>
</tbody>
</table>

UICC, 1987; AJCC, 1988
included reclassifying $T_{1b}$ carcinomas as $T_2$ carcinomas, reclassifying extension onto the medial wall of the pyriform sinus or postcricoid mucosa as $T_3$ carcinomas rather than $T_4$ carcinomas and changing $T_4N_0$ carcinoma from Stage III to Stage IV disease. These changes came about due to the redefinition of the laryngeal boundaries to include the lingual surface of the epiglottis and allowing tumour extension to the postcricoid mucosa and medial wall of the pyriform sinus to remain in the $T_3$ classification although they are extra-laryngeal. In addition the N classification was restructured, and bilateral and contralateral nodal metastases were changed from Stage III to Stage IV.

Johns et al (1982) critically evaluated these changes with prognosis by reviewing 178 patients with supraglottic laryngeal cancer treated between 1955-1976. Each case was classified using both the 1972 and 1977 AJC TNM system and Johns et al concluded (as have others workers (Black and Gluckman, 1983)) that all the changes made in the 1972 system were justified except changing $T_4N_0$ supraglottic cancers from Stage III to Stage IV.

On reviewing recent handbooks (UICC, 1982; AJCC, 1983) both the UICC and AJC recommended a TNM classification system for the nasopharynx, oropharynx, hypopharynx, larynx and oral
cavity but restrict it to carcinomas. Although the AJC included a classification for cancer of the nose and paranasal sinuses, the UICC states it had no recommendation to make. The salivary glands are included in the AJC classification but not in the UICC classification. The anatomical limits of regions, sites and sub-sites were the same in both systems except that the lingual surface of the epiglottis was classified as part of the supraglottic larynx by the AJC and oropharynx by the UICC; and the superior surface of the soft palate, part of the oropharynx by the AJC and nasopharynx by the UICC.

The T category descriptions were the same in both systems with tumour size forming the basis for oral cavity and oropharynx; and tumour site (with degrees of local extension) the basis for larynx, hypopharynx and nasopharynx.

The criteria for classification of nodal status was completely different in the two systems. The UICC classification since its inception has had four categories; $N_0$-$N_3$ (UICC, 1982). $N_2$ has always been used for bilateral mobile nodes and $N_3$ for fixed nodes on one, or both sides of the neck. The UICC recognised the significance of cervical node involvement and placed particular emphasis on mobility versus fixation and the importance of contralateral and bilateral
neck disease. The UICC also made recommendations (UICC, 1978) that the level of the cervical lymph node involvement be recorded but stated such procedures were not mandatory. Similar recommendations appeared four years later (UICC, 1982).

The AJC, however, although adopting similar symbols has always used a different classification. In 1961, the AJC brought out its classification for the larynx (Smith et al, 1961) and for the pharynx in 1963 (Smith et al, 1963). Patients with mobile nodes on one or both sides of the neck were classified as N₁ and all those with fixed nodes, also on one or both sides, were included in category N₂. Smith et al made a revision in 1967 and added a third category of N₃ for fixed or bilateral nodes. Of note, the staff of the M.D. Anderson Hospital in Houston, (Texas, USA) have used their own scheme since the late 1950's (Barkely et al, 1972). This classification is shown in Table 3 and it will be seen N₃b also covers two categories; nodes on both sides of the neck which may be fixed or mobile. This information is important when analysing data published from that Institution. Thus whatever system is used there were only two categories to cover three different possible combinations of side and fixation: mobile nodes on both sides of the neck; nodes on both sides, one or more being fixed; and fixed nodes on one side of the neck.
TABLE 3
THE M.D. ANDERSON HOSPITAL CLASSIFICATION FOR REGIONAL CERVICAL LYMPHADENOPATHY

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>N₀</td>
<td>No clinically positive node</td>
</tr>
<tr>
<td>N₁</td>
<td>Single clinically positive node less than 3 cm in diameter</td>
</tr>
<tr>
<td>N₂ₐ</td>
<td>Single clinically positive node greater than 3 cm in diameter</td>
</tr>
<tr>
<td>N₂ₐ</td>
<td>Multiple clinically positive ipsilateral nodes</td>
</tr>
<tr>
<td>N₃ₐ</td>
<td>Unilateral fixed node (s), clinically positive</td>
</tr>
<tr>
<td>N₃ₐ</td>
<td>Clinically positive bilateral nodes, fixed or not fixed</td>
</tr>
</tbody>
</table>

Barkely et al, 1972
Patients with nodes on both sides of the neck, one of which is fixed, have received little attention in the literature probably since such patients cannot be classified separately. In the first AJC classification for pharyngeal carcinoma, Smith et al (1963) described 1320 such patients and stated 'in a few instances of bilateral nodes in oropharyngeal and hypopharyngeal cancer patients, there were no five year survivals'.

It has been known for thirty years that the prognosis of a patient with a fixed cervical lymph node in the neck is extremely poor. Smith et al (1961) reported a series of 600 patients with laryngeal cancers and of these, fourteen (2.3%) had fixed nodes and of these only one patient of the 14 (7%) survived five years, compared with 40% of patients with mobile nodes. Two years later in a retrospective study of 1320 patients with hypopharyngeal cancer, Smith et al (1963) reported 216 patients (16.4%) had fixed nodes, 30 (2.3%) had nodes on both sides of the neck and 186 (14.1%) had nodes on one side. The effect of fixation was significant. The five year survival for unilateral fixed nodes was 7% and for bilateral nodes, 0%.

The word 'fixed' is open to interpretation in different ways by different surgeons. Spiro et al (1974a) used the term to describe lymph nodes which were immobile and fixed
to the underlying scalene muscles, prevertebral fascia or skull base. The term "reduced mobility" was applied if any restriction of mobility was present, that is, the node or nodes being neither freely mobile nor "fixed". Santos et al (1975) described fixation to indicate attachment to adjacent structures such as the mandible, the skull base, the carotid artery, the deep muscles of the anterior and posterior triangles, the clavicle and the skin of the neck. This difference in usage would explain the varying proportions of fixed nodes in different series from about 2-16% (Smith et al, 1961; Smith et al, 1963; Spiro et al, 1974a; Black and Gluckman, 1983; Stell, 1983; Stell et al, 1984) up to 35% (Snow et al, 1982). Indeed Spiro et al (1974a) showed the importance of the interpretation of the word "fixed". In this series Spiro et al showed for tumours of the oral cavity and oropharynx the five year cure rate for single nodes of "reduced mobility" was 40%, but only 13% for fully fixed nodes.

Because of the wide variation in the individual interpretation of the word "fixed", the AJC discarded its use (Chandler et al, 1976) and now relies on size and number of lymph nodes (Chandler et al, 1976; AJCC, 1977). Indeed it has been shown that a node is unlikely to be truly fixed until it is 6 cms in diameter (Santos et al, 1975).
The criteria, then, for the classification of nodal status were completely different in the two systems. The significance of cervical node involvement was recognised, but the AJC attached importance to the size and number of homolateral nodes whereas the UICC put particular emphasis on mobility versus fixation. Both attached importance to contralateral or bilateral neck node disease but not in the same way.

What then of patients with nodes on both sides of the neck, one of which is fixed? The AJC classification (AJCC, 1977) states that each side of the neck should be classified separately if both sides are invaded but no such criteria exists in the UICC classification (UICC, 1978). Stell (1983) suggested a fourth category which might be called $N_4$ (i.e. $N_3 + N_1$) to cover the patient with bilateral nodes, one of which is fixed, and he compared three groups of patients classified $N_2$ and $N_3$ under the UICC system and 'N4' as previously suggested. He was able to show that patients with bilateral nodes, one of which is fixed, constitute a separate group and few of them can be treated. Their prognosis is significantly worse than patients with bilateral mobile nodes or unilateral fixed nodes, and Stell concluded if this group is not to have a separate category it should be logically included with bilateral nodes as is done in the AJC system ($N_{3b}$) or in the M.D. Anderson System ($N_{3b}$), but not in the UICC system.
The UICC also made recommendations (UICC, 1978) that the level of cervical lymph node involvement be recorded but to date this has not been officially incorporated into the N category description.

The UICC and the AJC have used the TNM system to describe the anatomical extent of cancer at the time of diagnosis before the commencement of definitive treatment. In addition the AJC stated (1977) that classification and staging could be done at various times during the natural history of the cancer patients management. These various staging possibilities are cTNM (clinical diagnostic staging); sTNM (surgical evaluative staging); pTNM (post-surgical treatment pathological staging); rTNM (re-treatment staging) and aTNM (autopsy staging). The UICC included several additional descriptive terms for greater classification. The symbol "y" was introduced for cases where definitive surgery was used after treatment by other methods and employed a system to indicate the type of examination on which the TNM categories were based (UICC, 1982). This system was called the level of certainty or "C" factor and consisted of five categories, C1-C5. Categories C1-C3 were equivalent to cTNM, C4 was equivalent to pTNM and C5 was equivalent to aTNM. Distant metastases carry the same significance in both systems (UICC, 1982; AJCC, 1983) in which the symbol M₁ indicates evidence of their presence.
In view of the classification differences that existed between the two systems (AJCC, 1977; UICC, 1978), the question arises as to whether this has any effect on survival data? Looking at the two systems it seemed that data on the 'T' levels for the hypopharynx and oral cavity could be directly interchanged. Data on the oropharynx could also be directly interchanged (despite UICC and AJC anatomical overlap with the supraglottic larynx and nasopharynx) because the 'T' categories were based on size with no mention of upgrading if the level extended beyond the anatomical limits of the oropharynx. This principle could not be applied to the supraglottic larynx and nasopharynx because there is no such qualification.

Such effects were assessed (Black and Gluckman, 1983) by evaluating 156 cases of supraglottic laryngeal carcinoma treated at the University of Cincinnati (USA) from 1969 to 1979. Nine cases (6.8%) of $T_{1/2/3}$ tumours (AJCC) would have been classified $T_4$ under the UICC system because of involvement of the lingual surface of the epiglottis (i.e. extension beyond the larynx). While this was a significant number, comparison of survival based on the 'T' categories alone did not differ between the two systems. The same considerations were applied to the nasopharynx and no cases would have been classified differently. Although the soft palate was involved in six out
of 17 cases, all satisfied additional criteria of extension beyond the nasopharynx to be classified T₃ in both systems (i.e. spread to the upper pole of the tonsil or down the lateral pharyngeal wall). However, no firm conclusions were drawn from this data due to the small sample size. The authors also examined 373 cases of squamous cell carcinoma of the larynx, oropharynx, hypopharynx and oral cavity who presented with clinically involved cervical nodes. Of the N₁ (AJCC) group, 3.8% had fixed nodes and therefore would be classified N₃ UICC. Because of the different importance attached to bilateral and contralateral nodes no N₂ (AJCC) nodes were comparable. Seventy-five percent of nodes were reported as 'moveable' and hence would be N₁ UICC; and the remaining 25% which were fixed, N₃ UICC. Only 52% of N₃ AJC nodes were fixed, with the others being distributed between N₁ and N₂ UICC depending on the side (s) of involvement. All these differences were significant but there was no difference in the two year survival data by comparison of N levels. Such good correlations between the two systems, despite multiple differences in staging criteria, has been confirmed by other workers (Bataini et al, 1985).

However, Johns et al (1984) evaluated 356 patients with primary sites of the supraglottis, tonsil and pyriform sinus who were staged retrospectively using both the UICC and AJC staging criteria (UICC, 1982; AJCC, 1983). They showed
using the UICC classification, a statistical difference
was detected only between the presence or absence of cervical
metastases and that the system could not statistically
discriminate between $N_1$, $N_2$ or $N_3$ groups. The AJC system,
however, was highly statistically discriminatory between nodal
classes and the authors concluded the AJC system of staging
cervical lymph nodes was significantly more discriminating in
terms of estimating survival than the UICC system, and that
the UICC nodal staging system should be revised.

Johns et al (1984) also showed that when bilateral neck
disease was present, there was no significant difference in
five year survival between patients with $N_{3b}$ necks and those
with $N_{2a}$ and $N_{2b}$ necks, and that this was due to the presence
of early bilateral ($N_1$) disease.

One of the stated aims of the TNM system is to act as a
prognostic guide (UICC, 1974) and, in general terms for
most cancers, Stage I will have the best prognosis and
Stage IV the worst. A precise staging system for oral cancer,
with universal applications, would provide invaluable
information (Binnie et al, 1972) and a variety of different
TNM classifications have been proposed for carcinoma of the
oral cavity (AJCC, 1967; Schwab, 1968; UICC, 1968;
Indeed it was stated quite recently that there is no generally
accepted pre-therapeutic classification of oral carcinoma and
therefore some uncertainty about which classification is of most value in clinical practice (Platz et al, 1983). However, it is well recognised due to clinical experience together with statistical analysis that a proper judgement of any oral cavity tumour requires consideration of both lesion site and histology (Sakai and Masaki, 1971; Binnie et al, 1972; Fayos, 1972; Fayos and Lampe, 1972; Fries et al, 1973; Spiessel et al, 1973; Westbrook, 1974). Such considerations led some workers (Langdon et al, 1977; Rapidis et al, 1977) to propose a modification of the TNM system for oral cancer known as the STNMP (site, tumour, nodes, metastases, pathology) system. In this modification a weighted numerical score was ascribed not only to the tumour, nodes and metastases but also to the primary site and its pathological grade. Langdon et al found the STNMP system was more accurate than the TNM system in staging oral cancer patients with early disease and gave a better prediction of which patients would do badly. Despite such claims, further work (Rich and Radden, 1984) comparing the TNM and STNMP system in oral squamous cell carcinoma showed that although both staging systems separated patients into those with either a good or poor prognosis, the more sophisticated STNMP system did not provide any additional information. For this reason it was not adopted into current clinical practice and the alternative TNM classification was preferred (UICC, 1982; AJCC, 1983).
The TNM committees of the UICC and AJC have been working along similar lines with common objectives although both opinions and methodology have varied from time to time. In the search for common understanding, regular meetings have been held between representatives of the UICC and AJC regarding cancer classification in an attempt to unify the TNM staging system (Beahrs et al, 1977). Recently both groups have agreed on such a classification scheme (Sessions, 1986; Richard et al, 1987) and this is reflected in current publications (UICC, 1987; AJCC, 1988). However, controversy still exists as to whether or not the TNM system of classification represents a complete description of the disease status of the patient (Sessions, 1986).

Finally, at its meeting in Copenhagen in July, 1954, the UICC adopted "the realisation of a clinical atlas" but such a planned book of illustrations underwent a prolonged metamorphosis until the National Committee and International Organisations had officially recognised the classification of malignant tumours at various sites as presented in the third edition of the TNM booklet (UICC, 1978). The first edition of the TNM atlas appeared in 1978, the second edition seven years later (UICC, 1985) and the latest edition (UICC, 1989) is similar in structure to the current TNM booklet (UICC, 1987). The text is limited to bare
essentials so that consultation provides immediate information on the history, principles and general rules of the TNM system. Together the booklet and atlas aim to fulfill the utopian goal of making the clinical classification of malignant tumours as routine as their histopathological classification.
1.1.4. CURRENT STATUS

Both the UICC and the AJC have used the TNM system to describe the anatomical extent of head and neck cancer at the time of diagnosis and before the initiation of definitive treatment. More recently a joint UICC-AJC TNM and stage classification for each specific head and neck site has been proposed and the fourth edition of the TNM classification of malignant tumours (UICC, 1987) has rules of classification and staging exactly the same as those appearing in the third edition of the AJC manual for the staging of cancer (AJCC, 1988) and has the approval of all national TNM committees. Its general principles are described below.

**General Rules**

The TNM system for describing the anatomical extent of head and neck cancer is based on the assessment of three components, namely T, the extent of the primary tumour; N, the presence or absence and extent of regional lymph node metastases and M, the presence or absence of distant metastases. All cases are identified by T, N and M categories, which must be accurately determined and recorded before treatment is commenced. The system is confined to carcinoma for all sites (and to squamous cell carcinoma of the lips and oral cavity) and malignancy must be confirmed by histological examination.
Two classifications have been described for each site. There is a clinical classification (pre-treatment clinical classification), designated cTNM, and a pathological classification (post-surgical histopathological classification, designated pTNM). For cTNM, traditional staging demands that certain pre-requisite patient assessment be performed and its use reflects the level of certainty according to the particular diagnostic method used.

The UICC-AJC classification suggests that for each site the specific methods of investigation available for TNM classification should be listed. These include mandatory methods such as clinical examination and biopsy which should always be employed to establish the extent of a tumour, additional methods such as conventional radiography which may be of use at a particular site and special investigations such as computerised axial tomography (CAT) or magnetic resonance imaging (MRI) that are only available in some centres and which are written into a TNM classification only when generally available. The pTNM classification is based on evidence acquired before treatment, supplemented or modified by additional information acquired either surgically or pathologically. The pathological assessment of the primary tumour entails a resection or biopsy adequate to evaluate the highest pT category. The pathological assessment of the regional lymph nodes (pN) entails removal of sufficient nodes to adequately validate the absence of regional lymph node metastases (pN\textsubscript{0}) and to obtain the highest pN category.
Further information regarding the primary lesion may be recorded under the headings "G" for histopathological grading, "L" for lymphatic invasion and "V" for venous invasion. The presence or absence of residual tumour after treatment may be described by the symbol "R". The current cTNM, pTNM and histopathological staging systems are all available in current literature (UICC, 1987).

Within the current classification (UICC, 1987) each head and neck site is listed by a code number of the international classification of diseases for oncology (ICD-O, World Health Organisation (WHO), UICC, 1987, AJCC, 1988). Each site is described under a TNM heading (mandatory) and a cTNM and pTNM classification (optional). There are a number of additional descriptions available. In those cases where classification was performed during or following initial multinodal therapy, the TNM categories are identified by a "y" prefix. Likewise recurrent tumours are identified by the prefix "r". After being assigned various T,N and M categories, patients are grouped into a number of clinical stages (Table 4).

Primary Head and Neck Squamous Cell Carcinoma

Within the TNM classification (UICC, 1987), the lip and oral cavity, pharynx, larynx, maxillary sinus and salivary
TABLE 4

THE CURRENT STAGE GROUPING FOR HEAD AND NECK CARCINOMA

<table>
<thead>
<tr>
<th>CLINICAL STAGE</th>
<th>TNM GROUPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>T₁, N₀, M₀</td>
</tr>
<tr>
<td>II</td>
<td>T₂, N₀, M₀</td>
</tr>
<tr>
<td>III</td>
<td>T₃, N₀, M₀</td>
</tr>
<tr>
<td></td>
<td>T₁, T₂, T₃; N₁, M₀</td>
</tr>
<tr>
<td>IV</td>
<td>T₄, N₀ or N₁, M₀</td>
</tr>
<tr>
<td></td>
<td>Any T, N₂ or N₃, M₀</td>
</tr>
<tr>
<td></td>
<td>Any T, Any N, M₁</td>
</tr>
</tbody>
</table>

UICC, 1987; AJCC, 1988
glands are listed as primary sites, the pharynx being subdivided by convention into the nasopharynx, oropharynx and hypopharynx. Together with the cervical oesophagus this makes eight sites within the head and neck, the maxillary sinus and salivary glands being new additions to the current system.

Classification is limited to carcinoma at all sites except the lip and oral cavity (squamous cell carcinoma only), although it is well recognised that over 85% of all head and neck carcinoma (excluding skin) is squamous cell in origin (Cann et al, 1985).

Although squamous carcinoma of the external skin is the commonest head and neck malignancy (Levine et al, 1985) it is excluded from the above classification since it can be considered a different disease behaving in an entirely different manner, except in its later stages (Levine et al, 1985). The thyroid gland is listed as a primary site for head and neck carcinoma in the current UICC booklet. However, within the pathological distinctions that exist, squamous cell carcinoma would be included in the unclassified section and is mentioned here only to be dismissed although squamous cell carcinoma is a well recognised occurrence within the gland (Goldman, 1964; Korovin et al, 1989).
Numbers (i.e. $T_1$ or $T_2$) indicate degrees of increasing extent of the primary tumour, and the number of $T$ categories may vary from site to site. In general the UICC has recommended that there be four. There are a limited number of qualities of each tumour assessed on clinical examination such as direct size measurement or a "yes" or "no" distinction, mobility, non-mobility, fixation or non-fixation. Those tumours which are easiest to classify are those arising in a single organ such as the tongue where exact anatomical localisation is possible (Sessions, 1986). Alternatively there are some tumours such as those in the floor of the mouth where size and extent are not as easily ascertained (Fayos, 1972). These tumours can then be described as to the extent of invasion or involvement of the surrounding structures. Lastly there are those tumours, such as those involving the maxillary antrum, which are more difficult to diagnose without taking into account the operative findings (Sakai and Masaki, 1971). Multiple tumours should be classified independently and in the case of multiple synchronous tumours in one organ, the tumour with the highest $T$ category should be classified and the multiplicity or number of tumours indicated in parenthesis.

**Regional Lymph Nodes**

The regional lymph nodes of the head and neck are shown in Figure 2 and the current $N$ classification in Table 2
Regional Lymph Nodes
The regional lymph nodes are the submental nodes (1), the submandibular nodes (2), the cranial jugular (deep cervical) nodes (3), the medial jugular (deep cervical) nodes (4), the caudal jugular (deep cervical) nodes (5), dorsal cervical (superficial cervical) nodes along the accessory nerve (6), the supraclavicular nodes (7), the prelaryngeal and paratracheal nodes (8), the retropharyngeal nodes (9), the parotid nodes (10), the buccal nodes (11), and the retroauricular and occipital nodes (12).
The old classification is shown in Table 1 (AJCC, 1977; UICC, 1982). The current N classification is applicable to all head and neck tumours except the cervical oesophagus and the thyroid gland which have different classifications based on the presence (N₁) or absence (N₀) of regional metastases. Under the current joint classification, the clinical findings regarding regional cervical lymphadenopathy are defined for each site independent of the primary tumour. N₀ indicates no palpable adenopathy whilst a designation such as N₁, N₂, or N₃ describes the clinical state of the nodes that are palpable. Lymph nodes are described as ipsilateral, bilateral, contralateral or midline; as single or multiple; and by measured size, exact number and anatomical location. Midline nodes are considered ipsilateral. During clinical examination the actual size of the nodal mass should be measured, and allowance made for the intervening soft tissues (Sessions, 1986). It is well recognised that most masses over 3 cms in diameter are not single nodes but represent confluent nodes or tumour in the soft tissue compartments of the neck (Sisson and Pelzer, 1985). The current UICC classification recommends that, although the level of involvement of cervical lymph nodes is not incorporated into the current N-classification, it should be recorded wherever possible. The logic behind this is that the level of involvement may directly affect treatment and
prognosis (Trible and Dias, 1964; Farr and Arthur, 1971; Spiro et al, 1974a; Stell et al, 1983; Lefebvre et al, 1987). There are four levels of cervical lymph nodes within the current UICC classification and these are shown in Figure 3.

**Distant Metastases**

The joint staging recommendations of the UICC-AJCC (UICC, 1987; AJCC, 1988) for the classification of distant metastases states that the presence or absence of metastatic disease should be indicated by the letter M. Microscopic confirmation of metastatic carcinoma is mandatory. M₀ indicates that no metastasis can be detected clinically and M₁ indicates that metastases other than the regional lymph nodes are present. M₁ can be subdivided to include the anatomical area involved such as pulmonary (PUL), hepatic (HEP) or brain (BRA)
The Cervical Lymph Node Levels (UICC, 1985)

Level of Involvement of Cervical Lymph Nodes

The level of involvement may have a bearing on both treatment and prognosis. Although these levels are not incorporated in the N category definitions at present, it is recommended that they should always be recorded.

Level 1. Lymph nodes palpable in the submandibular and/or submental regions.

Level 2. Lymph nodes palpable distal to level 1 and confined to the region above the skin crease at or just below the level of the thyroid notch.

Level 3. Lymph nodes palpable distal to level 2 and confined to the anterior cervical triangle including those deep to the sternocleidomastoid muscle.

Level 4. Lymph nodes palpable distal to level 3 and confined to the posterior cervical triangle.
1.1.5. CRITICISMS OF THE SYSTEM

Many options are available in the treatment of head and neck cancer. The traditional roles of surgery (Schuller et al, 1979) and radiotherapy (Parsons et al, 1982) continue to be evaluated along with the planned sequential use of these therapeutic options (El Badawi et al, 1982; Kumar et al, 1987; Mendenhall et al, 1987). The addition of various chemotherapeutic protocols (Oster, 1981; Jacobs et al, 1987; Johnson et al, 1987) hyperthermia (Kim and Hahn, 1979) and immunotherapy (Vogl et al, 1982; Webster et al, 1982) all increase the armamentarium of the head and neck oncologist. However, the only means of assessing the role each of these modalities has to play is by the evaluation of properly controlled prospective trials and usually, but not always, sufficient numbers for valid statistical evaluation can only be obtained from large institutions and most successfully from multi-institutional co-operative trials.

The exchange of information occurs nationally and internationally and for direct comparison the same staging systems should be used. Until recently two major systems were in use (UICC, 1982; AJCC, 1983) but the need for a unified system has long been recognised (Beahrs et al, 1977)
due to recognised conflict between the two classification systems (Rubin, 1971; Johns et al, 1984). Such discrepancies have resulted in confusion when comparing different treatment regimes (Rubin, 1971; Brill, 1979; Black and Gluckman, 1983; Johns et al, 1984).

The TNM system provides head and neck surgeons with a common means of communication that is clinically orientated and which is based on pre-treatment diagnostic studies. No one system is perfect, and the criticisms that were aimed at the old UICC and AJC classifications focused on the numerous sub-categories which contained so few cases per category that statistical conclusions could not be drawn. In addition there was the lack of agreement on anatomical boundaries, the staging of cervical lymphadenopathy and the fact that host-tumour responses and histopathological findings were not taken into account (Harrison, 1979; Black and Gluckman, 1983; Johns et al, 1984). On reviewing the current handbook (UICC, 1987) a TNM classification is recommended for the head and neck sites but it is limited to carcinomas (squamous cell carcinoma for the lip and oral cavity). Emphasis is still placed on 'T', tumour size, although it is well recognised that size alone is of little prognostic significance in many head and neck carcinomas (Cachin et al, 1979; Black and Gluckman, 1983; Hibbert et al, 1983; Platz et al, 1983; Stell and McCormack, 1985a; Moore et al, 1986; Gavillan et al, 1987).
In addition, head and neck surgeons are aware of the difficulties in assessing the extent of primary disease in the oral cavity, pharynx, larynx or paranasal sinuses. For tumours of the oral cavity and oropharynx, the progressive assignment of levels $T_1$ through to $T_4$ is based on the assumption that the size of the tumour can be readily measured. The increasing severity with $T_4$ is reserved for tumours greater than 4 cms in diameter with any evidence of deep invasion into muscle, bone or other adjacent structures. There can be little difficulty in establishing the difference between $T_1$ and $T_4$ disease but treatment planning and prognostic significance become less clear when the tumour measures between 1.5 and 3.0 cms. Bony invasion of the mandible demonstrated radiographically is classified as $T_4$ disease. The question arises as to what constitutes bony erosion? A 2 cm lesion in the anterior floor of mouth that involves the alveolar ridge and which is adherent to the periosteum will not necessarily demonstrate bony erosion on radiographic evaluation. However, most surgeons agree that the underlying bone should be included in the surgical resection and therefore $T_2$ and $T_4$ disease may require essentially the same treatment. A similar problem is encountered in determining the depth of invasion of lesions into the soft tissues of the floor of the mouth. Superficial invasion of the sublingual area as opposed to invasion of the mylohyoid muscle can be subtle.
Again a small difference exists between $T_2$ and $T_4$ disease and the resultant surgical and prognostic implications. All the above measurements are open to subjective interpretation which is a recognised problem in the assessment of head and neck tumours (Brill, 1979).

In contrast, the nasopharynx, hypopharynx and larynx are classified according to the number of anatomical surfaces involved. However, in the past there has been considerable disagreement and inadequate definition of the anatomical boundaries of these regions and, in particular, the larynx and hypopharynx (Ogura and Mallen, 1965; Harrison, 1970; Black and Gluckman, 1983). Such confusion continues since, to date, neither classification (UICC, 1987; AJCC, 1988) defines the lateral limits of the posterior pharyngeal wall. In addition, the $T_3$ extension of a nasopharyngeal tumour into the oropharynx or a $T_3$ laryngeal lesion do not have the same grave consequences of a $T_3$ hypopharyngeal tumour with fixation of the hemilarynx. What then of a $T_3$ supraglottic tumour with fixation of the supraglottic larynx? Surely fixation of the supraglottic larynx suggests invasion of the soft tissues of the neck and should therefore qualify as $T_4$ disease. There is considerable inter-observer error not only in the accurate reporting of this finding (Sisson and Pelzer,
1985) but also other laryngeal pathology (Robinson and Weir, 1987) and, in addition, the use of ambiguous terms such as "fixation" (Stell et al, 1984) adds to the variable factors of human judgement and error.

In a patient with a head and neck squamous cell carcinoma, the spread of disease to the regional lymph nodes is one of the most important prognostic indicators (Cachin et al, 1979; Schuller et al, 1980; Yamamoto et al, 1984; Grandi et al, 1985; Sisson and Pelzer, 1985). Although the presence of metastatic lymph node disease is an established high-risk factor, opinions concerning the correlation between various extents of neck metastases and prognosis differ. Number, size, location, extranodal spread and immunological status have been evaluated by many authors and the conclusions reached have often been controversial. This is due to the large number of factors evaluated, the various therapeutic modalities adopted, and the various lengths of follow-up, each with a different patient selection. The biggest problem in the evaluation of neck disease is the subjective error associated with its clinical and pathological examination. This is such a large and important topic that it is studied in depth in a subsequent section (1.4.).
Within the current UICC classification (UICC, 1987) emphasis is based on both the number and the size of metastatic lymph nodes. Most authors agree that the number of nodes is of prognostic significance (Trile and Dias, 1964; Cachin et al, 1979; Schuller et al, 1980; Snow et al, 1982; Platz et al, 1983; Gavilan, 1987; Richard et al, 1987). Similarly, the prognosis decreases as the nodal size increases (Sessions, 1976; Grandi et al, 1985; Richard et al, 1987) although this is due, in part, to nodal number since the progression from \( N_1 \) to \( N_2 \) disease is based on the experience that most masses greater than 3 cms in diameter are not single nodes but confluent nodes or tumour in the soft tissues of the neck.

Criticism of the current system must centre around the fact that the \( N \) designation is not sophisticated enough since it does not provide a description of the level of nodal involvement which is a recognised prognostic indicator (Trile and Dias, 1964; Barrie et al, 1970; Spiro et al, 1974a; Shah et al, 1976; Stell et al, 1983; Lefebvre et al, 1987). Similarly the immunological and pathological status of the lymph nodes are not included in the current TNM system despite the fact that both the immunology of lymph nodes (Ortega et al, 1987), and the presence of extracapsular nodal invasion (Johnson et al, 1981;
Carter et al, 1985; Johnson et al, 1985; Snyderman et al, 1985) are important prognostic indicators. The inclusion of bilateral or contralateral disease as N₂ is confusing since it implies a better prognosis than N₃ disease. Such a statement is unjustified, particularly since a similar problem existed with previous UICC classifications (UICC, 1974; UICC, 1978; UICC, 1982) and was recognised by previous workers (Stell, 1976).

The word "fixation" has, at least, been removed from the current nodal classification since it was open to wide and varied subjective interpretation. However, the classification of N₃ disease includes nodes which are greater than 6 cms in size by which time they are invariably fixed (Santos et al, 1975). No mention is made of Stell's N₄ group (Stell, 1983) which includes patients with bilateral nodes, one of which is fixed. Since the word "fixation" has now been removed, it is difficult to know whether these patients should be placed in the N₂ or N₃ categories because, as stated previously, "fixed" usually means nodes which are 6 cms or more in size. It is an interesting observation that, despite all the criticisms aimed at the word "fixation" and its inclusion in recent UICC classifications (UICC, 1982), some authors have criticised the AJC for not including it in their system (Sisson and Pelzer, 1985). Although most authors agree that
pathological factors such as extracapsular spread are more reliable than their clinical counterparts in predicting prognosis in metastatic head and neck squamous cell carcinoma (Grandi et al., 1985), we must wait for their mandatory inclusion into the current TNM classification.

The classification of cervical lymph nodes is now the same for the two systems (UICC, 1987; AJCC, 1988) and puts emphasis on both the size and number of lymph nodes. However, this system includes contralateral and bilateral nodes as N₂ disease. This surely reflects a retrograde step, particularly for contralateral disease, especially when one considers previous classifications (AJCC, 1983). For bilateral disease, upstaging from N_{3b} to N_{2c} has occurred and this is probably due to the work of Johns et al. (1984), although such upstaging is based on the presence of early (N₁) bilateral disease. This represents a misinterpretation of the data (Michael Johns, Personal Communication, 1988).

Each side of the neck should be staged separately and the patient placed in the most advanced group. The presence of bilateral nodes does not make the neck automatically N₃ and, in fact, N₃ disease does indicate a poor prognosis. In addition, no specific recommendations are made concerning the separate staging of each side of the neck (UICC, 1987)
and there remains confusion about the level of lymph node involvement. The UICC defines four levels (Figure 3) but their inclusion into TNM staging is not mandatory. The AJC makes no mention of the lymph node level, although recommends that the position of any lymph node involvement should be recorded pictorially. However, the Memorial Sloan-Kettering Cancer Centre has used five levels of distribution for some years (Shah et al, 1981) and these are included in standard American textbooks (Sessions et al, 1986). Obviously there is a need to standardise cervical lymph node level nomenclature, and this has been discussed in recent publications (Suen and Goepfert, 1987). A controlled prospective study is needed to show whether the level of a node is more prognostic than its size and, if so, whether the difference is statistically significant.

Therefore the current UICC-AJC joint classification should be regarded as an improvement since it groups progressive lymph node involvement in a more logical manner when compared with the previous UICC classification (UICC, 1982). However, the present system would appear inferior to previous AJC classification (AJCC, 1983) since it classifies contralateral and bilateral nodes as $N_2$ disease rather than the $N_3$ grouping it deserves. The incorporation of early ($N_1$) bilateral disease as $N_{2c}$ could, however, be
justified (Johns et al, 1984) but this is not made clear in the classification and, as such, all bilateral disease is included.

Since Sebileau's original classification of maxillary sinus cancer some 80 years ago (Sebileau, 1906), there have been many different methods proposed for staging this disease (Ohngren, 1933; Sisson et al, 1963; Lederman, 1969; Rubin, 1972; Sakai et al, 1972; Harrison, 1978; Morton et al, 1985). Most of these classifications have employed the TNM system. A recent article (Willat et al, 1987) reviewed the above classifications and showed Harrison's to be the most valid. Willat et al concluded that Harrison's staging system produced the most balanced distribution of cases, staged the majority of tumours and correlated with both treatment and survival. Until recently, the UICC had no recommendations to make on the staging of maxillary cancer. The AJC (AJCC, 1977) has always included the maxillary sinus in its staging manual and used a system based on Ohngren's original classification. The current system (UICC, 1987) adopts AJC recommendations and is therefore similarly based on Ohngren's work although it now appears Harrison's classification is superior. One of the most confusing areas to a young head and neck surgeon is that maxillary sinus carcinoma with bony involvement of the medial or inferior walls receives only a T2 classification. However, when oral
carcinoma involves the antrum (erosion of the inferior bony wall of the sinus), the classification is $T_4$. Apparently this disparity is based on the discrepancy in behaviour of the two separate bone involvements (Sisson and Pelzer, 1985). In addition, cribiform plate involvement is $T_4$ disease but this is quite resectable.

For nasopharyngeal carcinoma, a number of different staging systems exist (Ho, 1978; UICC, 1987; AJCC, 1988). The UICC-AJC criteria are identical, except for the "N" category, and it is the most commonly used system. However, Ho's system has its proponents, and it probably relates more accurately with prognosis since it subclassifies bony involvement (Levendag et al, 1983; Michael Henk, Personal Communication, 1988).

Over 30 years ago, an acceptable classification system was introduced for the major salivary glands (Foote, Jr and Frazell, 1953). This together with further work (Spiro et al, 1974b; Spiro et al, 1975; Spiro et al, 1976; Spiro et al, 1978; Levitt et al, 1981) forms the basis for current classifications (UICC, 1987; AJCC, 1988). Within this TNM classification are clinical variables which include tumour size, local extension together with regional and distant metastases. Although the 'T' staging is based on size, all
categories are subdivided into (a) no local extension and (b) local extension. Local extension is defined as clinical or macroscopic evidence of invasion of skin, soft tissues, nerve and bone. A facial nerve paralysis associated with a parotid mass indicates malignancy and, moreover, it indicates a neoplasm with a poor prognosis. Eneroth (1964) found no cases of facial paralysis among 1780 benign parotid tumours whereas among 378 malignant ones he found 46 cases of facial paralysis. In the latter group, the average survival interval from the onset of paralysis was 2.7 years and all patients died within five years. A later multicentric trial (Eneroth et al, 1977) reviewed 1029 malignant parotid tumours. Fourteen percent had facial nerve paralysis and the five year survival was 9%. Other workers have confirmed this poor prognostic sign (Conley and Hamaker, 1975; Spiro et al, 1975). It becomes obvious that with the current classification system it is possible to have Stage I and Stage II parotid disease with a facial paralysis. However, this contradicts the above data, and surely the presence of a facial paralysis should mean automatic mandatory Stage III or IV classification.

\[ T_4N_0 \] supraglottic cancers were upstaged from Stage III to Stage IV disease in 1977 (AJCC, 1977). Johns et al (1984) stated this was unjustified and recommended \[ T_4N_0 \] carcinomas be returned to Stage III. Other workers (Mendenhall et al, 1984) have confirmed that favourable
subsets exist within Stage IV head and neck squamous cell carcinoma. Despite these recommendations, $T_4N_0$ supraglottic cancer is still Stage IV and no stage IV subsets appear within the current system (UICC, 1987; AJCC, 1988). Failure to mention the cervical trachea as a sub-site from the current lung staging system (UICC, 1987) is surely an omission on the part of the UICC since it is included within both the current AJC manual (AJCC, 1988) and the former TNM atlas (UICC, 1985). Until recently (AJCC, 1982; UICC, 1983), the 'T' classification for the cervical oesophagus was based on size. Although the current AJC system (AJCC, 1988) retains this classification the UICC now 'T' stages oesophageal carcinoma by tumour depth (UICC, 1987) despite the fact that size is one of the most important prognostic indicators in both oesophageal and hypopharyngeal disease (Razack et al, 1978). There is no mention in either system of a TNM classification for carcinoma of the external auditory meatus and middle ear, although one has recently been proposed (Stell and McCormack, 1985b). Age and general condition have proved important prognostic factors in some head and neck squamous cell carcinomas (Platz et al, 1983; Jacobs et al, 1985; Stell and McCormack, 1985b). Both the AJC and UICC have suggested recording the performance status of the patient. There are three different performance scales which have been used (Sessions, 1986) although none are mandatory with current classifications (UICC, 1987; AJCC, 1988).
All the above inconsistencies make the head and neck a complicated region in which to apply a singular concept of classification. However, the end result embraces the orderly description of disease of increasing size and extent and one which lends itself to incorporation into a staging system, so that comparisons of treatment results might be meaningful. The author believes the present TNM system, while fallible, is founded on sound principles and represents the combined work and experience of many physicians and surgeons who have spent years treating head and neck cancer. Any shortcomings or criticisms of the system must reflect the complexity of the disease rather than any actual deficiencies within the staging classification although it is interesting, however, to note that of the five United Kingdom representatives on the current UICC Committee, not one is a head and neck surgeon.

Given that one of the biggest problems in staging head and neck cancer is inter-observer variation and subjective error, then, if all clinicians discipline themselves to apply the philosophy and guidelines of the new classification, useful data will be acquired over the next decade. Only then can the UICC and AJC committees evaluate any discrepancies within the present system and make intelligent changes so that a classification with so many complexities and variables can be regularly revised.
Given that the presence, or absence, of cervical lymph nodes is one of the most important prognostic factors in head and neck squamous carcinoma, their accurate staging becomes of paramount importance with both therapeutic and prognostic implications. Current methods to detect not only occult primary disease but also local cervical and distant metastases are notable by their absence (Section 1.4). In particular, techniques to detect occult cervical lymphadenopathy would be valuable in the evaluation of the clinically N\textsubscript{0} neck. The search is on for new imaging modalities to increase diagnostic sensitivity and specificity and, subsequently, to improve the way we diagnose, stage and treat head and neck squamous carcinoma.

With the concept of a new joint UICC-AJC TNM classification, head and neck clinicians are embarking on a new era in the staging of head and neck cancer. Perhaps it is prudent to reiterate the words of Pierre Denoix (Anon, 1967) - 'There can be no argument that TNM is here to stay and that the system deserves the attention of all specialists in the cancer field'.
1.2. THE CERVICAL LYMPHATICS

1.2.1. Introduction

1.2.2. History

1.2.3. Methods of Study

1.2.4. General Anatomy

1.2.5. Natural History and Evolution of Neck Disease
1.2.1. INTRODUCTION

Lord Moynihan, who was one of the greatest surgeons of this century, once wrote 'The surgery of malignant disease is not the surgery of organs, it is the anatomy of the lymphatic system (Moynihan, 1908). Over the ensuing eighty years, very little progress has been made towards the clinical realisation of such a profound statement. Very few surgeons have seen the lymphatics which transport metastatic disease and fewer fully understand the routes, number and position of the regional cervical lymph nodes and their relationship with primary head and neck cancer. In addition, such information is notable by its sparcity in modern day literature.

Leaf's English translation of Poirier, Cunéo and Delamere was published in 1903 and Tobias' English translation of Rouvière's work in 1938. Since then very little of the modern data concerning the pathways and distribution of head and neck lymphatic metastases had been published in single works. However, recent classic studies (Welsh and Welsh, 1966; Fisch, 1968; Feind, 1972; Lindberg, 1972; Molinari et al, 1977; Bataini et al, 1985) have all made notable contributions.
With advances in modern day anaesthesia and surgical techniques, the head and neck surgeon can today perform dissections of the cervical regional lymph nodes which were neither conceivable nor practical in times gone by. Indeed the surgeon can remove virtually all lymph nodes where surgical access and the probability of metastatic disease make the operation a reasonable proposition.

The subsequent discussion makes no attempt to describe the general function of the cervical lymphatics with regard to oedema, infection and immunity. Instead, it outlines the history, methods of study and general anatomy of the cervical lymph nodes and discusses the spread of cancer and evolution of neck disease as they pertain to head and neck squamous cell carcinoma. Such knowledge is crucial to any physician or surgeon involved in the ongoing management of patients with this disease.
1.2.2. HISTORY

The ancient Greeks, including Hippocrates, observed the mesenteric lymphatics but made no comments as to their function. During the stimulating period of the Renaissance, the practice of dissecting dead bodies became well established and it was Galen, and then Aselli, an Italian anatomist, who made important observations leading to the discovery and description of the lymphatics (Aselli, 1627). The true route of the lacteal fluid into the blood was discovered by Jean Pecquet who published a book called 'Experimenta Nova Anatomica' (Pecquet, 1653) which contained details of the receptaculum chyli and the thoracic duct as observed in the dog. In Scandinavia, both Olof Rudbeck and Thomas Bartolin studied animal lymphatics and it is Bartolin who is credited in 1653 with the first usage of the term "lymphatic" (Maar, 1916). In the same year Rudbeck (1653) published a book entitled 'Nova Excercitatio Anatomica' (Nielson, 1942) in which he described lymphatic vessels in various parts of the body.

In the latter part of the seventeenth century, important advances were made in the use of mercury for injecting lymphatics. Until this time anatomists had used water, ink, a variety of coloured fluids and wax to inject vessels of all types. By using mercury it was possible to inject the smaller
lymphatics and Anton Nuck was the first to use it, publishing a description of the lymphatics of the uterus in the last year of his life (Nuck, 1692).

Despite the availability of better microscopes, few advances were made in the knowledge of lymphatics until a century after Rudbeck. In the middle of the eighteenth century, William Hunter and his brother John, together with William Cruikshank and William Hewson, all worked on the anatomy and physiology of the lymphatics at William Hunter's School of Anatomy in Windmill Street, London. They performed a variety of experiments and dissections on birds, reptiles, fish, mammals and man and their outstanding work culminated in a monograph entitled "The Anatomy of the Absorbing Vessels of the Human Body" (Cruikshank, 1786).

One year later, Paolo Mascagni published a large atlas of the lymphatics (Mascagni, 1787). So extensive was this work that it represented a significant advance in the knowledge of the lymphatic system and the quality of both Cruikshank's and Mascagni's work was such that it remains unsurpassed in modern times. In the nineteenth century, the knowledge of both the gross and histological anatomy of the lymphatic system was perfected. This was largely due to the work of Sappey who published an Atlas of the Lymphatics
(Sappey, 1878). This contained pictures of the cutaneous lymphatics which are still of use today as aide-memoires. Sappey's methods of injections were improved by Gerota (1896) who used a colour-mercury method and his subsequent work added greatly to our topographical knowledge of lymph vessels.

Study of the histology of the lymphatic system had been enhanced by Von-Recklinghausens work (1862) which demonstrated the endothelium of lymph vessels stained black with silver nitrate. This work was supplemented by subsequent studies (Newmann, 1873; Ranvier, 1897; MacCullum, 1903) and, at the beginning of this century, knowledge of the embryology of the lymphatic system developed rapidly (Sabin, 1911; Sabin, 1916).

The elegant preparations made by both Mascagni and Cruikshank using intralymphatic injections of mercury remain unparalleled in modern times. Their beautiful drawings together with those of Gerota and Sappey paved the way for later classic systematic investigations of the human lymphatic system (Bartels, 1909; Jossifow, 1930; Rouvière, 1932). Later contemporary descriptions of the cervical lymphatics in man (Fisch, 1968; Feind, 1972) added very little to Rouvière's original work. Indeed Feind used Rouvière's original selection of names for the regional groups of head and neck lymph nodes except he substituted "submandibular" for "submaxillary".
Our knowledge of the physiology of the lymphatic system has lagged far behind its anatomical counterpart. Pioneering work by Hunter (1837) and by Starling (1896) at Guy's Hospital provided evidence that lymph was formed from blood by filtration through the capillary membrane. Further work (Drinker et al, 1922; Drinker, 1942) inspired the comprehensive discussions that are available on this subject today (Allen, 1967; Yoffey and Courtice, 1970; Kinmoth, 1982). Recent advances in immunology coupled with the advent of monoclonal antibodies (Köhler and Milstein, 1975) and the use of immunocytochemistry (Shi et al, 1984) together with the electron microscope (Leak, 1980) have added greatly to our understanding of both the structure and function of the human lymphatics (Robb-Smith and Taylor, 1981; Roit et al, 1985).
1.2.3. METHODS OF STUDY

Knowledge of the topographic anatomy of the cervical lymphatics has been obtained by using, either singly or in combination, post mortem studies and injections, in-vivo injections and information obtained from surgical specimens.

Post Mortem Studies

Using oil colours, dissolved in turpentine and ether, our understanding of the gross anatomy of the cervical lymphatic system was completed in the early part of this century by German and French anatomists. Gray used thorotrast, a colloidal preparation of thorium dioxide, to perform post mortem injections of lymphatics (Gray, 1938). He injected the thorotrast into fresh warm tissues and then massaged the area of injection. Using this technique he obtained some of the best fine detail studies of the lymphatics that have ever been made. More recently, Neoprene Latex has been used (Ottaviani, 1954). This hardens after injection, and when the tissues have been macerated and cleared away, detailed models of the lymphatics can be obtained. This technique has been criticised (Haagensen, 1972) since it is debatable whether or not the thin walled lymphatics are dilated by the Neoprene. The most detailed study of the topographical
distribution of the cervical lymph nodes in man was carried out by Rouvière on human cadavers (Rouvière, 1932). Subsequently very little has been added to our knowledge of this region although recent studies have helped to clarify some finer details (Fisch, 1968; Feind, 1972; Marks, 1984).

**In-Vivo Injections**

Dalmady (1911) was the first person to demonstrate human lymphatic vessels *in-vivo* by using cutaneous injections of adrenaline (Fisch, 1968 p 1). Following the discovery of X-rays, many authors successfully attempted radiological demonstration of the human lymphatics using thorium dioxide to perform indirect lymphography. Injections were made intra-peritoneally (Menville and Ané, 1932) intra-pleurally (Bortolotti and Torelli, 1934), intra-articularly and subcutaneously (Bignami, 1939). Similar experiments were carried out in mice, rabbits and man using the vital dye, Direct Sky blue (McMaster and Hudack, 1932; Hudack and McMaster, 1933). Their experimental methods were applied to human studies (Butcher and Hoover, 1955), but this kind of indirect lymphography proved to be of little value in the limbs since the deeper collecting trunk lymphatics were not identified. However, others used the technique to demonstrate visceral lymphatics, and those of the breast (Haagensen, 1972).
Although the subcutaneous injections of Thorotrast were successful in animals, tests performed on humans were disappointing (Arnulf, 1958). Moreover, it was discovered that not only did Thorotrast have local irritating effects, it also produced malignant haemangioendothelioma of the liver and fatal blood dyscrasias (Horta et al, 1965). Attempts to perform direct lymphography by either lymph node puncture (Carvalho et al, 1931) or direct transcutaneous lymph vessel puncture (Servelle, 1945) proved unsuccessful. Kinmoth (1952) described a simple method for injecting contrast medium directly into the human lymphatics. Following a short metamorphosis, his experimental technique of lymphography was rapidly introduced into clinical practice (Kinmoth et al, 1955) and an authoritative collection of his lifetime experience was published in 1982. The introduction of oily contrast media made the technique more applicable since these were not diluted by the lymph as easily as the water soluble media (Sheehan et al, 1961).

Some workers have criticised the use of lymphography to investigate metastatic cancer as the high pressures necessary to inject the contrast medium may result in the dissemination of tumour cells (Haagensen, 1972). Engzell et al (1968) evaluated the rabbit lymph node barrier to Vx-2 squamous carcinoma cells before, and after, lymphography.
They showed that the lymph node barrier was effective against cultured Vx-2 cells injected into an afferent lymphatic. They also showed that cells introduced prior to lymphography were not transported through the node by the contrast medium and that the barrier function of lymph nodes is severely impaired after lymphography, especially when oily contrast media are used.

During this period the cervical lymphatics received little attention using lymphographic techniques due to the practical difficulties of cannulating the smaller lymphatics of the head and neck. Luccherini (1936) and Sangiovanni (1937) were the first to attempt direct cervical lymphography by injecting Thorotrast into the tonsils. Others were also able to demonstrate the cervical lymph nodes by a transcutaneous injection of Thorotrast into a hyperplastic lymph node (Lenzi and Cucchini, 1940). Some 20 years later Battezzati et al (1960) systematically evaluated the superficial cervical lymphatics radiologically by direct cannulation of a superficial retroauricular lymphatic vessel. Others used the same method to study the cervical lymphatic drainage in patients with carcinoma of the larynx (Pietrantoni et al, 1960) and subsequently Yannoulis and Sfoungaris (1963) demonstrated the cervical lymph nodes in man using an oily contrast medium. In the same year both Jackson et al, and Fisch and Del Buono published studies on the cervical lymphatic system using an oily contrast medium (Fisch and Del Buono, 1963; Jackson et al, 1963). Larson et al studied the lymphatics of
the mouth and neck in health and disease (Larson et al, 1965) and Fisch subsequently evaluated his technique by performing over 200 cervical lymphographies, the results of which are published in his monograph on the subject (Fisch, 1968). Although he added little to the knowledge already available on the topography of the cervical lymphatics, he did make important observations on normal cervical lymphatic flow, and the alterations which can occur following surgery and irradiation.

Hultborn et al (1955) used radioactive colloidal gold to evaluate the lymphatic drainage of the breast. Others adopted this technique to study the cervical lymphatics in both health and disease (Welsh, 1964; Welsh and Welsh, 1966; Schwab, 1967; Gruart, 1977). Although these studies made important contributions to our knowledge of normal and abnormal lymphatic flow, very little was gained regarding topographical distribution. Gruart demonstrated areas of low uptake of colloidal gold in lymph node metastases and suggested this method could be used to detect their presence in the neck. However, later studies have shown cervical lymphoscintigraphy to be unreliable in the detection of metastatic disease (Parell et al, 1981; Blakeslee et al, 1985).
Study of Surgical Specimens

Although most of our knowledge regarding the topographical anatomy of cervical lymph nodes has come from cadaveric dissections, the most important source of information regarding lymph node metastases has come from the study of surgical specimens. In the past, however, this method has been largely neglected due to the fact that most pathologists were not keen to spend the time required to study the specimen adequately. In addition, a considerable number of nodes would be missed due to the time factor and consequently only six nodes, on average, would be obtained from a neck dissection. However, using the clearing technique i.e. dissolving the fat, Feind examined 1500 radical neck dissections, and contributed greatly to our knowledge of the patterns of cervical metastases in head and neck squamous cell carcinoma (Feind, 1972). Since then others have recognised the need to examine neck dissections in an orderly and systematic manner (McKelvie, 1976; Van Der Waal and Delemarre, 1982; Rhys Evans et al, 1987).

The normal anatomy of the cervical lymph nodes has been studied using CAT and MRI (Mancuso et al, 1983a; Stark et al, 1984a). However, the resulting information has added
little to our topographic knowledge and these techniques are discussed in Section 1.3. on the investigation of head and neck squamous cell carcinoma.
1.2.4. GENERAL ANATOMY

The head and neck constitutes one of the most anatomically complicated structures of the body. The lymphatics of the entire area drain ultimately into the head and neck lymph nodes and, as such, the surgeon should fully understand their normal topographic distribution.

Comparative Anatomy

The lymphatic vessels are phylogenetically the oldest part of the lymphatic system as lymph nodes appear in only birds and mammals (Fisch, 1968 p 44). A comprehensive discussion of the mammalian lymphatics is available in the current literature (Arvy, 1973). In both structure and function they resemble their human counterparts and, as such, have been used extensively as models for cancer research (Engzell et al, 1968). Of interest to the present work, the topographic distribution of the head and neck lymph nodes of the rabbit is shown in Figures 4 and 5.

Embryology

Controversy surrounds the embryology of the cervical lymphatics and, to date, there are two different views
Figure 4

THE HEAD AND UPPER NECK LYMPH NODES OF THE RABBIT

THE CERVICAL LYMPH NODES OF THE RABBIT

Submental

Upper cervical

Submandibular

Lower cervical

Axillary

regarding its origin. One hypothesis is based on the publications of Ranvier and Sabin (Ranvier, 1897; Sabin, 1911; Sabin, 1916). Sabin states that the jugular sac and lymphatic vessels originated by sprouting from the venous endothelium so that a relation between lymphatic and venous vascular systems exist from the beginning (centrifugal growth). In contrast, others assume a centripetal growth pattern which proceeded from fusion of mesenchymal clefts (Huntingdon, 1908; Kampmeier, 1912; Huntingdon, 1914; Zimmerman, 1940). Opinions since have always been divided regarding these theories of origin. Some favour the centripetal theory (Rusznyak et al, 1960; Godart et al, 1964) while others favour the centrifugal one (Yoffey and Courtice, 1970). The balance of evidence now suggests that all but the earliest channels of the lymphatic system originate independently of the venous system, and only connect with it at a later date (Kampmeier, 1969).

Lymphatic vessels first appear in the human embryo at the 10 to 11 mm stage within the cervical area forming paired jugular lymphatic sacs. From these sacs the cervical and axillary lymphatic vessels branch off as do the cranial parts of the primary paired thoracic ducts. However, the cranial part of the right thoracic duct remains rudimentary whereas its caudal portion runs into the left thoracic duct
by transverse connections. At the end of the second month, valves are present in the primary lymph spaces of the neck and upper thoracic duct (Kampmeier, 1928). The lymph nodes are developed in the third foetal month by aggregation of mesenchymal elements which is followed by the collection of lymphoid cells around convolutions of lymphatic vessels. The differentiation of the lymph nodes starts only after the 50 mm stage (Hamilton et al, 1962). At birth the lymph nodes are completely formed with the exception of the secondary nodules, which differentiate post-natally.

**Structure of the Cervical Lymphatic System**

The cervical lymphatic system consists of the following distinct morphological and functional structures; lymph capillaries, lymphatic vessels, lymph nodes and terminal lymph collectors (Becker, 1963). Also included are the circulating lymphocytes and collections of lymphoid tissue within Waldeyer's internal ring (1884) and the thymus gland.

**Lymphatic Capillaries**

The lymphatic capillaries are a closed system of endothelial vessels forming an intricate network in the interstitial spaces (Yoffey and Courtice, 1970). They often
commence with a dilated, bulb-like, blind extremity and their calibre is greater, and less regular, than that of capillaries. The wall of a lymph capillary consists of a single wall of endothelial cells. It resembles that of a blood capillary, but the basement membrane is often lacking and specialised attachments between endothelial cells are infrequent (Gray, 1980). During embryonal development only one lymphatic plexus is found in the skin immediately below the corium (Sabin, 1904). Later on, lymphatic capillaries bud off this primary skin plexus so that in the adult there are two skin plexuses; a superficial plexus in the corium, and a deeper dermal plexus. There are no lymphatic capillaries in the epidermis.

In the mucosa, and in the transition area between the mucosa and the skin, dense networks of lymphatic capillaries are present. A rich lymphatic plexus is found in the nasal mucosa. The lymph from the capillaries of the submucosa of the upper respiratory tract and the oesophagus drains into the deep cervical lymphatics. Lymph capillaries are present in most tissues of the body but are absent from avascular structures. Within the head and neck these include the epidermis, hair, cornea, the central nervous system and the membranous labyrinth (Rouvière, 1932).
The main function of the lymphatic capillaries is to reabsorb excess interstitial body fluid and extravascular protein (Yoffey and Courtice, 1970). Under normal conditions the lymph stream originating from the skin of the head and cervical region is rather small. However, the capillaries of the skin play an important role in the removal of extracellular protein and subcutaneous oedema occurs quite rapidly should they become blocked.

**Lymphatic Vessels**

The lymphatic vessels originate from a plexus of lymphatic capillaries and are characterised by their thicker walls, and by valves. They can be distinguished from veins by their greater number, much thinner walls, less sinuous course and a large number of valves. Kampmeier described in detail the development and structure of the lymphatic valves (Kampmeier, 1928). The lymphatic vessels show a definite three layer partition of the vascular wall (tunica.intima, tunica media and tunica adventitia (Kaindl et al, 1960)). This has been confirmed by electron microscopic studies (Boggon and Palfrey, 1973) although these authors failed to demonstrate muscle fibres in the adventitia. There are no major differences between the lymphatic vessels of the head and neck and those in other parts of the body. The lymph flow
on each side of the body is, usually, completely separate although organs in the midline, such as the tongue and larynx, may possess lymphatic vessels that cross the midline (Sappey, 1878; Jamieson and Dobson, 1920; Taillens, 1960; Welsh and Welsh, 1966). Within the head and neck the lymphatic vessels are divided into superficial and deep, depending on their position in relation to the superficial fascia. The former are more numerous, and longer than the latter, and tend not to run with blood vessels. In contrast, the deep lymphatics usually lie next to the vessels and nerves.

As a general rule, the lymph of any given area of the head and neck is transported via a number of lymphatic vessels to a particular lymph node or group of nodes (regional nodes). The lymphatic area drained by a node or group of nodes is called a tributary zone (Becker, 1963). The function of the lymphatic vessels is to transport lymph, fats, proteins, vitamins and hormones.

**Lymph Nodes**

The lymph nodes of the head and neck do not differ in either development or structure from the nodes of other regions of the body, except in their dimensions. The importance of the cervical lymphatic system is such that
approximately 30% of the 500 to 1000 lymph nodes that exist in the human are located within the cervical area (Policard, 1963; Million et al, 1982 p 301). Their dimensions are such that a normal size adult node may vary in size from 1 mm to 3 cm (Rouvière, 1932; Hendrick, 1967), and all adult head and neck lymph nodes are totally or partially embedded in fat (Som, 1987). Depending on their position in relation to the superficial fascia the lymph nodes can be divided into superficial and deep, the latter being the more numerous. The lymph nodes which drain lymph from a given head and neck region are called regional lymph nodes (lymph nodes of the first order; 'ganglions du premier echelon' of Rouvière, 'bulbons satellites' of Taillens: Rouvière, 1932; Taillens, 1962). In addition to the lymph nodes, Rouvière also described small aggregations of lymphatic tissue interposed between the lymphatic capillary plexus and the lymph nodes proper. These lymphatic formations have been called intermediary lymph nodules (nodules intercalaires) and may have the potential to develop further into lymph nodes proper.

It is not known whether lymph nodes regenerate after regional lymph node dissection and this problem has been studied in animals. Rouvière and Vallette excised the popliteal node in adult rabbits and reported that no regeneration occurred (Rouvière and Vallette, 1937 p 111). However, Furuta removed
the popliteal node in rabbits and reported it did not regenerate in the adult, but did in the young (Furuta, 1947). Sigel and Fisch (1965) showed lymphographically, and histologically, in rabbits that had undergone modified radical neck dissection that cervical lymph nodes are capable of regeneration. Subsequently, other workers have substantiated these findings (Baker et al, 1969). In man, the lymph node apparatus is much more complex and the overall view is that, after a careful and complete lymph node dissection, regeneration does not usually occur (Haagensen, 1972). As age advances, lymph nodes tend to become smaller although the actual number often remains constant.

Lymph nodes are small, oval or reniform bodies situated in the course of lymph vessels. Each presents on one side a slight depression termed the hilum, through which blood vessels enter and leave the node. The efferent lymph vessel (usually single) also emerges at this spot while the afferent vessels enter at different parts of the periphery.

A lymph node has a highly cellular cortex and a darker medulla. It consists of a continuous framework which includes a capsule, trabecula, reticular fibres and cells entangled within this framework.
The capsule is composed mainly of collagen fibres, a few fibroblasts and some elastin. The reticulum is a meshwork of fine reticulin fibres and attendant cells, which permeates the spaces outlined by the capsule and trabeculae providing mechanical support for the adjacent cell masses. The lymphatic channels permeate each node ensuring the maximum exposure of the lymph to the cells within the node. Each lymph node is supplied by a lymphatic blood system, the features of which are described in the literature (Gray, 1980).

The majority of the entangled cells within the node are B- and T-lymphocytes, with some macrophages. In the cortex, the cells are densely packed into isolated masses called lymphatic follicles, the central area of which is called a germinal centre and contains lymphoblasts. These lymphoblasts, by mitosis, produce small lymphocytes which congregate in the marginal zones around the germinal centre before entering the lymph sinuses to be conveyed through the medulla to the efferent lymph vessel. Within the medulla, the lymphocytes are more loosely packed into irregular branching medullary cords.

There are also a number of non-lymphocytic cells found within lymph nodes. These are endothelial cells, fibroblasts,
macrophages, perivascular cells and dendritic cells (Steinman et al, 1974). The general architecture of a cervical lymph node is depicted in Figure 6.

The Terminal Collecting Lymphatic Trunks

The lymph from the entire body is channelled into a small group of large terminal collecting lymphatic trunks which return it to the venous stream at the confluence of the internal jugular and subclavian veins (venous confluence) on each side of the base of the neck. The largest of these collecting trunks is the thoracic duct. This is one of the largest vessels in the human body and, as such, its existence was recognised early on (Mascagni, 1787). The anatomy of the thoracic duct is well described (Rouvière, 1932), and details of its anatomical aberrations, particularly within Waldeyer's triangle in the neck are available in the literature (Feind, 1972). Early anatomists depicted the great collecting lymphatic trunks from the head and neck, axilla and mediastinum emptying into the confluence of veins as single vessels. However, later work has shown them to be irregular in both number and distribution (Rodrigues and Pereira, 1930). Apart from the thoracic duct, they are the subclavian, internal mammary, and anterior mediastinal trunks. There are usually several trunks on each side from the paratracheobronchial and posterior mediastinal groups of nodes, and sometimes a short collecting
Figure 6
THE STRUCTURE OF A HUMAN CERVICAL LYMPH NODE

Gray, 1980. p. 769
trunk from the posterior intercostal lymph nodes of the first and second interspaces which ascends behind the great vessels and empties into the venous confluence. Lastly, instead of a single jugular trunk there are usually one or two quite separate trunks from the transverse cervical nodes. The findings of Rodrigues and Pereira are summarised in the literature (Haagensen, 1972).

All seven of these groups of collecting lymphatic trunks terminate finally in the venous confluence on each side of the neck. On the right side the collecting trunks either empty separately or form a short common trunk called the right lymphatic duct. On the left side the various trunks empty into the internal jugular vein, the subclavian vein or into the thoracic duct. Of interest, the right mediastinal duct carries lymph from all of the right lung as well as that from the inferior lobe and lingula of the left. The right mediastinal duct connects with the right subclavian lymph nodes which may be of value in the early diagnosis of pulmonary disease (Pre-scalenic biopsy of Daniels, 1949). Other authors have demonstrated connections between the thoracic duct and the left subclavian lymph nodes, and this emphasises the significance of left supraclavicular lymph node involvement (Virchow's node) in the diagnosis of abdominal disease. Although all lymph from the head and neck ultimately passes back into the venous system, there is experimental evidence that
direct communications do exist between cervical lymph nodes and veins (Pressman and Simon, 1961).

**The Thymus**

The thymus is a primary central organ of the lymphoid system. It is derived from the endoderm of the ventral part of the third pharyngeal pouch on each side. The development of thymic tissue from the ventral recess of the fourth pharyngeal pouch probably occurs in a proportion of embryos (Van Dyke, 1941), although this has been disputed by others (Weller, 1933; Norris, 1938). Thymic tissue developing from this site is usually found outside the thyroid gland in close association with the superior parathyroid gland. An ectodermal contribution to the thymus, probably of placodel origin, occurs in some mammals but its existence in man is far from proven (Garret, 1948).

Although the thymus is usually described as a single bilobed, unpaired organ, each lobe is derived from the third pharyngeal pouch on each side and, as such, there are two thymic bodies. Each lobe is surrounded by a delicate fibrous capsule and consists of an outer cortex and an inner medulla. Essentially, throughout both these parts, there are two principal tissue components which differ quantitatively in the
two regions and these are the lymphocytic cells, and the epithelial cells which form the reticular framework (Gray, 1980). By far the most important are the densely packed cortical lymphocytes which form 90% by weight of the thymus gland. The majority of thymic lymphocytes have a short life span (3-5 days), and undergo degeneration while still within the gland while the remainder leave the thymus to form part of the circulating pool of lymphocytes. Within the medulla are found a number of true macrophages, plasma cells and the concentric corpuscles of Hassall. The lymphatic connections of the thymus gland together with the regional thymic nodes have been extensively investigated (Afanassiew, 1877; Severeanu, 1909) and a summary of their work is available in the literature (Weinberg, 1972). The thymus is concerned with the production of immunologically T-cells (thymocytes), the control of lymphopoiesis and in the production of hormones called thymosins.

**Circulating Lymphocytes**

A cervical lymph node is a peripheral lymphoid organ. It constantly receives lymphocytes from the vascular system and from its afferent lymphatic supply and, in return, continually provides lymphocytes to its efferent drainage. In foetal life, and to a lesser extent through postnatal development, these
lymphocytes arise from the central lymphoid organs of the bone marrow and thymus gland. Once in a lymph node, these lymphocytes may proliferate when stimulated and, in the case of B-lymphocytes can form plasma cells. These may rejoin the circulatory system and pass to sites of inflammation (Gray, 1980).

Topographic Anatomy of the Cervical Lymphatics

Rouvière (1932) made the first attempts to systemise the topography of the human lymphatics. The subsequent nomenclature used in this section is based on his work supplemented by the clinical investigations of others (Taillens, 1962; Fisch, 1968; Feind, 1972). No attempt is made to describe the anatomical triangles of the neck, details of which are available in the literature (Sessions et al, 1986).

The cervical lymphatic system is organised into three connected lines of defence (Taillens, 1962): Waldeyer's tonsillar ring is a ring of lymphoid tissue at the transition between the head and neck, and the cervical lymph nodes proper. Waldeyer's ring forms the first line of defence of the upper air and food passages and consists of a ring of lymphatic tissue surrounding the pharynx. This includes special lymphoid epithelial organs such as the palatine, pharyngeal, tubal and lingual tonsils as well as diffuse lymphatic collections
in the submucosa of the pharynx and salpingopharyngeal folds. The lymph nodes at the transition between the head and neck regions are arranged in a circular manner around the cervical visceral organs. They are the occipital, postauricular, parotid, submandibular, sublingual, retropharyngeal and submental lymph nodes. They are sometimes called "anneau ganglionnaire cervical" of Taillens (Taillens, 1962) and the 'cercle ganglionnaire pericervical' of Poirier and Cunéo (Poirier and Cunéo, 1903). They are also sometimes called Waldeyer's external ring although he did not describe them. Both Waldeyer's internal ring and the transitional lymph nodes ultimately drain into the cervical lymph nodes proper. All the lymph nodes of the head and neck (most of which are situated in the neck) are confined between the superficial cervical and prevertebral fascia. They consist of the transitional nodes and the cervical lymph nodes proper. Rouvière divided these nodes into 10 principal groups and a modification of his classification is shown in Table 5. His original selection of group names has remained the same over the last fifty years except "submandibular" now replaces "submaxillary" (Feind, 1972), and a junctional group is included within the lateral cervical nodes (Fisch, 1968). The topographical distribution of the transitional and superficial cervical lymph nodes is shown in Figures 7 and 8.
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<th>TABLE 5</th>
<th>THE REGIONAL GROUPS OF HEAD AND NECK LYMPH NODES</th>
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<td><strong>Post-auricular Nodes</strong></td>
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Rouvière, 1932; Taillens, 1960; Fisch, 1968; Lindberg, 1972
Figure 7

THE TOPOGRAPHICAL DISTRIBUTION OF THE HEAD AND NECK TRANSITIONAL LYMPH NODES IN MAN

A OCCIPITAL NODES
B POSTAURICULAR NODES
C PAROTID NODES
D SUBMANDIBULAR NODES
E SUBMENTAL NODES
F RETROPHARYNGEAL NODES

ROUVIERE, 1932
Figure 8

THE TOPOGRAPHICAL DISTRIBUTION OF THE SUPERFICIAL CERVICAL LYMPH NODES IN MAN

A MEDIAL SUPERFICIAL
B LATERAL SUPERFICIAL

ROUVIÈRE, 1932
Occipital Nodes

The occipital nodes consist of two subgroups: the superficial and the deep nodes. The superficial nodes usually number one or two. They are flat and vary from 0.5 to 1.0 cm in size. They are most commonly found between the superficial cervical fascia and the splenius muscle, lying between the insertions of the sternomastoid and trapezius muscles at the apex of the posterior triangle. They receive lymph from the occipital area of the scalp and skin of the upper part of the back of the neck. Their efferent drainage goes either to the deep occipital, or to the deep upper spinal accessory nodes.

There is only usually one deep occipital node. A consistent finding is that the node is elongated, measuring up to 0.5 x 0.2 x 0.2 cm. It lies below the splenius capitis muscle, receives lymph from the superficial occipital nodes and drains into the deep spinal accessory nodes.

Postauricular Nodes

The postauricular nodes may number 1 to 4, although 1 is the most common number. They are more prevalent in the young and are often atrophic or absent in the elderly (Feind, 1972). The single postauricular node is a flat oval node measuring 0.5 to 0.7 cm or smaller. It is situated behind the auricle just below the posterior auricular muscle.
The postauricular nodes are often non-existent, being absent in one third of foetuses and half the adult and aged (Rouvière, 1932). Their afferent vessels originate from the posterior portion of the parietal and mastoid regions of the scalp, together with the skin over the posterior aspect of the auricle. Efferent vessels pass either to the infra-auricular parotid nodes or to the junctional deep cervical nodes.

**Parotid Nodes**

Rouvière divided the parotid nodes into extraglandular (or periglandular) and intraglandular (Rouvière, 1932). The extraglandular group is subdivided into the preauricular and infra-auricular nodes. These nodes are contained in the parotid sheath or are adjacent to the parotid gland with the sheath blending in with the node capsule. The preauricular group lies over the superficial aspect of the parotid gland. There may be 1 to 4 nodes (0.1-0.5 cm in size) in the pretragal area, and these are the most superficial nodes. The remaining nodes, when present, become part of the intraglandular division. The infra-auricular nodes consist of 1-4 nodes. They lie adjacent to the posterior facial vein at the lower pole of the parotid gland. The preauricular nodes receive afferent vessels from the lateral and frontal aspects of the scalp and from the cranial vault. They also receive vessels from the auricle, external auditory canal, the lateral half of the lower eyelid, the upper eyelid and the root of the nose. The infra-auricular group receives vessels
from the cheek, buccal mucosa, parotid gland, nose and upper eyelid (Feind, 1972). There has been some controversy about the actual number of lymph nodes which occur within the parotid gland. Rouvière numbers the intraglandular nodes 4-10 with a maximum of 14. This has been confirmed by others (Graham, 1965; Marks, 1984), but others have quoted three times this figure (Conley and Arena, 1963; Pope and Lehman, 1967). Feind found an average of 20 nodes (Feind, 1972) and it is his work, together with Rouvière's, that is frequently quoted by others. The intraglandular nodes are found embedded within the parotid, and lie chiefly in the superficial portion of the gland alongside the posterior facial vein. They receive afferent vessels from the parotid gland, the skin of the side of the head above the parotid gland, the lateral aspect of the eyelids, the conjunctiva, the external auditory meatus, the Eustachian tube and tympanic membrane. They also receive efferent trunks from the extraglandular parotid nodes. Both the intraglandular and extraglandular lymph nodes should be regarded as a complete entity as they function as one unit (Feind, 1972). Efferent vessels from the parotid nodes pass from the preauricular extraglandular nodes to both the intraglandular and infra-auricular nodes, and then on to the upper deep lateral cervical nodes.
Submandibular

There are prevascular and postvascular, preglandular, retroglandular and intracapsular submandibular nodes (Rouvière, 1932; Feind, 1972). The prevascular group consists only usually of one node (0.4-0.7 cm in size) which sits in front of the anterior facial vein on the facial artery, and which is in contact with the mandibular branch of the facial nerve. The postvascular group may be made up of one or two nodes situated along the edge of the anterior facial vein. The preglandular group consists of one or two nodes in front of the submandibular gland, superficial to the mylohyoid muscle, which measure 0.2-0.5 cm in size and which are intimately related to the submental vein. The retroglandular node is a rare finding which has been found in surgical specimens but not injected cadaver specimens. It is probably a deeply situated postvascular node or a laterally placed "sublingual" node (Feind, 1972). The intracapsular submandibular nodes probably do not exist. Feind stated that he had not seen a lymph gland completely engulfed by the submandibular salivary gland although he had seen a node protrude from, or be partially embedded in, an interlobar fissure on the deep side of the gland. He concluded these nodes should be included as the retroglandular group and the intracapsular group be abandoned. The afferent vessels of the submandibular lymph
nodes take origin from the efferent vessels of the submental nodes and the facial node. The following are considered sources of lymphatic drainage: submandibular salivary gland, lower and upper lip, cheeks, nose, nasal fossae mucosa, gums, teeth, medial parts of eyelids, posterior palatine fossae, anterior faucial pillar, soft palate and many of the collecting vessels of the anterior two thirds of the tongue. Efferent pathways have been described to the upper deep lateral cervical nodes (Rouvière, 1932; Feind, 1972), lower internal jugular nodes (Rouvière, 1932), and to the spinal accessory nodes (Sassier, 1927).

Facial Nodes

Rouvière divided the facial nodes into four main groups: buccinator, infraorbital, malar and inferior maxillary (Rouvière, 1932). To avoid confusion between maxillary and mandibular, the inferior maxillary group has been renamed mandibular (Feind, 1972). There is only usually one mandibular facial node (the node of Stahr), but occasionally there may be three. It is the most constant facial node and its usual position is over the mandible, in front of the facial vessels, superficial to the insertion of the buccinator muscle on the mandible. The mandibular facial nodes are located on the collecting lymphatics from the medial part of the eyelids, the medial orbit, the nose, the nasolabial
fold, the upper lip, cheek and anterior part of the zygomatic region. The buccinator group is often absent. They lie superficial to the buccinator muscle below the line of Stenson's duct, and lie along the lymphatic vessels heading for the mandibular facial nodes. The infraorbital node is rarely present (Feind, 1972). It can be anywhere along the nasolabial fold from the inner canthus to the alar, and it is situated on the afferent lymphatic vessels draining this area. The malar node has only been reported twice (Watzold, 1927; Rouvière, 1932). It was found just below the outer canthus on the lymphatic pathway draining the upper lid (Feind, 1972). All the facial nodes drain into the submandibular group of nodes.

**Submental Nodes**

There has been much discrepancy in the descriptions of the submental lymph nodes (Stahr, 1898 p 444; Poirier and Cunéo, 1903; Sassier, 1927; Rouvière, 1932). Rouvière divided the submental nodes into three groups: anterior, middle and posterior and further divided the middle group into medial and lateral. These nodes may number 2 to 8, the average being about 5. They vary in size from 0.2-0.6 cm and lie in the adipose tissue of the submental triangle. The nodes receive superficial lymphatics from the chin, middle of the
lower lip and the cheeks. The deeper afferent pathways drain the gums, anterior floor of the mouth and anterior third of the tongue. The nodes themselves are interconnected and give efferent pathways to ipsilateral or contralateral neck nodes. These efferent pathways go to either the submandibular nodes or to the upper deep lateral cervical nodes via vessels which accompany the venae comitans nervi hypoglossi (Feind, 1972).

Sublingual Nodes

Although Rouvière included these nodes within his classification, subsequent workers have failed to demonstrate their presence in clinical specimens (Feind, 1972). They consist of interrupting nodules along the collecting trunks of the tongue and sublingual salivary gland. They probably should not be grouped as head and neck nodes (Feind, 1972), and are not discussed further.

Retropharyngeal Nodes

These nodes lie behind the pharynx and in front of the prevertebral fascia. Most and Rouvière reported them only to the level of the greater cornu of the hyoid bone (Most, 1900; Rouvière, 1932), although others have shown they can occur from the skull base to the thoracic inlet (Feind, 1972). They are divided into lateral and medial groups.
The lateral group is invariably present in the infant, but absent in the adult. They are true nodes, and usually three in number, which lie at the level of the atlas behind the nasopharynx. The medial group lies in the midline at the same level as the lateral group. The afferent vessels of both groups come from the posterior nasal cavity, the sphenoid and posterior ethmoid sinuses, the hard and soft palate, middle ear, Eustachian tube, nasopharynx and posterior pharyngeal wall. The upper medial nodes drain into the lateral group, and both groups then drain to the upper deep lateral cervical nodes.

**The Cervical Lymph Nodes**

Data in the literature concerning the topography and nomenclature of the cervical nodes is often confusing, especially with regard to the deep lateral cervical lymph nodes which are the most important group to the head and neck surgeon. The nomenclature used to describe the cervical lymph nodes in this study is based on the publications of Rouvière, Taillens and Fisch (Rouvière, 1932; Taillens, 1960; Fisch, 1968). This is because their work coincides with data gathered from surgical experience and the study of surgical specimens (Fisch, 1968; Feind, 1972).
The cervical lymph nodes can be divided according to their anatomical location into medial and lateral lymph nodes. The medial group is situated in a region corresponding to the trigonum colli mediale, i.e. in a triangle having a base formed by a horizontal line at the level of the hyoid bone and its sides along the inner sides of the sternocleidomastoid muscle (Becker, 1963). The superficial layer of the deep cervical fascia forms the root of the triangle while the prevertebral fascia forms the floor. Rouvière divided this medial group into two subgroups. The first he called the anterior jugular chain, being superficial to the strap muscles, while the second deeper chain he called the juxtavisceral group.

The anterior jugular chain receives its name from its relationship to the corresponding vein. The nodes occur infrequently although the lymphatic collecting vessels are constant. The chain receives lymph from the skin and muscles of the anterior part of the neck and efferent pathways pass through the superficial fascia into the deep lateral cervical lymph nodes. The deep medial lymph nodes of the neck are closely related to the visceral organs, i.e. the pharynx, cervical oesophagus, larynx and trachea. Rouvière named this group the juxtavisceral chain, and according to their topographic relationships they are called the prelaryngeal and prethyroidal, the pretracheal and paratracheal lymph nodes.
The paratracheal group is also sometimes known as the recurrent nerve chain group (Feind, 1972). Rouvière (1932) further subdivided the prelaryngeal group into three subgroups which was to justify the finding of a single prethyroidal lymph node placed over the middle of the thyroid cartilage. There are two main subgroups: the superior one lying in the vicinity of the thyrohyoid membrane and the inferior over the cricothyroid membrane. These subgroups are called the thyrohyoid and cricothyroid nodes. The former were described by Most (1905 p 96) although Rouvière reported later they were rare. The latter group is a more constant finding and usually consists of one node, although there may be three. It usually measures 0.2-0.5 cm in size. The pretracheal group consists of those nodes found from the isthmus of the thyroid gland down to the left inominate vein. Six to eight nodes are usually present. The paratracheal nodes are situated along the recurrent laryngeal nerve. They number between four and 12 and vary in size from 0.1-0.4 cm. These deep medial nodes chiefly drain the lymph from the subglottic region, from the anterior part of the trachea and cervical oesophagus and also the thyroid gland. Their efferent vessels pass mainly to the deep lateral cervical lymph nodes. On the left side the lymph from the paratracheal nodes drains into the thoracic duct but on the right side it drains into the jugular trunk or into the anterior mediastinal nodes (Lennert, 1961).
The lateral lymph nodes of the neck are situated in a region corresponding to an extension of the lateral (posterior) triangle of the neck (trigonum colli laterale) and the sternocleidomastoid region. The limits to this area are formed in front by the anterior margin of the sternomastoid muscle, below by the clavicle, and behind by a line from the mastoid process to the acromial end of the clavicle over the trapezius.

The superficial lateral cervical nodes are located chiefly along the external jugular vein. They form a small group of one to four lymph nodes draining skin areas lying above sternomastoid. The efferent lymphatics pass under the superficial fascia to end in the deep lateral cervical nodes.

The deep lateral cervical lymph nodes are situated in a large connective tissue space that lies between the deep and superficial cervical fascia within the lateral triangle of the neck. This space communicates anteriorly with the large medial connective tissue space, and posteriorly with a similar space under the trapezius muscle in the nuchal area. It is closed superiorly by the base of the skull. Its lower limit, at the thoracic entrance, is formed dorsally by the pleural domes.
The deep lateral cervical nodes consist mainly of three chains (Figure 9). The internal jugular chain, the chain of the spinal accessory nerve and the supraclavicular lymph nodes.

The lymph nodes of the internal jugular vein are the most important nodes in the neck and are situated along the internal jugular vein from the posterior belly of the digastric muscle to the angle between the internal jugular and subclavian veins. There is much confusion about the precise topography and terminology of the jugular lymph nodes. Taillens (1960) described three parallel chains of jugular nodes following the anterior or posterior margins of the sternomastoid muscle or running deep to the muscle (Figure 10). Others have divided the nodes into cranial, middle and caudal or only into cranial and caudal nodes (Figure 10), the limits between these subgroups being either the level of the common facial vein and the crossing of the omohyoid muscle with the internal jugular vein, or the latter limit by itself.

Rouvière divided the internal jugular nodes into anterior and lateral divisions (Figure 10). The anterior group are located anterior to the internal jugular vein and are found predominantly in the upper half of the chain. It consists of a larger subdigastric group above the common facial vein and a smaller thyroid group below. The uppermost node of
Figure 9

THE TOPOGRAPHICAL DISTRIBUTION OF THE DEEP CERVICAL LYMPH NODES IN MAN

A JUNCTIONAL NODES
B INTERNAL JUGULAR NODES
C SPINAL ACCESSORY NODES
D SUPRACLAVICULAR NODES
E NUCHAL NODES
F DEEP MEDIAL VISCERAL NODES

Figure 10
THE VARIOUS CLASSIFICATIONS OF THE INTERNAL JUGULAR LYMPH NODES IN MAN

TAI LLENS, 1960
ROUVIERE, 1932

A ANTERIOR GROUP
B LATERAL GROUP

I CRANIAL NODES
II CAUDAL NODES
III MEDIAL NODES

PERNKOPF, 1952 BECKER, 1963 SPITALIER AND COLONNA D'ISTRIA, 1961
the subdigastric group is the so called principal node of Küttner (1898) or 'ganglion superieur jugulaire of Taillens' (1960). This node may extend behind the digastric muscle and, when involved with disease, can extend up to the jugular foramen. These subdigastric nodes vary in number from two to ten and reach up to 1.5 cm in size.

Both Ducuing (1932) and Rouvière (1932) noted an accumulation of deep lateral cervical lymph nodes in the upper part of the neck at the junction between the jugular and the spinal nodes. Rouvière called this collection 'amas ganglionnaire de la junction' (junctional agglomeration of nodes). Subsequent comparative radiological and gross anatomical examination of surgical specimens (Fisch and Sigel, 1964; Fisch, 1968) has confirmed that these nodes correspond to the upper deep lateral cervical nodal group which includes the subdigastric node of Küttner. These nodes have been called junctional nodes (Fisch and Sigel, 1964). They form a kind of crossroads for the cervical lymph as they not only receive regional lymph from the oropharynx, tonsils, nasal sinuses, palate, base of tongue, hypopharynx and laryngeal vestibule but also efferent lymph from the transitional nodes. Using cervical lymphography, Fisch demonstrated the average number of lymph nodes in the deep lateral cervical lymph group was
48 and of these, nine were junctional and 11 were jugular. He also stated it would be anatomically inaccurate to refer to the junctional nodes as jugular, since very few of them were associated in his experience with the internal jugular vein. Other workers have made similar observations and grouped the junctional nodes with the lower parotidean and postauricular lymph nodes (Spitalier and Colonna D'Istria, 1961).

Rouvière's lateral internal jugular group extended from the digastric muscle to the subclavian vein. These nodes are part of an elongated network of nodes and vessels which vary greatly in pattern and number. Some nodes measured 2-3 cm and varied in number from 10 to 20. In some cases there is more than one lymphatic trunk, particularly in the caudal part of the chain. The jugular chain proper receives lymph from the transitional and junctional nodes, the larynx, the thyroid and parathyroid glands and communicates with the spinal accessory and supraclavicular nodes. In modern textbooks the jugular lymph nodes proper are described as either superior and inferior (Sessions et al, 1986) or as upper (subdigastric or jugulodigastric), middle (jugulo-omohyoid) and lower (Million and Cassisi, 1984).
The lymph nodes of the spinal accessory nerve are situated in the region of the lateral triangle of the neck and are distributed along the course of the eleventh cranial nerve. Superiorly they are closely connected with the junctional nodes. Rouvière (1932) described six to 10 while Fisch demonstrated an average of 19 (Fisch, 1968). They drain the parietal region of the scalp, the nuchal skin, and the skin of the lateral cervical areas, as well as that of the shoulder. The spinal nodes constitute regional stations for the lymph from the occipital postauricular and suprascapular lymph nodes. Efferent lymph passes to the supraclavicular lymph nodes.

The supraclavicular nodes lie below the jugular nodes in the gap between the omohyoid and the clavicle. They surround the transverse cervical artery and vein and are sometimes called the transverse cervical chain ("chaine cervicale transverse") of Taillens. Rouvière described six to 12 nodes while Fisch demonstrated nine. Others have called these nodes lower jugular nodes (Becker, 1963). They form the regional station for the lymphatic vessels of the skin of the anterolateral part of the neck and thoracic wall and are connected with lymphatic channels from the spinal chain and axillary, infraclavicular and mediastinal nodes. Efferent lymphatics meet with those from the axillary nodes to end as a short subclavian trunk.
The existence of the nuchal lymph nodes is controversial. They were not mentioned by Rouvière, but have since been described by others (Taillens, 1960; Fisch, 1968). They consist of four to five lymph nodes lying under the origin of trapezius and which run down parallel to its midline. The afferent and efferent pathways of these nodes are poorly understood (Becker, 1963).

The Cervical Skin

In contrast to the deep lateral cervical lymph nodes, there is less controversy concerning the lymphatic drainage of the cervical skin (Rouvière, 1932; Fisch, 1968; Feind, 1972). The most important of the regional lymph nodes of the cervical skin are the deep lateral cervical lymph nodes (jugular, spinal accessory and supraclavicular), and the lower lymph nodes of the parotid gland, the submental nodes, the lymph nodes along the external jugular vein and the medial superficial cervical nodes. The lymphatics of the cervical skin are considered in five regions (Figure 11). These are the nape, lateral, supraclavicular, suprahyoid and infrahyoid regions. The posterior neck vessels run anteriorly and obliquely downward to the lower end of the spinal accessory chain. The lateral skin of the neck drains to the lower parotid nodes or the external jugular chain.
Figure 11

THE CUTANEOUS AFFERENT LYMPHATICS OF THE NECK IN MAN

Feind, 1972
The supraclavicular area is usually drained by means of short collecting vessels into the transverse cervical or spinal accessory chain. The skin of the suprathyroid region drains into the submental and lower parotid nodes. Vessels from this region occasionally drain to the contralateral neck. The infrahyoid skin has a complex lymphatic network with no true midline and the collecting vessels drain into the internal jugular nodes.

The above description of the cervical lymph nodes is based predominantly on the publications of Rouvière supplemented by those of Taillens and Fisch. Although other classifications have been suggested (Kopsch, 1957), they are confusing and have not been adopted into the current literature. The deep lateral cervical nodes have accordingly been divided into junctional, jugular, spinal accessory, supraclavicular and nuchal groups. The jugular nodes have been divided further (Figure 10 and Table 5) into upper (cranial), middle (medial) and lower (caudal) although there is still some confusion surrounding the anatomical boundaries, if any, between the upper jugular and junctional nodes, and the lower jugular and supraclavicular nodes. Despite such classifications and distinctions, Rouvière suggested that the deep lateral cervical lymph nodes function as a single unit and Fisch confirmed a uniform reaction of the system under certain pathological
conditions (Fisch, 1968). The deep lateral cervical lymph nodes must also be considered as one functional unit by the head and neck surgeon since they contain the last filtering stations before the lymph from most of the head and neck organs enters the venous circulation. Hence the aim of modern head and neck cancer surgery, where appropriate, is the removal of the primary tumour, together with the en bloc removal of all the deep lateral cervical lymph nodes (radical neck dissection).
1.2.5. THE NATURAL HISTORY AND EVOLUTION OF NECK DISEASE

The utopian goal when treating any cancer within the head and neck should be to manage the entire tumour burden. The ultimate satisfaction the oncologist derives from developing the best treatment approach should be based on a sound understanding of the patterns and probabilities of spread of cancer from any one particular head and neck site. The state of the art no longer necessitates maximal treatment to all possible areas but therapy should be adjusted according to the known natural history of the disease process in question.

Most head and neck squamous cell carcinoma, with the exception of nasopharyngeal carcinoma, behaves in a similar pattern (Sessions et al, 1986). Cancer from each site has its own particular pattern of spread which is dictated by size and location (Lindberg, 1972), tumour thickness (Mohit-Tabatabai et al, 1986; Moore et al, 1986; Spiro et al, 1986) histology (Broders, 1926; Million, 1984; Close et al, 1987), perineural spread (Soo et al, 1986), the presence or absence of recurrence (Million, 1984) together with the abundance of capillary lymphatics. Areas such as the nasopharynx, tongue, tonsil and hypopharynx have dense capillary lymphatics and the rate at which cancer metastasises from them is high (Pietrantoni and Fior, 1958). In contrast,
areas such as the laryngeal glottis and the paranasal sinuses have a more sparse lymphatic network, and consequently exhibit a proportionately lower metastatic rate when involved by squamous cell carcinoma (Lindberg, 1972; Willis, 1973). One of the most prominent features of head and neck squamous cell carcinoma is its propensity to metastasise through lymphatic channels to the regional lymph nodes (Willis, 1973; Del Regato, 1977; Van de Velde and Carr, 1977). The glands most often involved are the deep lateral cervical lymph nodes since these are the regional nodes for many of the visceral organs in the upper neck and, as such, form the secondary stations for almost all the lymph originating within the head and neck. Peltier et al (1951) identified metastases in the deep lateral cervical lymph nodes of 65% of patients who had died from head and neck squamous cell carcinoma. Subsequently other workers have confirmed a similar high incidence of cervical metastatic disease (Feind, 1972; Lindberg, 1972; Molinari et al, 1977; Mendenhall et al, 1980; Bataini et al, 1985). Hence the early recognition of cervical metastases becomes a prerequisite for the successful treatment of patients with head and neck cancer. Indeed, over one hundred years ago when the concept of surgery to treat cervical metastases was in its infancy, Billroth performed the first total laryngectomy for cancer only to be frustrated by the
demise of the patient several months later due to cervical lymph node metastases which had been undetectable at the time of operation (Gussenbauer, 1874).

The majority of metastases within the lymph glands are due to detached tumour emboli transported by lymphatic flow. It has been suggested that lymphatic deposits from breast cancer (Handley, 1922) and head and neck squamous cell carcinoma (Fisch, 1968) may be due to continuous lymphatic permeation. It is now well recognised that local recurrences following surgical removal of cancers of the tongue and breast occur at either the primary site or within the lymph glands themselves. Growths at intermediate sites along the course of lymphatic vessels are rarely observed. Stiles (1899), in his classic account of the extension of breast cancer, gave support to the embolic origin of metastatic lymph gland deposits since he was unable to demonstrate any growths in the main lymphatics connecting the breast with the axilla. In cases of carcinoma of the tongue, Ewing (1940 p 916) similarly failed to identify cancer in the lymphatics connecting the primary growth with metastatic glands.

The release of malignant cells from the primary tumour results in their passive entry into lymph (Carr and Carr, 1982). Neoplasms are often the site of high interstitial fluid pressures due to the absence of a well formed lymphatic
system (Gullino, 1966; Butler et al, 1975). Since lymphatic vessels are not surrounded by a thick basal lamina or basement membrane, increased numbers of malignant cells may be forced through lymphatic junctions (Casley-Smith, 1977), and the frequent forcible movements of chewing and swallowing help to propel lymph and tumour emboli (McQuarrie et al, 1986). Other workers have studied the passage of metastatic tumour cells into lymphatic vessels using electron microscopy (Van de Velde and Carr, 1977). They concluded that single migrating tumour cells easily penetrated the lymphatics by reverse diapedesis through open interendothelial junctions.

When cancer cells enter lymph they are passively transported to the first eschelon lymph node. Such nodes have been proposed to act as effective "barriers" to the passage of cancer (Baker et al, 1969; Van de Velde and Carr, 1977) although most workers recognise that such a barrier, should it exist, is only temporary since most cancer cells rapidly pass through the first lymph node that they encounter (Zeidman and Buss, 1954; Fisher and Fisher, 1965; Fisher and Fisher, 1966 a-b).

McKelvie (1976) examined cleared radical neck dissection specimens to study the passage of squamous cell carcinoma through the neck lymphatics. He showed that the
slope of the metastatic tree is extremely steep unlike other cancers which metastasise via lymph nodes, e.g. breast cancer. He suggested that some original metastases are segmental, there are some fast long-range pathways involved especially by postcricoid and tongue squamous cell carcinomas and confirmed that separate levels of involved nodes indicate a poor prognosis. He was also able to show that squamous cell carcinoma within the neck spends little time in the occult state and has a rapid doubling time, being explosive to approximately 3 cm. While McKelvie's observations (1976) suggested that the metastatic involvement of various lymph node regions usually progresses from superior to inferior in an orderly fashion, others have shown that lymph node groups can be bypassed even in the normal lymphogram (Larson et al, 1965; Fisch, 1968). Toker (1963) demonstrated discontinuous metastases or "skip" lesions in 28% of neck dissection specimens and others have confirmed his findings (Drroulias and Whitehurst, 1976; Johnson et al, 1980). Chu and Strawitz (1978) found that neck metastases occurred lower in the neck following approximately 30% of negative suprahyoid lymph node dissections.

Once tumour cells arrive at a draining lymph node, they can proliferate, die, remain dormant or enter the blood circulation through blood vessels in the node (Nicolson, 1986).
The process of metastasis is not a random phenomenon although random events may be important (Weiss, 1983). In an attempt to explain the apparent non-random nature of tumour metastases, Paget (1889) proposed the "seed and soil" hypothesis. In his paper, Paget stated that the distribution within the body of particular tumour cells ("seeds") was affected by the micro-environment of any one organ or tissue ("soils"). Paget's theory has subsequently been substantiated by experimental data, albeit in a modified form (Kinsey, 1960; Sugarbaker et al, 1971; Hart, 1982). In the regional cervical lymph glands, pathological changes often appear prior to the appearance of demonstrable tumour deposits. Such changes include an increase in the number of follicles, a proliferation of the reticulum cells and sinus endothelium, and desquamation of the latter into the lymph sinuses which may be partly or completely filled by large pale multiplying cells and this condition is known as sinus histiocytosis or "sinus catarrh" (Willis, 1973). Although some workers have supposed that such changes indicate a specific pre-metastatic process due to soluble toxic tumour products and that successful metastases can only develop after the lymph node "soil" has been so prepared, there is no evidence to support such a hypothesis (Willis, 1973). However, a number of important properties have recently been assigned to tumour cells or metastatic "seeds". These include cell growth,
chemotaxis, immunological, metabolic and hormonal factors. Similar host environment "soil" factors include the tissue and stromal environment, hormones, inflammatory and immunological responses, and the presence or absence of vital nutrients (Nicolson, 1986; Schantz et al, 1988).

There are several stages of metastases via lymphatic pathways. Premetastatic invasion of the epithelial basal lamina of the primary tumour is followed by subsequent encroachment, penetration and translocation of cells through a lymphatic (Batsakis and Medina, 1986). This is followed by intranodal settling, proliferation and destruction of the lymph node. Secondary metastases to other lymph nodes soon develop although their occurrence is not always accompanied by the destruction of the primary echelon node. Metastatic squamous cell carcinoma within a cervical lymph node can stimulate the stroma in a variety of ways and a number of histological and immunological patterns have been described (Berlinger et al, 1976; Ferlito and Polidoro, 1979; Schuller et al, 1985).

Grundman (1984) stated that it is not possible to make any valid histologic, histomorphometric and immunocytochemical parameters for the prognostic immunostaging of resected lymph nodes. However, others have shown that certain histological and immunological patterns are prognostically significant (Yamamoto et al, 1984; Ring et al, 1985; Ortega et al, 1987).
Toker (1963) observed that there were certain anatomical relationships displayed by the terminal portions of the afferent lymphatics en route to the subcapsular sinuses of cervical lymph nodes. He demonstrated that lymphatics traversed the nodal capsule with marked obliquity and that valves were a common occurrence within the intracapsular segment of the afferent lymphatic. Toker then elegantly showed there were four distinct growth patterns of squamous cell carcinoma within cervical lymph nodes. In the first pattern, following original cancerous deposits in the subcapsular sinus, growth within the affected node proceeds to a considerable extent before extranodal spread occurs. Ultimately extranodal extension occurs by the direct penetration and destruction of the capsule, or by the arrest of further emboli in capsular or juxtacapsular lymphatics. In the second pattern of behaviour, extranodal spread occurred at an earlier stage in the genesis of the tumour growth within the node. A third less common pattern involved the deposition of a malignant embolus within the subcapsular sinus, together with the simultaneous arrest of tumour within capsular or juxtacapsular lymphatics. This resulted in the coincident and equivalent proliferation of cancer, both within and outside the node. The last and least common growth pattern showed capsular or juxtacapsular emboli with no
intranodal cancer. Toker concluded that metastatic squamous cell carcinoma within cervical lymph nodes occurred not only within the subcapsular sinus to involve the nodal substance proper, but also within the capsule or the tissue external to it. Subsequently extranodal spread can occur much earlier in the natural history of the disease process and such a phenomenon is important when conservative neck surgery is contemplated or "at risk" necks are managed conservatively by adopting a policy of 'wait and see'.

Distant metastases from head and neck cancer were said to occur in approximately 1% of cases (Crile, 1906). More recent studies show this incidence is now much higher (Braund and Martin, 1941; Peltier et al, 1951; O'Brien et al, 1971; Stefani and Eels, 1971; Merino et al, 1977; Kotwall et al, 1987; Watkinson et al, 1988). Metastases via haematological spread in head and neck cancer can occur by direct invasion into blood vessels (Poland, 1885), or by spread to regional lymphatic nodes and subsequent entry into the circulation via blood vessels or lymphatico-venous communications (Pressman and Simon, 1961; Perez-Tamayo et al, 1963; Willis, 1973). Vascularisation of tumours usually occurs when growths are greater than 0.1-1 mm in size (Nicolson, 1986) and, following this, rapid rates of neoplastic growth, and increased rates of vessel
invasion can occur. Willis (1973) examined 500 necropsy samples from all types of cancer and found evidence of extensive blood-vessel invasion in approximately 30%. Once inside blood vessels, some cancers can extend by permeation for long distances within the unoccluded vessels, whereas others detach and circulate as single cells or cell emboli without apparent growth at the initial site of entry into the blood vessel (Nicholson, 1986).

The rates at which malignant cells are shed into the circulation have been determined. Butler et al (1975) used a rat mammary carcinoma model to show tumours in the range 2-4 g released up to $4 \times 10^6$ cells/g of tumour tissue per day. In some tumour systems, the rates of malignant-cell release into the blood correlate with the size of the primary tumour, whereas in others they do not (Nicolson, 1986). Circulating tumour emboli consist occasionally of single cells, more often of cell "clumps" and, more often still, of thrombus fragments containing tumour cells. It is now generally accepted that considerable fragments of tumour are required to cause blood borne metastases and that such phenomenon are rare with isolated cells (Willis, 1973). Reports of finding free tumour cells in the blood date back for over one hundred years. Ashworth (1869) claimed to see "cells similar to those
in the tumours" in blood taken at post-mortem from the saphenous vein of a patient with multiple skin cancers. Circulating tumour cells are rapidly eliminated in the circulation, mostly by non-specific mechanisms (Butler et al, 1975; Fidler, 1976). Salsbury (1975) stated that the mere presence of tumour cells within the circulation is of little prognostic significance, and does not constitute a metastasis since most tumour cells fail to form distant metastases and Malmgren (1967) found there was no correlation between the incidence of distant metastases and the presence of tumour cells in the blood. Thirty years ago there were many publications (Malmgren, 1968) which claimed to have identified "tumour cells" in concentrated blood samples of a large number of cancer patients and that such examinations may prove useful in assessing operability and prognosis. However, it was subsequently realised that a large number of these "tumour cells" were atypical blood cells, detached vascular endothelial cells and megakaryocytes and that the proportion of cases with genuine tumour cells in the blood is small (Malmgren, 1967; Willis, 1973).

The success of lymphatic or blood borne metastases is determined by both tumour and host factors. Some tumour cells are capable of producing both lymphatic and blood-borne metastases, while others only metastasise via one of these
routes. It has been shown that lymphatic metastases are favored when tumour cells are shed into the surrounding fluid at a high rate, when cells invade blood vessels at low rates and lymph vessels at high rates, and lastly, when tumour cells die quickly in the circulation (Nicolson, 1986). Neri et al (1982) used an experimental adenocarcinoma in syngeneic Fischer 344 rats and showed that distant lung metastatic tumour cells were derived from a subpopulation that had first colonized a draining lymph node from the primary tumour site. Subsequent work in the same tumour model (North and Nicolson, 1985) has shown lymph node colonisation to be a necessary pre-requisite for distant metastases to occur.

Current neck staging (UICC, 1987; AJCC, 1988) is based on clinical evaluation and the assignment of a neck to an N\textsubscript{0} category does not rule out histological or subclinical disease within the cervical lymphatics. The management of the clinically N\textsubscript{0} neck in head and neck squamous cell carcinoma is one of the most controversial topics in head and neck oncology and the majority of the available treatment options are based on the probability of metastases from any one site. The proportion of clinically negative necks that harbour occult metastatic cancer depends on the site, extent and histopathological grade of the primary lesion. The number of clinically negative necks which are pathologically positive can be obtained either by examining specimens from prophylactic neck dissections, or by observing those N\textsubscript{0} necks
which subsequently become positive with no elective treatment. The latter depends on control of the primary lesion with the greatest number of neck failures being observed in those patients whose primary lesions are not controlled. Mendenhall et al (1980) reviewed the literature and compiled statistics (Table 6) to show the percentage of each type of head and neck primary tumour that appears with positive clinical neck disease, the percentages that appear as $N_0$ but following neck dissection turn out to be histologically positive and the percentages of each type of primary tumour that presents as $N_0$ and, when left untreated, become clinically positive. This collective data shows the high incidence of both clinical and subclinical disease within the neck in head and neck squamous cell carcinoma, particularly in those sites with a rich lymphatic network such as the tongue, nasopharynx, tonsil and hypopharynx.

The size of the primary tumour is an important factor in the evolution of neck disease (McGavran et al, 1961; Biller et al, 1971; Farr and Arthur, 1971; Spiro and Strong, 1971; Lindberg, 1972; Cachin, 1983; Teichgraeber and Clairmont, 1984; Bataini et al, 1985). Lindberg compiled an exhaustive analysis of nodal metastases from seven head and neck sites and related them to the stage of the primary sites of 2044 patients (Table 7). All necks were staged using the
### Table 6

**The Incidence of Metastatic Disease from Head and Neck Primary Squamous Cell Carcinoma Sites**

<table>
<thead>
<tr>
<th>Site</th>
<th>Initially Clinically Positive (%)</th>
<th>Clinically Negative; Pathologically Positive (%)</th>
<th>Clinically Negative; Untreated and Became Clinically Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floor of Mouth</td>
<td>30-59</td>
<td>40-50</td>
<td>20-35</td>
</tr>
<tr>
<td>Gingiva</td>
<td>18-52</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>Hard Palate</td>
<td>13-24</td>
<td>-</td>
<td>22</td>
</tr>
<tr>
<td>Buccal Mucosa</td>
<td>9-31</td>
<td>-</td>
<td>16</td>
</tr>
<tr>
<td>Oral Tongue</td>
<td>34-65</td>
<td>25-54</td>
<td>38-52</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>86-90</td>
<td>-</td>
<td>19*-50</td>
</tr>
<tr>
<td>Anterior Tonsillar Pillar/Retromolar Trigone</td>
<td>39-56</td>
<td>-</td>
<td>10-15</td>
</tr>
<tr>
<td>Soft Palate/Uvula</td>
<td>37-56</td>
<td>-</td>
<td>16-25</td>
</tr>
<tr>
<td>Tonsillar Fossa</td>
<td>58-76</td>
<td>-</td>
<td>22+</td>
</tr>
<tr>
<td>Base of Tongue</td>
<td>50-83</td>
<td>22</td>
<td>-</td>
</tr>
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</table>
TABLE 6 (CONT)

<table>
<thead>
<tr>
<th>SITE</th>
<th>INITIALLY CLINICALLY POSITIVE (%)</th>
<th>CLINICALLY NEGATIVE; PATHOLOGICALLY POSITIVE (%)</th>
<th>CLINICALLY NEGATIVE; UNTREATED AND BECAME CLINICALLY POSITIVE (%)</th>
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<tr>
<td>Pharyngeal Walls</td>
<td>50-71</td>
<td>66</td>
<td>-</td>
</tr>
<tr>
<td>Supraglottic Larynx</td>
<td>35-54</td>
<td>16-26</td>
<td>33</td>
</tr>
<tr>
<td>Hypopharynx</td>
<td>52-72</td>
<td>38</td>
<td>-</td>
</tr>
</tbody>
</table>

Mendenhall et al, 1980; Sessions et al, 1986
*\(T_1N_0\) patients only + patients received pre-operative radiation
TABLE 7
THE PROBABILITY OF CERVICAL METASTASES (N) RELATED TO PRIMARY (T) STAGING IN PATIENTS WITH HEAD AND NECK SQUAMOUS CARCINOMA

<table>
<thead>
<tr>
<th>PRIMARY SITE</th>
<th>T STAGE</th>
<th>N₀ %</th>
<th>N₁ %</th>
<th>N₂-N₃ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral Tongue</td>
<td>T₁</td>
<td>86</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>T₂</td>
<td>70</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>T₃</td>
<td>52</td>
<td>16</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>T₄</td>
<td>24</td>
<td>10</td>
<td>66</td>
</tr>
<tr>
<td>Floor of Mouth</td>
<td>T₁</td>
<td>89</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>T₂</td>
<td>71</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>T₃</td>
<td>56</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>T₄</td>
<td>46</td>
<td>10</td>
<td>43</td>
</tr>
<tr>
<td>Retromolar Trigone</td>
<td>T₁</td>
<td>88</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Anterior Faucial Pillar</td>
<td>T₂</td>
<td>62</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>T₃</td>
<td>46</td>
<td>21</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>T₄</td>
<td>32</td>
<td>18</td>
<td>50</td>
</tr>
<tr>
<td>Soft Palate</td>
<td>T₁</td>
<td>92</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>T₂</td>
<td>64</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>T₃</td>
<td>35</td>
<td>26</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>T₄</td>
<td>33</td>
<td>11</td>
<td>56</td>
</tr>
<tr>
<td>Tonsillar Fossa</td>
<td>T₁</td>
<td>30</td>
<td>41</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>T₂</td>
<td>32</td>
<td>14</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>T₃</td>
<td>30</td>
<td>18</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>T₄</td>
<td>10</td>
<td>13</td>
<td>76</td>
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<th>PRIMARY SITE</th>
<th>T STAGE</th>
<th>( N_0 % )</th>
<th>( N_1 % )</th>
<th>( N_2-N_3 % )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Base of Tongue</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
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<td>( T_1 )</td>
<td>30</td>
<td>15</td>
<td>55</td>
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<tr>
<td>( T_2 )</td>
<td>29</td>
<td>14</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>( T_3 )</td>
<td>26</td>
<td>23</td>
<td>52</td>
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</tr>
<tr>
<td>( T_4 )</td>
<td>16</td>
<td>8</td>
<td>76</td>
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<tr>
<td><strong>Oropharyngeal Walls</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>( T_1 )</td>
<td>75</td>
<td>0</td>
<td>25</td>
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<tr>
<td>( T_2 )</td>
<td>70</td>
<td>10</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>( T_3 )</td>
<td>33</td>
<td>22</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>( T_4 )</td>
<td>24</td>
<td>24</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td><strong>Supraglottic Larynx</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( T_1 )</td>
<td>61</td>
<td>10</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>( T_2 )</td>
<td>58</td>
<td>16</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>( T_3 )</td>
<td>36</td>
<td>25</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>( T_4 )</td>
<td>41</td>
<td>18</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td><strong>Hypopharynx</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( T_1 )</td>
<td>37</td>
<td>21</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>( T_2 )</td>
<td>30</td>
<td>20</td>
<td>49</td>
<td></td>
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<tr>
<td>( T_3 )</td>
<td>21</td>
<td>26</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>( T_4 )</td>
<td>26</td>
<td>15</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td><strong>Nasopharynx</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( T_1 )</td>
<td>8</td>
<td>11</td>
<td>82</td>
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<td>( T_2 )</td>
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<td>72</td>
<td></td>
</tr>
<tr>
<td>( T_3 )</td>
<td>12</td>
<td>9</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>( T_4 )</td>
<td>17</td>
<td>6</td>
<td>78</td>
<td></td>
</tr>
</tbody>
</table>

Lindberg, 1972; Sessions et al, 1986
M.D. Anderson system. Lindberg showed that in many primary sites such as the oral tongue, floor of mouth, retromolar trigone and anterior faucial pillar, soft palate, oropharyngeal walls and supraglottic larynx, the small $T_1$ primary tumours were associated with a high incidence of $N_0$ necks. In contrast, primary sites such as the tonsillar fossa, base of tongue, hypopharynx and nasopharynx had low incidences of $N_0$ necks in the small primary tumours. Such small primaries were often characterised by a large proportion of advanced neck disease and often developed nodal disease as the first clinical symptom. Although Lindberg's data showed there was a direct correlation between the $T$ stage and $N$ stage for all sites, it is now recognised that the nasopharynx and hypopharynx are notable exceptions (Million, 1984). Lindberg's data correlated with that from Biller et al in 1971 (supraglottic larynx), and from Spiro and Strong (1971) and Teichgraebar and Clairmont in 1984 (tongue). The relationship between $T$ stage and distant metastases is less clear cut (Spiro et al, 1974a). However, reports suggest a direct relationship between distant metastases and $T$ stage (Probert et al, 1974; Merino et al, 1977; Marechal et al, 1986; Kotwall et al, 1987), $N$ stage (Peltier et al, 1951; Merino et al, 1977; Kotwall et al, 1987), total stage (Merino et al, 1977; Kotwall et al, 1987) and the presence of recurrent disease (Million, 1984). The $N$ stage has a greater influence on the rate of distant metastases than the $T$ stage and the lungs and bones are the most common first sites of metastases (Merino et al, 1977; Kotwall et al, 1987).
The incidence, by site, of distant metastases in head and neck squamous cell carcinoma is shown in Table 8. Several clinical studies have analysed the metastatic neck patterns that exist in patients with head and neck squamous cell carcinoma (Feind, 1972; Lindberg, 1972; Molinari et al, 1977; Bataini et al, 1985). Lindberg's classic study is the most frequently quoted (Sessions, 1986) while the other studies serve to corroborate his findings. Lindberg reviewed the locations of clinically metastatic lymph nodes found at the time of initial presentation of 2044 previously untreated patients with head and neck squamous cell carcinoma. All nodes were assigned to one of 10 nodal neck regions. The subdigastric region in this classification included the upper jugular nodes as well as the tonsillar node of Küttner; it approximated to the junctional nodal region described by Fisch (1968). The spinal accessory and supraclavicular nodes were grouped together as the posterior cervical chain. Abnormal nodes existed in 57% of patients. Lindberg's findings are summarised in Table 9 and from them some notable patterns have emerged. The subdigastric nodes were the most commonly involved group for all the primary tumours studied. The jugular chain was involved more frequently than the posterior cervical chain, the latter being only commonly involved in nasopharyngeal disease. Contralateral neck node metastases were more common than involvement of the ipsilateral posterior cervical chain.
TABLE 8

THE INCIDENCE OF DISTANT METASTASES, BY SITE,
IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

<table>
<thead>
<tr>
<th>PRIMARY SITE</th>
<th>DISTANT METASTASES (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypopharynx</td>
<td>60</td>
</tr>
<tr>
<td>Tongue Base</td>
<td>53</td>
</tr>
<tr>
<td>Anterior Tongue</td>
<td>49</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>47</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>45</td>
</tr>
<tr>
<td>Tonsil</td>
<td>45</td>
</tr>
<tr>
<td>Supraglottic Larynx</td>
<td>44</td>
</tr>
<tr>
<td>Glottic Larynx</td>
<td>44</td>
</tr>
<tr>
<td>Floor of Mouth</td>
<td>43</td>
</tr>
<tr>
<td>Paranasal Sinuses</td>
<td>38</td>
</tr>
<tr>
<td>Oral Cavity</td>
<td>32</td>
</tr>
</tbody>
</table>

TOTAL 46

Kotwall et al, 1987
### TABLE 9

**PATIENTS WITH HEAD AND NECK SQUAMOUS CARCINOMA AND CLINICALLY NODAL METASTASES ON ADMISSION TO THE M.D. ANDERSON HOSPITAL 1948-1965**

<table>
<thead>
<tr>
<th>PRIMARY SITE</th>
<th>PATIENTS</th>
<th>NODE NEGATIVE (%)</th>
<th>NODE POSITIVE (%)</th>
<th>JUGULAR CHAIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral tongue</td>
<td>302</td>
<td>65</td>
<td>35</td>
<td>5</td>
</tr>
<tr>
<td>Floor of mouth</td>
<td>258</td>
<td>69</td>
<td>31</td>
<td>6</td>
</tr>
<tr>
<td>Retromolar trigone/Anterior faucial pillar</td>
<td>227</td>
<td>55</td>
<td>45</td>
<td>1</td>
</tr>
<tr>
<td>Soft palate</td>
<td>80</td>
<td>56</td>
<td>44</td>
<td>3</td>
</tr>
<tr>
<td>Tonsillar fossa</td>
<td>140</td>
<td>24</td>
<td>76</td>
<td>1</td>
</tr>
<tr>
<td>Base of tongue</td>
<td>185</td>
<td>22</td>
<td>78</td>
<td>1</td>
</tr>
<tr>
<td>Oropharyngeal walls</td>
<td>149</td>
<td>41</td>
<td>59</td>
<td>2</td>
</tr>
<tr>
<td>Supraglottic larynx</td>
<td>267</td>
<td>45</td>
<td>55</td>
<td>1</td>
</tr>
<tr>
<td>Hypopharynx</td>
<td>267</td>
<td>24</td>
<td>76</td>
<td>1</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>169</td>
<td>13</td>
<td>87</td>
<td>2</td>
</tr>
</tbody>
</table>

*Lindberg, 1972; Cummings, 1986
+Totals do not add up to 100% since many patients had nodes in more than one area.
### TABLE 9 (CONT)

**PATIENTS WITH HEAD AND NECK SQUAMOUS CARCINOMA AND CLINICALLY NODAL METASTASES ON ADMISSION TO THE M.D. ANDERSON HOSPITAL 1948-1965**

**NODAL SITES INVOLVED (percentage of node positive patients+)**

<table>
<thead>
<tr>
<th>PRIMARY SITE</th>
<th>PATIENTS</th>
<th>NODE NEGATIVE (%)</th>
<th>NODE POSITIVE (%)</th>
<th>UPPER POSTERIOR</th>
<th>MID-POSTERIOR</th>
<th>LOW-POSTERIOR</th>
<th>SUPRA-CLAVICULAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral tongue</td>
<td>302</td>
<td>35</td>
<td>35</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Floor of Mouth</td>
<td>258</td>
<td>69</td>
<td>31</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Retromolar trigone/Anterior faucial pillar</td>
<td>227</td>
<td>55</td>
<td>45</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Soft palate</td>
<td>80</td>
<td>56</td>
<td>44</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tonsillar fossa</td>
<td>140</td>
<td>24</td>
<td>76</td>
<td>9</td>
<td>7</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Base of tongue</td>
<td>185</td>
<td>22</td>
<td>78</td>
<td>6</td>
<td>6</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Oropharyngeal walls</td>
<td>149</td>
<td>41</td>
<td>59</td>
<td>10</td>
<td>8</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Supraglottic larynx</td>
<td>267</td>
<td>45</td>
<td>55</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Hypopharynx</td>
<td>267</td>
<td>24</td>
<td>76</td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>169</td>
<td>13</td>
<td>87</td>
<td>36</td>
<td>26</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

*Lindberg, 1972; Cummings, 1986*

+Totals do not add up to 100% since many patients had nodes in more than one area
Patients with floor of mouth tumours metastasise consistently to the submandibular and subdigastric nodes. Submental nodes are infrequently involved as are the lower jugular and posterior cervical groups. Lesions of the oral tongue commonly involve the submandibular and subdigastric nodes. Mid-jugular nodes are occasionally involved while submental, low-jugular and posterior cervical involvement is notable by its absence. Oropharyngeal squamous cell carcinoma spreads to the subdigastric nodes whereas submandibular and submental involvement is uncommon. Within the oropharyngeal group notable variations exist. Cancers of the retromolar trigone/anterior faucial pillar region spread to the submandibular and subdigastric regions. Palatal lesions metastasise to the subdigastric nodes. Tonsillar squamous cell carcinoma spreads to the mid and lower jugular chain with a notable incidence of posterior cervical metastases. The tongue base involves the subdigastric and middle jugular nodes with the low jugular and posterior cervical nodes rarely involved. The posterior pharyngeal wall spreads to the subdigastric and mid-jugular nodes while posterior cervical nodal involvement is common. Nasopharyngeal squamous cell carcinoma spreads most commonly to the subdigastric region. There is a high incidence of posterior cervical involvement though submental and submandibular nodes are rarely involved. Spread from the
supraglottic larynx is along the jugular chain to involve the subdigastric and mid-jugular regions. Posterior cervical nodes are seldom involved. Hypopharyngeal squamous cell carcinoma spreads to the upper, mid and lower jugular chain in decreasing frequency. Posterior cervical nodes are not uncommon. The ear, scalp and associated skin lesions metastasise to the parotid and upper jugular nodes (Feind, 1972; McQuarrie et al, 1986), while the submental and submandibular nodes are involved with lip cancer. Anterior neck skin lesions drain to the internal jugular nodes while posterior lesions usually drain to the spinal accessory nodes. Some workers have shown that posterior lesions can occasionally drain to the internal jugular nodes (Feind, 1972), a phenomenon which is in direct conflict with anatomical data. The nose and paranasal sinuses drain to the submandibular, subdigastric and retropharyngeal nodes and the parotid gland drains via the parotid nodes to the upper jugular nodes. Thyroid metastases occur within the mid and low jugular, the prelaryngeal and the tracheo-oesophageal nodes while both the cervical oesophagus and subglottis involve the lower jugular and tracheo-oesophageal nodes. Molinari et al (1977) examined 2,500 patients with previously untreated head and neck squamous cell carcinoma and allocated nodes
to similar neck regions as those used by Lindberg in 1972. They applied Bayes theorem for probability calculus to the maps of metastatic distribution and calculated the probability of any one primary tumour site based on given patterns of both single and multiple malignant cervical adenopathy. While their findings correlated well with those of Lindberg, they concluded that spread to the subdigastric and middle jugular region is associated with so many primary tumour sites that it is almost impossible to interpret them and, as such, they can be judged as non-specific.

When discussing the probability of metastases in head and neck cancer, spread to the contralateral neck must always be considered. Contralateral spread of metastases can occur in a number of ways. Lymph flow can occur by passing through afferent lymphatic vessels or by actual spread across the midline via efferent lymph vessels after regional nodes become extensively involved. It may occur within certain anatomical areas where midline boundaries are indistinct or can follow surgical intervention, however small, within the neck. Generally those lesions that are anatomically well lateralised such as the pyriform sinus tend to metastasise to the homolateral neck,
whereas those more midline structures such as the supraglottic larynx, base of tongue, nasopharynx and posterior pharyngeal wall all exhibit a higher incidence of bilateral spread (Table 10). However, exceptions do exist. Any head and neck primary squamous cell carcinoma with homolateral metastases creates a risk for contralateral spread (Million, 1984). The risk increases not only with the size and number of the homolateral nodes but also with increasing primary T stage (Biller et al, 1971; Lindberg, 1972). In patients with laterally placed primary lesions, unilateral cervical adenopathy is rarely contralateral and, if it is, ipsilateral nodes develop soon afterwards. In such situations, unilateral spread occurs in the subdigastric, mid and low-jugular nodes in decreasing order of frequency (Million, 1984). Much emphasis has been placed on the bilaterality of spread from the tongue base although there is ample evidence, both anatomically (Jamieson and Dobson, 1920) and clinically (Spiro et al, 1974a; Drroulias and Whitehurst, 1976; Johnson et al, 1980) that many lymphatic channels exist anteriorly which drain to both sides. Feind and Cole (1969) demonstrated contralateral disease in four out of 21 (21%) patients who had had bilateral neck dissections for anterior tongue squamous cell carcinoma.
**TABLE 10**

INCIDENCE OF CONTRALATERAL METASTASES IN PATIENTS WITH HEAD AND NECK SQUAMOUS CELL CARCINOMA

<table>
<thead>
<tr>
<th>SITE</th>
<th>INCIDENCE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower Lip</td>
<td>25</td>
</tr>
<tr>
<td>Floor of Mouth</td>
<td>14-17</td>
</tr>
<tr>
<td>Oral Tongue</td>
<td>7-15</td>
</tr>
<tr>
<td>Base of Tongue</td>
<td>25-37</td>
</tr>
<tr>
<td>Tongue (overall)</td>
<td>30</td>
</tr>
<tr>
<td>Soft Palate</td>
<td>25-37</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>11*-56</td>
</tr>
<tr>
<td>Tonsil, Faucial Arch and Vallecula</td>
<td>14</td>
</tr>
<tr>
<td>Supraglottis</td>
<td>13-33</td>
</tr>
<tr>
<td>Glottis (fixed cord)</td>
<td>2</td>
</tr>
<tr>
<td>Hypopharynx</td>
<td>10-14</td>
</tr>
</tbody>
</table>

* Patients received pre-operative radiotherapy

Using cervical lymphography, Fisch and Sigel (1964) and Sigel and Fisch (1965) described the normal pattern of cervical lymph flow in man (Figure 12). They then studied the flow changes that occurred following surgery and irradiation (Fisch, 1965; Sigel and Fisch, 1965). Every surgical procedure, however, small, within the head and neck destroys the anatomical integrity of the lymphatic system at the operation site. Despite the extent of the operative procedure (biopsy, node enucleation, neck dissection etc) there is always extensive interruption of the normal lymphatic flow. Consequently, lymphatic stasis and ultimately frank lymphatic oedema develops in the region of a surgical wound. Fisch (1965) showed that even minor surgical procedures within the neck, such as lymph node biopsy, are followed by extensive blockage of the lymphatics at the operation site for up to one year. After a classical radical neck dissection, he was able to demonstrate in man a complete block of contrast material within the surgical area for up to one year after operation. Similar findings have been observed in rabbits (Sigel and Fisch, 1965). Subsequent studies (Fisch, 1965; Fisch, 1968) showed a collateral circulation developed submentally in man which used pre-existing ipsilateral superficial and contralateral deep lymphatics and following bilateral neck dissection or unilateral neck
Figure 12

THE NORMAL CERVICAL LYMPH FLOW IN MAN
VISUALISED BY LYMPHOGRAPHY

Note that the homolateral deep medial cervical lymph nodes do not fill with contrast and that contralateral lymphatic flow does not occur under normal conditions.

Fisch, 1968
dissection combined with irradiation, retrograde flow to subcutaneous dermal lymphatics occurs. Similar collateral submental lymph diversion following radical surgery was observed in rabbits (Sigel and Fisch, 1965). For up to two months following radiotherapy to the neck in man, there are no recognisable changes in either the cervical lymph nodes or lymphatics (Fisch, 1965; Fisch, 1968; Burge, 1975). Later, both the size and number of lymph nodes and vessels decreases and such changes are accompanied by structural changes within the node (Burge, 1975). Changes appear directly proportional to the dose of radiation administered. Complete blockage of the cervical lymphatics following radiotherapy is never observed (Fisch, 1968), although the barrier function of nodes is partly damaged since up to 25% of cells which would normally be held up within nodes pass through after irradiation (Engeset, 1964).

Biller et al (1971) studied 411 patients with laryngeal and laryngopharyngeal squamous cell carcinoma of whom 63 (15%) had contralateral neck disease. Within these 63, nine (13%) patients had developed contralateral disease following dissection of a histologically negative ipsilateral neck. Skolnick et al (1980) showed that contralateral failure rates in patients who had undergone ipsilateral radical neck dissection were higher in those who were histologically
negative than in those who were histologically positive. The effects of ipsilateral neck treatment on the incidence of contralateral disease are both noteworthy and worrying. Physicians and surgeons must be aware of the distinct possibility of contralateral neck disease and, as such, should not only vigorously search for it but also be prepared to offer treatment to those patients who are at risk of developing bilateral disease.

Other factors which influence the natural history and evolution of neck disease such as nodal size, number, level and pathological status have been discussed in Section 1.1.5.
1.3. THE INVESTIGATION OF HEAD AND NECK SQUAMOUS CARCINOMA

1.3.1. Introduction

1.3.2. Current Methods

1.3.3. Primary Disease

1.3.4. The Occult Primary

1.3.5. The Second Primary

1.3.6. Distant Metastases

1.3.7. Residual and Recurrent Disease
1.3.1. INTRODUCTION

Cancer of the head and neck is a major disease with significant morbidity and mortality in the adult population of England and Wales (3.4% of newly diagnosed cancers (excluding skin), Cancer Statistics 1984; 2.4% of total deaths (excluding skin), Mortality Statistics, 1985). The initial assessment, subsequent investigation and final staging of a patient with a head and neck squamous carcinoma are crucial, since predicting prognosis is directly related to accurate staging at the time of presentation. This section outlines the current methods available to evaluate patients with primary disease (excluding the neck), together with those with an occult primary, a second primary, distant metastases, or with residual and recurrent disease following surgery and irradiation. The evaluation of the neck is such an important topic that it is discussed separately in the next section (1.4.).
1.3.2. **CURRENT METHODS**

The evaluation of a patient with a suspected head and neck malignancy begins with a full history and examination. A head and neck data sheet (Figure 1) and detailed diagrams are essential. A comprehensive head and neck examination covers an area equivalent to 13% of the total body surface (Browne, 1986), and takes approximately ten minutes to perform properly. It is unnecessary to discuss methods of head and neck examination, details of which are available in standard textbooks (Million et al, 1982; Browne, 1986). However, where appropriate, pertinent comments are made in each section regarding the examination of any one site or sub-site.

Following the initial clinical examination, the physician will proceed with pre-treatment evaluation which will include, in the first instance, full blood count and sedimentation rate, urea and electrolytes, serum calcium and liver function, treponemal serology and a chest radiograph (Swann and Blakeslee, 1988). Many substances have been evaluated as tumour-markers in head and neck squamous carcinoma. These include serum carcinoembryonic antigen (CEA) (Silverman et al, 1976), serum ferritin
(Maxim and Veltri, 1986), serum B$_2$-microglobulin (Wennerberg et al, 1984), serum vitamin A (Mugliston and Coe, 1986) and the serum enzymes phosphohexose isomerase, aliesterase, adenosine deaminase and 5-nucleotidase (Goel et al, 1986; Lal et al, 1987a; Lal et al, 1987b; Lal et al, 1989). However, none of these have any proven use and, as such, there is no reliable tumour marker with any practical value in the diagnosis and management of head and neck squamous carcinoma.

Histological diagnosis is a pre-requisite to the treatment of head and neck cancer and biopsy (under local or general anaesthetic) is mandatory. Aspiration biopsy (fine needle or tru-cut) has added a new dimension to the pre-treatment evaluation of patients with tumours of the head and neck. It is a safe technique with a high diagnostic accuracy, minimal complication rate and may be performed in the clinic or at endoscopy (Wilson et al, 1985; Shaha et al, 1986; Siodlak et al, 1986). Diagnostic sensitivity can be increased by using CAT, MRI or ultrasound guided probes (Gatenby et al, 1984; Lufkin et al, 1987; Baatenburg De Jong, 1988). Following initial assessment and preliminary investigations, the surgeon will proceed to endoscopy (fibreoptic and/or rigid) and biopsy, so as to
confirm or refute his, or her, suspicions of malignancy, obtain a histological diagnosis and stage the cancer(s). Histological diagnostic sensitivity is increased by using immunocytochemistry (Shi et al, 1984; Cortesina et al, 1988). Accurate staging is facilitated by pre-treatment imaging to include CAT, MRI, ultrasound and radionuclide scanning. Imaging should be performed before endoscopy and only as appropriate, since it is neither necessary or financially feasible in all cases and should only be carried out if it will significantly alter patient management (Cantrell, 1984; Stell, 1987).

Such imaging techniques are of value not only in the evaluation of the primary, occult primary and local and distant metastases, but also in assessing the second primary and residual and recurrent disease following surgery and irradiation. The merits of each investigation in evaluating these conditions at each of the head and neck sites and subsites are discussed in the subsequent sub-sections (1.3.3.-1.3.7.).

Computerised axial tomography was introduced in the early 1970's, beginning with first generation head scanners (Ambrose, 1973). These first scanners were limited in their
otolaryngological applications by the presence of a water bag between the head and the scanning gantry. The water bag was essential for accurate density measurements but limited the range of the study to the orbits and the brain. These problems were resolved by the development of second generation scanners which then began to make important contributions to the diagnosis and management of otolaryngological problems (Mancuso et al, 1977; Wortzman et al, 1978). Continued development has provided third (Sagerman and Chung, 1981) and fourth generation scanners (Smith and Noyek, 1988) with improved resolution, decreased scanning time and applicability to all parts of the body.

Computed tomography of the head and neck provides two- and three- dimensional information and allows a visual scaled demonstration of normal anatomical structures and their geometrical relationships. It can also locate and demonstrate tumour masses and, so long as the mass is within the resolution of the unit and other technical factors are controlled, tumour size and extension, encroachment onto major vessels, the aero-digestive passages and soft tissues and attachment to, and invasion of, bone and cartilage can all be demonstrated. In addition, distant metastases in organs such as the lung and liver can be accurately evaluated.
Computerised tomography does have distinct disadvantages. It involves X-ray irradiation, is operator and observer dependent and is expensive. In addition, it only provides anatomical, and not physiological information. The CT signal intensity is dependent on a single variable (attenuation coefficient) and streaking artefacts often exist due to bone, metal dental fillings and motion abnormalities related to respiration and deglutition. The subsequent reconstruction of coronal and sagittal images from the transverse sections results in a prolonged imaging time (Kean and Smith, 1986), and although direct coronal and sagittal CT images can now be performed this is not always possible due to technical limitations, or an unco-operative or unfit patient. Also, the low contrast levels in CAT means that iodinated contrast materials are often used which subsequently block the thyroid gland and this can preclude radionuclide thyroid imaging for up to six months.

However, CAT is the most valuable of the imaging modalities currently available to investigate the head and neck and, in appropriate cases, has revolutionised the work up and staging of patients with head and neck squamous carcinoma (Sagerman and Chung, 1981; Muraki et al, 1983; Mancuso and Hanafee, 1985; Schaeffer et al, 1985a).
Magnetic resonance imaging was first discovered in 1946 by Bloch and Purcell for which they received the Nobel Prize in 1952 (Wilhelm, 1983, p 89). Over the next 25 years, MR spectroscopy became a major analytical tool to identify the various different chemical states of elements. Damadian (1971) demonstrated that a particular MR parameter ($T_1$) of tumour samples, measured in-vitro was significantly higher than normal tissues. This discovery, coupled with the impact of CAT on medical imaging, paved the way for MR imaging and its subsequent introduction into clinical practice (Lauterbur, 1973), and then otolaryngology (Baker, 1986).

MRI provides both two- and three-dimensional information and demonstrates normal anatomical structures and their geometric relationships in a similar manner to CAT. MRI has a number of advantages and disadvantages when compared with CAT.

MRI uses non-ionising radiation and the signal intensity is dependent on three variables ($T_1$, $T_2$ and proton density), unlike the single variable in CAT (attenuation coefficient). There is direct availability of sagittal and coronal imaging and high contrast levels make the use of conventional contrast agents unnecessary.
in most instances. The anatomy displayed on transverse head and neck MR sections corresponds closely to that displayed by CAT but there are several important differences. There is more sensitive soft tissue contrast resolution and a very high level of grey-white matter contrast of neural tissue, particularly on inversion recovery, and this is even greater than that of fourth generation CAT scanners. Compact bone gives no signal on MRI. Therefore, there are no streaking artefacts from bone and soft tissue structures so that the facial nerve can be demonstrated without being obscured by surrounding bone. However, this means that pathological bony destruction will not be demonstrated. Although bone does not cause streaking artefacts, these can occur with stainless steel dental pins (Hinshaw et al, 1988).

MRI is an expensive technique, more so than CAT, and is at present only available in major teaching centres. It has poor spatial resolution and prolonged imaging times when compared with CAT, and although it is superior to CAT in imaging intracranial anatomy (Kean and Smith, 1986), its exact role, when compared to CAT, in the evaluation of head and neck squamous carcinoma is still being assessed.
The ultrasonic echo technique, which is now used in all areas of modern medicine, was first developed by Langevin during World War I for the detection of submarines (Kitamura et al, 1969). Ultrasound energy is reflected whenever the beam passes from one medium to another having a different acoustic impedance. The amount of energy reflected is dependent upon the difference in acoustic impedance of the two substances and the angle between the reflecting interface and the ultrasound beam.

B-mode high resolution echography has been widely investigated in the diagnosis and management of all aspects of otolaryngology (Scheible and Leopold, 1978; Scheible, 1981). It is cheap, non-invasive and does not use ionising radiation. However, it is observer dependent and accuracy is directly related to the experience of the operator. It is principally used within the head and neck, in conjunction with radionuclide scanning and fine needle aspiration biopsy (FNAB) to evaluate thyroid swellings (Scheible et al, 1979). It has also been claimed to be of value in the evaluation and follow-up of patients with head and neck neoplasms (Baker and Krause, 1981; Westhofen, 1987) since it can demonstrate the site, size and structure of a neck mass. However, it cannot reliably distinguish benign from malignant disease and, therefore,
has no established role in the evaluation and staging of head and neck squamous carcinoma although it may be of value in the assessment of cystic neck swellings, the diagnosis and drainage of abscesses and ultrasound guided FNAB.

Malignant conditions affecting the mucosal surfaces of the upper aerodigestive tract, adnexal organs such as the thyroid gland, salivary glands and cervical lymph nodes as well as the cartilaginous and bony structures of the larynx and skull can often be diagnosed with ease using traditional methods of history and examination combined with conventional radiology. The recent advances in imaging techniques (CAT, ultrasound and MRI) increase diagnostic sensitivity and specificity but suffer from distinct disadvantages since they can only provide anatomical information. Radionuclide scanning can add a physiological dimension to diagnostic imaging within the head and neck and has been of particular value in the evaluation and treatment of thyroid neoplasms (Watkinson and Maisey, 1988).

The accumulation of Mercury-197 chlormerodrin at sites of known head and neck squamous carcinoma was first reported by Johnson et al in 1965. Since then physicians
and surgeons have employed a variety of radiopharmaceutical agents to investigate head and neck squamous carcinoma in an attempt to identify primary and occult tumour with cervical metastases together with residual or recurrent disease following surgery and irradiation.

Approximately 20 years ago Edwards and Hayes (1969) investigated the potential of Gallium-67 (Ga\(^{67}\))-Citrate as a bone scanning agent and noted its concentration in the cervical lymph nodes of a patient with Hodgkin's disease. Ga\(^{67}\) was subsequently described as "tumour-seeking", not only for head and neck malignancy (Kashima et al, 1974) but for tumours in general (Andrews and Edwards, 1975). It has been extensively evaluated as a tumour imaging agent in head and neck squamous carcinoma and its uptake has been reported and confirmed in a variety of primary sites and sub-sites including metastatic lymphadenopathy within the neck (Kashima et al, 1974; Kornblut et al, 1974; Silberstein et al, 1974; Smith et al, 1975; Higashi et al, 1977a-b; Teates et al, 1980).

However, Ga\(^{67}\) has distinct disadvantages. It has a normal biodistribution within the head and neck which includes the nasopharynx, the lacrimal glands and the salivary glands (Kashima et al, 1974), the uptake in the
latter two being permanently enhanced by radiotherapy (Kashima et al, 1974; Bekerman and Hoffer, 1976). Uptake also occurs, not only in a variety of malignant tumours including lymphoma, but also in benign tumours and inflammatory tissue (Kashima et al, 1974; Kornblut et al, 1974; Ohta et al, 1984a; Ohta et al, 1988). Lesions less than 2 cm in size are not usually detected with planar scintigraphy (particularly cervical lymph nodes), by which time they are usually clinically palpable (Cummings et al, 1981). Sensitivity for all lesions is significantly increased for masses greater than 3 cm in size (Teates et al, 1980) and is also affected by site (Bland and Rose, 1981), being highest for lesions not situated in or near either the salivary glands or the nasopharynx. Such phenomenon all contribute to a low sensitivity and specificity which varies from 53% and 89% (Silberstein et al, 1974), 56% and 64% (Teates et al, 1980) and 85% and 51% (Endo et al, 1985). In Silberstein's paper all the patients (52) had proven squamous carcinoma. However, in Teates' paper, only 51% had proven head and neck squamous carcinoma (the rest were unspecified, except 8% had lymphoepithelioma (lymphomas were excluded)), and in Endo's paper 75% of patients had squamous carcinoma (the rest were unspecified; lymphomas were excluded). In addition, Ga$^{67}$ is expensive with a prolonged blood
clearance which delays the scanning time up to 48-72 hours. Consequently, its current clinical use in head and neck tumour imaging is largely confined to the evaluation of lymphoma (Watkinson, 1990), although uptake in squamous carcinoma continues to be evaluated (Solfanelli et al, 1987).

Many other radiopharmaceutical agents have been used to evaluate squamous carcinoma of the head and neck. Cobalt-57 (Co$^{57}$)-Bleomycin (Poulouse et al, 1975; Sawas-Dimopoulou et al, 1978; Woolfenden et al, 1979; Cummings et al, 1981); Indium-III (In$^{III}$)-Bleomycin (Goodwin et al, 1981; Höfer et al, 1987); In$^{III}$-Transferrin (Goode et al, 1973); Mercury-197 (Hg$^{197}$)-Dichloride (Cl$_2$) (Johnson et al, 1965; Aversa et al, 1978); Tc$^{99m}$-Bleomycin (Lin et al, 1974) and Tc$^{99m}$-TcO$_4^-$ (Grant and Smith, 1974) have all been evaluated but, like Ga$^{67}$, have distinct disadvantages. They all exhibit a low sensitivity and specificity, considerable cost and a prolonged blood clearance (except Tc$^{99m}$-TcO$_4^-$). Of these radiopharmaceuticals, Co$^{57}$-Bleomycin is the one most extensively evaluated and the one which has been shown to be more sensitive than both Ga$^{67}$-Citrate and In$^{III}$-Bleomycin, with a sensitivity and specificity of 84% and 50% respectively (Cummings et al, 1981). Although
it has no normal biodistribution in either the nasopharynx or the salivary glands, it is accumulated by normal thyroid cartilage (Cummings et al, 1981). In addition, it has a half-life of 270 days and since 80% of the radioactivity is excreted via the urine within 24 hours, the urine must be collected during this period to reduce environmental contamination (Woolfenden, 1979). It is not possible to demonstrate lesions, particularly lymph nodes, which measure less than 2 cm in size with Co$^{57}$-Bleomycin (Cummings et al, 1981). In view of their limitations, neither Co$^{57}$-Bleomycin nor any of the other radiopharmaceutical agents evaluated in head and neck squamous carcinoma (except Ga$^{67}$) have been adopted into routine clinical practice and, therefore, play no role in staging or the detection of residual or recurrent disease.

One of the major criticisms of both Ga$^{67}$-Citrate and Co$^{57}$-Bleomycin has been their unreliability in the detection of lesions less than 2 cm in size. One of the reasons for this is that planar scintigraphy provides a two-dimensional image of a three-dimensional object and contrast is compromised not only by superposition of activity above, and below, the area of interest, but also tissue superimposition and attenuation, scatter, motion artefacts, image noise or statistics, and spatial resolution (Heller and Goodwin, 1987).
Recent advances in scanning techniques have allowed tomographic nuclear medicine studies using single photon emitters such as Tc$^{99m}$ (Sousaline, 1982). This technique is called Single Photon Emission Computerised Tomography (SPECT) and uses a rotating gamma camera which acquires 64 projections over $360^\circ$, each view for a $5.6^\circ$ rotation of the camera. This information can be combined with data already available from conventional planar views and using a data processor, mathematically reconstructed tomographic images can be produced in the coronal, sagittal and transaxial planes by utilising a filtered back-projection technique (Piez and Holman, 1985). This is based on algorithms developed in radio-astronomy to reconstruct a two-dimensional brightness distribution over a source from fan-beam scans taken in various position angles (Bracewell and Riddle, 1967). SPECT improves depth interpretation and reduces tissue superimposition artefacts and leads to an increase in sensitivity, image quality and spatial resolution (Heller and Goodwin, 1987).

Tc$^{99m}$ Dimercaptosuccinic Acid (DMSA) is a new radiopharmaceutical which has been used to evaluate head and neck squamous carcinoma and, for which, an increased sensitivity has been reported using SPECT (Ohta et al, 1985a
Aw et al, 1986; Ohta et al, 1988). Although its uptake has been reported at a number of primary head and neck sites and sub-sites and early reports are encouraging no studies have been made comparing tumour stage and size with sensitivity. Further studies are necessary. The history of Tc $^{99m}$ (v) DMSA is discussed in Section 1.6.

Techniques such as lymphoscintigraphy have been used to demonstrate the cervical lymphatics in health and disease and are discussed in Section 1.4.

The concept of using antibodies to identify tumours was conceived by Paul Erlich who invented the term "magic bullet" (Himmelweit, 1960). The magic bullets used today are monoclonal antibodies, first isolated by Köhler and Milstein at Cambridge in 1975 using hybridoma technology. Tranter et al (1984) used $^{131}$ monoclonal anti-CEA antibodies to evaluate five patients with primary and metastatic head and neck squamous carcinoma. Although positive uptake was demonstrated at the primary sites, at the sites of metastatic cervical lymphadenopathy, and at the sites of pulmonary metastases, no lesions were demonstrated which measured less than 2 cm in size and residual and recurrent disease was not detected.

Epidermal growth factor (EGF) may have an important role to play
in the development and regulation of human cancer and there are increased levels of EGF receptors, both in tumour biopsies, and in a number of established cell lines derived from head and neck squamous carcinoma (Gusterson, 1984; Cowley, 1986). Soo et al (1987) used In-III labelled EGFRI, (a monoclonal antibody against the EGF receptor (Waterfield, 1982)) to evaluate 11 patients with primary and cervical metastatic disease. Although he obtained a sensitivity of 73%, no lesions less than 3 cm were identified.

Monoclonal antibodies have made a huge impact on the \textit{in-vitro} diagnosis of head and neck squamous carcinoma (Price, 1987). In theory, \textit{in-vivo} radio-immunoscintigraphy provides the ideal answer to both tumour imaging and target directed therapy, but in practice, it suffers from distinct problems such as high background activity, low target to non-target ratios and non-specific accumulation in organs such as the liver and spleen. There is, at present, a variety of techniques attempting to solve some of these problems (Davies, 1985), but images are far from ideal and lesion uptake is well below that required for effective radioimmunotherapy (Vaughan et al, 1987). Therefore monoclonal antibodies, at present, play no role
in the evaluation and staging of head and neck squamous carcinoma, nor in the detection of residual and recurrent disease following surgery and irradiation.
1.3.3. PRIMARY DISEASE

The majority of the anatomical boundaries and pathological staging criteria using CAT, MRI and ultrasound have been based on AJC criteria (AJCC, 1978; AJCC, 1983). It is not the intention of this section to discuss the indications for sophisticated imaging techniques (usually CAT or MRI) at each head and neck site. However, in general terms, their use is only indicated if the result will significantly alter management, or where an accurate knowledge of tumour volume is mandatory for radiotherapeutic purposes. The normal head and neck anatomy, together with patterns of spread of malignant disease, as seen on CAT and MRI have been described and details are available in the literature (Mancuso et al, 1980a; Osborn et al, 1982; Reede et al, 1982a-b; Virapongse et al, 1982; Felson, 1983; Gamsu and Webb, 1983; Muraki et al, 1983; Silverman and Korobkin, 1983; Mancuso and Som, 1984; Stark et al, 1984a; Castelijns et al, 1985; Mancuso and Hanafee, 1985; Quint et al, 1985a-b; Unger, 1985; Lufkin et al, 1986a; Tubman, 1986; Casselman and Mancuso, 1987; Castelijns, 1987; Castelijns et al, 1987a-c; Mandelblatt et al, 1987; Teresi et al, 1987a; Som et al, 1988).
The Oral Cavity

The key to examining the oral cavity is systematic examination of the sub-sites with bimanual palpation (where possible) followed by examination under anaesthetic (EUA), panendoscopy and biopsy. Dysplastic lesions may be evaluated with toluidine blue (Strong et al, 1968). This technique can identify exposed areas of tumour to include tissue margins (Yco and Cruikshank, 1968), but it cannot delineate deep submucosal extension.

The smaller ($T_1$ and $T_2$) oral tumours are best evaluated by direct inspection since this is the most accurate method of assessing mucosal disease (Lufkin et al, 1986a). Larger tumours ($T_3$ and $T_4$) are best evaluated using complimentary techniques such as panoramic tomography, CAT or MRI.

Computerised tomography is a proven adjunct to the detection and staging of oral cancer (Muraki et al, 1983; Schaefer et al, 1985a). CAT can identify tumour volume, midline extension and can assess lingual artery, hypoglossal nerve, soft tissue and bony invasion more accurately than either palpation, panoramic tomography or endoscopy.
(Larsson et al, 1982; Osborn et al, 1982; Schaefer et al, 1985a). However, artefacts produced by the mandible and dental amalgam are a problem and coronal and sagittal images are difficult to obtain. MRI is more sensitive than CAT in the evaluation of tongue cancer (Lufkin et al, 1986a). MR imaging consistently produces superior soft tissue detail and artefacts are uncommon. Direct coronal and sagittal image planes allow recognition of intrinsic tongue musculature together with the assessment of tumour volume and soft tissue spread. Although the dense cortical bone of the mandible produces no signal on MR images, MRI can still resolve bony tumour involvement. Where rapid imaging times are essential, CAT is superior to MRI in the unco-operative patient (Lufkin et al, 1986a). Neither CAT or MRI detect histological features or microscopic disease and as many inflammatory, reactive and malignant conditions have similar appearances, biopsy is essential.

The normal anatomy of the tongue has been evaluated by ultrasound (Bruneton et al, 1986). Small tumours in the tongue tip and lateral border are difficult to visualise but most T2 and T3 lesions can be precisely defined in three dimensions. The depth of invasion can be accurately assessed as can extension across the midline (Bruneton et al, 1986)
Larger T4 tumours are difficult to evaluate due to the field of the scanner and these are better evaluated by CAT. Despite these findings, ultrasound imaging of oral cancer is inferior to clinical examination, CAT and MRI (Lufkin et al., 1986a) and, as such, has no role in either diagnosis or staging.

The uptake of Ga$^{67}$-Citrate and Tc$^{99m}$ (v) DMSA (planar and SPECT) has been reported in oral carcinoma (Kornblutt et al., 1974; Smith et al., 1975; Higashi et al., 1977a; Teates et al., 1980; Ohta et al., 1985a; Ohta et al., 1988). Ga$^{67}$ is associated with a low sensitivity and specificity when compared to clinical examination and lesions less than 2 cm in size are not usually detected. Because of this it plays no role in diagnosis or staging. The uptake of Tc$^{99m}$ (v) DMSA in oral carcinoma is encouraging. However, further studies are necessary to evaluate sensitivity against tumour size and assess whether bony uptake into the mandible occurs specifically with tumour invasion, or also with inflammation. Bone scanning with Tc$^{99m}$ methylene diphosphonate (MDP) has been claimed to be of value in the assessment of bony invasion of the mandible by squamous carcinoma (Noyek, 1979). Although bone scanning is extremely sensitive and will demonstrate lesions before they are visible on plain radiographs,
the findings are often non-specific and bony extension within the mandible from squamous carcinoma cannot be reliably distinguished from benign dental disease (Matteson et al, 1980).

**The Nasopharynx**

Conventional mirror examination of the nasopharynx is one of the most difficult clinical procedures to perform in otolaryngology. Direct inspection using fibre-optic endoscopy is now possible and is the investigation of choice when available (Million et al, 1982, p 305). Suspicious lesions should be evaluated and biopsied under general anaesthetic and the head and neck surgeon should be aware that in the absence of a mucosal abnormality, he or she may miss a predominant submucosal lesion (Mancuso and Hanafee, 1983).

There are two main reasons to investigate the nasopharynx radiologically. One is to evaluate the extent of a diagnosed nasopharyngeal mass and the other is to search for an occult primary. Until the advent of CAT, nasopharyngeal tumour staging was based on clinical examination combined with plain radiography and polytomography (Yamashita et al, 1985). CAT accurately demonstrates the air-mucosal interface and the deep fascial
planes below. Contrast agents are not used routinely, but may be of value when intracranial extension is suspected, to differentiate benign mucosal masses from deep infiltrative lesions, and to evaluate parapharyngeal extension (Mancuso and Som, 1984; Mancuso and Hanafee, 1985). Until the advent of MRI, CAT was the most reliable method of detecting and determining the extent of nasopharyngeal carcinoma (Mancuso and Som, 1984). The hallmark of nasopharyngeal carcinoma is deep infiltration. CAT can demonstrate soft tissue and bony extension, assess tumour volume and, when compared with clinical and conventional radiographic findings, is the most reliable and accurate method available to stage nasopharyngeal carcinoma (Yamashita et al, 1985).

The normal nasopharyngeal anatomy as seen on MRI is better demonstrated than on CAT (Dillon et al, 1984; Teresi et al, 1987a). MRI is superior to CAT in distinguishing soft tissue from tumour extension, tumour/muscle and tumour/brain interfaces due to better contrast resolution (Teresi et al, 1987b). Tumour infiltration of bone marrow is best seen with MRI, but CAT is superior in demonstrating bony changes (Teresi et al, 1987b).
Ultrasound has not been evaluated in nasopharyngeal carcinoma and therefore plays no role in staging. The uptake of Ga$^{67}$ and Tc$^{99m}$ (v) DMSA has been reported in nasopharyngeal carcinoma (Teates et al, 1980; Aw et al, 1986; Solfanelli et al, 1987). Ga$^{67}$ has no current role in diagnosis or staging due to a low sensitivity and specificity since it has a normal biodistribution which includes the nasopharynx and cannot detect lesions measuring less than 2 cm in size. Tc$^{99m}$ (v) DMSA requires further evaluation but initial reports suggest that it may also suffer from similar limitations (Aw et al, 1986).

**The Oropharynx**

Squamous cell carcinoma of the oropharynx is often associated with a poor prognosis which is primarily due to the advanced stage of the disease at the time of initial presentation. Although there are a number of techniques available to evaluate the oropharynx (such as digital palpation, indirect and direct laryngoscopy), only a small proportion of these carcinomas will be detected without cervical lymph node metastases.
Early radiological detection of oropharyngeal malignancy is similarly difficult. Lateral soft tissue radiographs can identify gross exophytic lesions and both contrast laryngopharyngography and double-contrast pharyngeal radiography can provide more information, but may be difficult to perform (Apter et al, 1984).

CAT of the oropharynx can assess tumour size and the extent of local deep infiltration and midline extension. It can evaluate lingual artery and hypoglossal nerve involvement, spread to contiguous sites and sub-sites and assess the submucosa in the presence of intact mucosa. Artefacts due to dental amalgam are not a problem and submucosal spread is best evaluated using intravenous contrast (Mancuso and Hanafee, 1985).

The indications for MR imaging of the oropharynx are the same as for CAT. MRI can identify all the features described on CAT, but has an increased sensitivity for defining intrinsic tongue musculature and for detecting tumour volume and deep plane extension. The lack of artefacts from dental amalgam and the availability of direct coronal and sagittal imaging means that MRI, when available, is the imaging modality of choice for the oropharynx (Lufkin et al, 1986a).
Both the tongue base and the tonsil have been evaluated with ultrasound and the normal anatomy defined (Mettler et al, 1979; Bruneton et al, 1986). It has been claimed that ultrasound can accurately define T2 and T3 tongue base squamous carcinomas in three dimensions, identify midline extension and accurately assess anterior spread of tonsillar cancer into the tongue base (Bruneton et al, 1986). However, ultrasound cannot visualise superficial lesions and has a reduced accuracy for evaluating large tumour (T4) extension and posterior pharyngeal extension when compared with CAT (Bruneton et al, 1986). For these reasons, ultrasound plays no part in the routine staging of oropharyngeal squamous carcinoma.

Radionuclide scanning using Ga_67-Citrate, Tc_99m (v) DMSA and Tc_99m-MDP has been used to evaluate oropharyngeal squamous carcinoma (Smith et al, 1975; Teates et al, 1980; Ohta et al, 1985a). However, Ga_67 cannot reliably detect lesions less than 2 cm in size and Tc_99m-MDP bone scanning cannot reliably distinguish tumour involvement from benign disease. As such, radionuclide scanning of oropharyngeal squamous carcinoma has no current role in diagnosis or staging. Tc_99m (v) DMSA requires further evaluation.
The Hypopharynx

Squamous cell carcinoma of the hypopharynx often presents late with advanced local disease and cervical metastases. In the past, tumours have been evaluated using direct endoscopy and contrast studies but these techniques on their own have distinct disadvantages since they can only infer deep-seated abnormalities from changes in surface contour. The commonest tumours are those affecting the post-cricoid region and the pyriform sinus. As these areas are often difficult to assess endoscopically, CAT and MRI have added a new dimension to their evaluation.

CAT does not reveal mucosal detail, so the best way to evaluate the hypopharyngeal mucosa is at endoscopy. CAT can identify mucosal abnormalities but these may be due to tumour, fibrosis, oedema, haemorrhage or inflammation. CAT complements endoscopy and biopsy since it reveals direct extension and can identify cartilage invasion (Mancuso and Hanafee, 1985, p 253). Accurate evaluation of the pyriform sinus can be improved by performing phonation scans (Gamsu et al, 1981), and the assessment of laryngeal involvement and vocal cord mobility is discussed in subsequent sections. MRI is superior to
CAT in identifying soft tissue definition and tumour extension and sensitivity can be increased by using a surface coil (Lufkin et al, 1986b). In some institutions surface coil MR imaging is the investigation of choice to evaluate the hypopharynx (Lufkin et al, 1986b).

Ultrasound imaging of the hypopharynx has not been evaluated. The uptake of Ga $^{67}$-Citrate has been reported in hypopharyngeal carcinoma (Kornblutt et al, 1974; Smith et al, 1975; Teates et al, 1980). However, it cannot identify lesions less than 2 cm and since it is less accurate than endoscopy compared with CAT or MRI, it has no role in either diagnosis or staging.

The Larynx

All patients with squamous carcinoma of the larynx should have a direct laryngoscopy and biopsy. The subsequent radiological evaluation of the larynx has undergone significant changes in the last decade and conventional techniques of plain film tomography and laryngography have been supplemented by CAT and MRI since, combined with direct endoscopy, they are more accurate.
(Archer et al, 1981; Lewis and Carter, 1987) and permit precise tumour localisation so that the surgeon can decide between radiotherapy and partial or total laryngectomy.

CAT provides helpful information about areas that may be hidden from visual inspection by bulky tumours, such as the subglottis, the apex of the pyriform sinus and the laryngeal ventricle, and sensitivity is increased by performing phonation scans (Gamsu et al, 1981). It reveals deep extension and helps to clarify suspected tumour extension in submucosal laryngeal structures where overlapping structures prevent a full two-dimensional evaluation. Minor mucosal abnormalities will not be seen on CAT, but these are much better evaluated at laryngoscopy. Caution must be used when diagnosing cartilage invasion on CAT due to the random distribution of calcification and ossification which occurs within normal cartilage (Archer et al, 1983). Although CAT may be of value in the assessment of vocal cord fixation (Mancuso et al, 1980b; Mancuso and Hanafee, 1985), it cannot define a transition zone from the true to the false cords and spread of tumour from the true to the false cords (or vice-versa) can be difficult to assess (Castelijns, 1987, p 30). Lastly, CAT may over-estimate tumour size due to oedema and inflammation, or under-estimate tumour size due to failure to identify microscopic disease (Silverman et al, 1984).
The demonstration of normal and normal variant anatomy using MRI can be improved by using a surface coil (Castelijns et al, 1985; McArdle et al, 1986). Employing standard head and body coils, the structures of the neck are particularly difficult to image using MRI (Castelijns, 1987). Head coils will not cover the middle and inferior regions of the neck and body coils are inefficient due to the small size of the region of interest. The application of specially designed surface coils has solved these problems, and the demonstration of normal laryngeal anatomy using an MRI surface coil is superior to that obtained with CAT.

MRI using a surface coil has potential advantages over CAT although patients with difficulty in swallowing, or with excessive coughing, are unsuitable due to motion artefacts (Castelijns, 1987). In addition, due to the long scanning times, it is not yet possible to image the larynx while performing phonation manoeuvres without getting motion artefacts.

By using frontal images, MRI can show the cranio-caudal extension of a tumour, particularly subglottic extension, and the relationship between the caudal margin of the tumour and the upper border of the cricoid is more
clearly demonstrated than on CAT. On sagittal images, infiltration of the base of the tongue is well visualised so that the distance between the caudal margin of a supraglottic tumour and the anterior commissure can be assessed. Although it was thought initially that MRI was no better than CAT in predicting laryngeal cartilage invasion (Rothberg et al, 1986), it has now been shown to be superior (Castelijns et al, 1987b-c). MRI of the larynx is the investigation of choice, when available, in the diagnostic imaging of laryngeal squamous carcinoma (Castelijns, 1987, p 150). However, if MRI fails due to motion artefacts, claustrophobia, metallic implants, or is impossible to perform due to a pacemaker or surgical clips, CAT may still be necessary. If imaging laryngeal carcinoma can be accomplished without the use of intravenous contrast, then this alone would make MRI the investigation of choice (Mancuso and Hanafee, 1985).

Ultrasound has been used to evaluate the larynx and has been used principally to evaluate tumour invasion of the thyroid cartilage. Although sensitive images can be obtained regarding cartilage thickness and integrity, they are non-specific and require close clinical correlation (Miskin et al, 1978). However, ultrasound laryngeal high resolution imaging may be of value in isolated cases.
It can identify tumour invasion into the thyroid gland, the internal jugular vein and the carotid artery. Where CAT or MRI findings of cartilage destruction are equivocal, ultrasound can effectively delineate cartilage integrity in $T_3$ lesions and cartilage destruction in $T_4$ lesions (Rothberg et al, 1986).

Radionuclide imaging in the primary evaluation of laryngeal squamous carcinoma has limited applications. The uptake of Ga $^{67}$-Citrate and Tc $^{99m}$ (v) DMSA has been reported at sites of laryngeal cancer (Kornblutt et al, 1974; Smith et al, 1975; Teates et al, 1980; Ohta et al, 1985a; Ohta et al, 1988). Ga $^{67}$ suffers from a low sensitivity and specificity since it cannot distinguish between tumour involvement and inflammation when there is positive cartilage uptake, and is also unable to detect lesions less than 2 cm in size. It therefore plays no current role in diagnosis or staging. Tc $^{99m}$ (v) DMSA requires further evaluation.

The Cervical Trachea and Oesophagus

Patients with squamous carcinoma of the cervical trachea and oesophagus should all have an endoscopy and biopsy, and contrast barium studies may help in staging lesions (Felson, 1983).
CAT is inaccurate in the pre-operative assessment of oesophageal carcinoma. While it can define deep extension into the neck and cervical trachea (Thompson et al, 1983; Mancuso and Hanafee, 1985) it consistently understages tumours, over-estimates tumour length and its major limitations are an inability to define the individual layers of the oesophageal wall, to evaluate peri-oesophageal spread due to the paucity of fat surrounding the cervical oesophagus (Quint et al, 1985a-b), and to correctly identify malignant lymphadenopathy (Thompson et al, 1983).

Squamous carcinoma of the cervical trachea is uncommon and 25% of patients have a secondary primary of the upper aerodigestive tract (Felson, 1983). CAT is of value in assessing tumour size and regional extension into surrounding structures (Felson, 1983; Gamsu and Webb, 1983; Mancuso and Hanafee, 1985), but is less accurate in predicting malignant lymphadenopathy.

The pattern of malignant tumours of the middle and lower oesophagus have been identified with MRI and both spatial resolution and image quality were superior to CAT (Quint et al, 1985b). MRI constantly understaged these tumours since the technique suffers from similar limitations
to CAT, and there is great difficulty in detecting tumour invasion through the muscle layer into the peri-oesophageal fat. The same should certainly be true for tumours of the cervical oesophagus. Tumours of the cervical trachea have not been evaluated.

Ultrasound imaging is of no value in the evaluation of squamous cell carcinoma of the cervical oesophagus and trachea.

The uptake of Ga$^{67}$ at sites of squamous carcinoma of the cervical trachea has not been reported although it is taken up by squamous carcinoma of the lung (Smith et al, 1975). Although it would be expected that lesions of the cervical trachea would show positive uptake with Ga$^{67}$, primary tumours at this site are best evaluated by endoscopy and CAT and, as such, radionuclide imaging has no role in either diagnosis or staging.

Ga$^{67}$ uptake has been reported in squamous cell carcinoma of the oesophagus and has been claimed to be of value in pretreatment staging, since it can predict extra-oesophageal spread and lymph node metastases to include the supraclavicular fossa (Pearlman, 1981; Kondo et al, 1982a). It
may be of particular value in the assessment of strictures since, although oesophagoscopy and contrast radiography may suggest a benign lesion, intense focal accumulation of Ga$^{67}$ indicates malignancy (Pearlman, 1981).

The Paranasal Sinuses

Malignant tumours of the paranasal sinuses constitute less than 1% of all malignancies. 80% of sinus tumours involve the maxillary antrum and the most common type is the squamous cell carcinoma. The initial examination of all patients with suspected paranasal sinus disease should be plain radiography. Soft tissue masses and bone destruction may be assessed in the sinuses as well as in the nasal cavity, nasopharynx and skull base. Any further investigation should be performed using CAT rather than conventional tomography (Tubman, 1986).

High resolution CAT provides superior bony resolution when compared to conventional tomography and has the added advantage of excellent soft tissue delineation. It can distinguish between primary and secondary chronic sinusitis, accurately identify extension into adjacent
structures, delineate intracranial extension and is a proven adjunct to clinical staging, pre-operative planning and the measurement of tumour volume prior to irradiation (Parsons et al, 1979; Kondo et al, 1982b; Kondo et al, 1986; Tubman, 1986). The use of intravenous contrast is usually mandatory (Mancuso and Hanafee, 1985).

MRI is more accurate in distinguishing tumour from inflammatory tissue (Som et al, 1988), and in the extension of intracranial extension (Mancuso and Hanafee, 1985). However, its lack of signal from bone limits its use in the staging of paranasal sinus disease although improvements in the future allowing greater tissue specificity may offer a major breakthrough in the early diagnosis of paranasal sinus disease.

Ultrasound has been used to evaluate paranasal sinus tumours. Although it is claimed the technique can differentiate between tumour, infection or an abscess, and detect bony dehiscence in sinus walls (Edell and Isaacson, 1978; Miskin et al, 1978), the investigation requires close co-operation between clinician and the radiologist.
The uptake of Ga\(^{67}\) and Tc\(^{99m}\) (v) DMSA has been reported at the sites of paranasal sinus tumours, the majority of which were squamous cell carcinomas of the maxillary sinus (Smith et al, 1975; Higashi et al, 1977a; Teates et al, 1980; Ohta et al, 1985a; Ohta et al, 1988). Since the paranasal sinuses are included in the normal biodistribution of Ga\(^{67}\), and positive bony uptake due to malignant disease cannot be reliably distinguished from inflammatory changes, Ga\(^{67}\) has a limited role to play in the diagnosis and staging of paranasal sinus tumours. Tc\(^{99m}\) (v) DMSA requires further evaluation. However, Ga\(^{67}\) scanning may be of value in the differentiation of maxillary sinus carcinoma from chronic maxillary sinusitis (Higashi et al, 1977b).

**The Temporal Bone**

The radiological investigation of the temporal bone can be broken down into three main categories. These are inner ear studies for sensorineural hearing loss, middle ear and mastoid studies for conductive hearing loss and generalised studies for tumours, trauma, congenital lesions and a variety of systemic disorders. The most common carcinoma of the middle ear is a squamous cell carcinoma. All patients should have a biopsy followed by a CAT scan which has now replaced conventional tomography as the investigation of choice for tumours of this region.
CAT plays a dominant role in diagnosis and staging since it can accurately identify bony destruction and bony canal enlargements. It can assess the status of the carotid canal and the lateral extremity of the internal auditory meatus. Direct invasion may occur through the dura, and coronal scanning of reformatted images following intravenous contrast may demonstrate reactive changes in the adjacent brain (Mancuso and Hanafee, 1985). CAT is superior to MRI due to its ability to identify bony destruction, although MRI may play a role in future imaging because of its ability to demonstrate reactive changes in adjacent bone, and the ability to perform coronal scans when middle cranial fossa invasion has occurred. Neither ultrasound or radionuclide imaging has any current role to play in the diagnosis and staging of squamous cell carcinoma of the temporal bone.

The Salivary Glands

The favoured modality for imaging salivary gland masses has evolved from plain films with intraductal contrast to radionuclide scanning to CAT scanning and MRI over the last ten years. Aspiration cytology has significantly altered the management of salivary gland lesions and all other diagnostic modalities should be reviewed in the light
of this examination. Although the demonstration of calculus or ductal disease is best evaluated with plain film or plain film sialography, all suspected neoplastic disease should be evaluated with a fine resolution CAT scan with contrast or an MRI scan. The information required from the investigation is whether a tumour is intrinsic or extrinsic, benign or malignant and what is its relationship to surrounding structures (i.e. the parotid and the facial nerve).

Parotid tumours are usually seen as areas of increased density compared to the surrounding less dense parotid parenchyma. Tumour location, infiltration, extraglandular extension, approximate facial nerve anatomy and any lymphadenopathy are all well shown (Tubman, 1986). Squamous cell carcinomas are poorly defined lesions with an infiltrative pattern which often extends beyond the gland. In high density parotid glands, tumours may be iso-dense and therefore not visualised with infused CAT. If the gland is noted to be high density, CAT sialography is indicated and this will not only identify any tumour but is extremely sensitive in differentiating intrinsic from extrinsic tumours, and appraising the location of the facial nerve in respect to intrinsic parotid lesions (Stone et al, 1981.).
The position of the submandibular gland tends to make clinical examination highly accurate. Extension of masses into the lateral low density fat plane is well shown by CAT (Tubman, 1986). Because of the relative frequency of malignancy in the gland (50%) when compared with the parotid (20%), many submandibular masses tend to be evaluated by FNAB and subsequent surgical removal without proceeding to radiological investigation (Tubman, 1986). Masses in the parapharyngeal space may also be evaluated as to site of origin due to the presence of an intact fat plane between the mass and the deep lobe of the parotid (Tubman, 1986).

CAT and MRI have the same diagnostic potential in neoplastic mass lesions of the parotid gland (Casselman and Mancuso, 1987). However, MRI can be considered the investigation of choice with superior resolution and soft tissue contrast, intravenous contrast is not necessary and the technique can accurately delineate the extent of any facial nerve involvement (Schaefer et al, 1985b; Teresi et al, 1987c). Where inflammatory or neoplastic aetiology is unclear, or if bone detail or calcification are potentially important, then CAT is the initial investigation of choice (Casselman and Mancuso, 1987).
Ultrasound has been used to evaluate salivary gland swellings (Ballerini et al., 1984), and the parotid is the gland most frequently investigated (Gooding, 1980; Ballerini et al., 1984). The normal ultrasonic anatomy has been defined (Gooding, 1980) and the different ultrasonic patterns for a variety of malignant lesions described (Ballerini et al., 1984). Ultrasound can detect a mass lesion and define whether it is intrinsic or extrinsic. Although malignancy should always be suspected when there is a clearly echogenic mass with irregular echo distribution, posterior attenuation and rough margins (Ballerini et al., 1984), the technique is poorly specific and since it cannot reliably distinguish benign from malignant disease, it is of limited value in the diagnosis and staging of salivary gland neoplasms (Gooding, 1980).

Radionuclide scanning has been used to evaluate salivary gland swellings. Scanning with Tc$^{99m}$-TcO$_4$ provides physiological images of the salivary glands assessing function and drainage, since pertechnetate is secreted by the ductal epithelium into saliva. Relative blood flow as well as excretory function can be assessed (Watkinson, 1990). A mass lesion may be either vascular or non-vascular, and functioning or non-functioning. Although malignant neoplasms are generally vascular but
non-functioning on the scan, so are benign mixed tumours and abscesses and, as such, the test is of limited value in distinguishing benign from malignant disease. The uptake of Ga$^{67}$ has been reported in parotid squamous cell carcinoma (Higashi et al, 1977a). However, Ga$^{67}$ localises faintly in normal salivary tissue and although focal increased accumulation occurs in malignancy, it is also seen in infection, benign conditions such as sarcoidosis and following radiotherapy (Bekerman and Hoffer, 1976). Therefore Ga$^{67}$ (and Tc$^{99m}$-TcO$_4^-$) scanning play no role in the diagnosis and staging of parotid squamous cell carcinoma.
1.3.4. The Occult Primary

Failure to detect the primary tumour site in patients presenting with metastatic squamous carcinoma in cervical lymph nodes represents 2-3% of the total number of patients with head and neck cancer (Nordstrum et al, 1979). A number of diagnostic algorithms exist to evaluate the occult primary (Muraki et al, 1984; Mancuso and Hanafee, 1985, p 187). All patients should have a full history and examination to include FNAB and the situation of the cervical lymphadenopathy may help in predicting the primary tumour site (Section 1.2.5.). If the FNAB confirms squamous cell carcinoma (or if the pattern of malignancy is indeterminate), the patient should have a CAT scan (prior to panendoscopy) to search for the primary site and stage the neck. This is important since some occult primary tumours are submucosal, but have characteristic CAT appearances (Mancuso and Hanafee, 1983). CAT should precede panendoscopy to avoid false-positive results due to the oedema which may follow a biopsy.

If the CAT does not demonstrate an obvious primary site, the patient should have a panendoscopy and biopsy of all suspicious mucosal lesions, biopsy of silent areas (nasopharynx, retromolar trigone, base of tongue, pyriform
sinus and post-cricoid region) and a tonsillectomy on the side of the neck node. If this regimen fails to identify a lesion then the neck should be treated as appropriate with radiotherapy to include presumed occult sites (Bataini et al, 1987). Such a rationale avoids excisional neck biopsy which was thought to decrease significantly patient survival by increasing the local complication and recurrence rate and the incidence of distant metastases (Comess et al, 1959; McGuirt and McCabe, 1978; Barakat et al, 1987). However, current opinion now shows that previous neck node biopsy does not adversely influence the incidence of local neck recurrence or affect survival as long as adequate treatment is subsequently given (Razack et al, 1977; Robbins et al, 1986).

Ga $^{67}$ has been used to search for occult primary malignancy (Smith et al, 1975; Lentle et al, 1976). Smith et al evaluated one patient with cervical lymphadenopathy and failed to identify the primary site. Lentle et al examined 50 consecutive patients with occult primary malignant disease and correctly identified the primary site in 17 patients (34%). Of these 17, 11 (65%) primary sites were within the lung (9 bronchogenic carcinomas (histology not specified), three lymphoma) and one patient had a nasopharyngeal primary. Ga $^{67}$ has not been adopted into the routine evaluation of patients
with an occult primary head and neck squamous carcinoma. However, it may be of value in patients who have supraclavicular nodes with a negative chest radiograph, CAT scan and endoscopy, and who have persistent chest symptoms. This is because it localises in approximately 85% of patients with bronchial and lung carcinomas (Greyson and Noyek, 1983).
1.3.5. The Second Primary

In patients with head and neck squamous carcinoma, silent second synchronous and subsequent second metachronous primary lesions are not uncommon. The incidence of synchronous disease ranges from 11-17% (Vrabec, 1979; McGuirt, 1982; McGuirt et al., 1982; Shons and McQuarrie, 1985; McQuarrie, 1986; Shaha et al., 1988) and the incidence of metachronous disease from 7-17% (Vikram et al., 1984; McQuarrie, 1986; Shaha et al., 1988). Even third and fourth primaries have been reported (McGuirt et al., 1982; McQuarrie, 1986). Many of these lesions are asymptomatic and are not discovered on clinical examination without a routine chest radiograph and full panendoscopy. These investigations have been shown to be cost-beneficial in evaluating all patients with head and neck squamous carcinoma to exclude synchronous lesions (McGuirt, 1982; McGuirt et al., 1982; Black et al., 1983; Leipzig, 1983; Shons and McQuarrie, 1985; McQuarrie, 1986; Shaha et al., 1988). Although oesophagoscopy and laryngoscopy are the most productive endoscopic examinations (McGuirt, 1982; McGuirt et al., 1982), all patients should have a full panendoscopy (McGuirt, 1982).
Radionuclide scanning using Ga$^{67}$ has detected a synchronous second primary tumour (Smith et al, 1975). However, this is an isolated report in a series primarily designed to evaluate primary and cervical metastatic disease and, as such, the technique has not been assessed in the detection of synchronous primaries. It therefore plays no role in current staging.

The number of metachronously developing lesions increases with the length of follow up with some tumours developing 20 years on (Black et al, 1983; Vikram et al, 1984). All patients should be regularly followed up in a head and neck clinic and have a full head and neck examination. Any new symptoms should be regarded with suspicion and the patient carefully evaluated with a chest radiograph and full panendoscopy to exclude a new primary lesion (Black et al, 1983).
1.3.6. **Distant Metastases**

Distant metastases have been said to be present in 10-12% of patients with head and neck squamous carcinomas (Merino et al, 1977), and the risk increases for each stage grouping to approximately 20% for Stage IV (Vikram et al, 1984; Marechal et al, 1986). These figures may be higher since not all patients dying without clinically evident disease have post-mortem examinations (Narula et al, 1988). Recent reports based on post-mortem examinations give an overall incidence of 46% (Kotwall et al, 1987) and in this series, 80% of patients had lung metastases, 31% had bony metastases and 31% had liver metastases. All patients with advanced head and neck squamous carcinoma (Stage IV) should be initially staged with a chest radiograph, serum calcium and panendoscopy (Marechal et al, 1986; Swann and Blakeslee, 1988).

Despite the high incidence of bony and liver metastases, in the absence of any clinical or biochemical evidence of metastatic disease, bone and liver/spleen scintigraphy and abdominal ultrasound play no role in the staging of advanced head and neck squamous carcinoma (Wolfe et al, 1979; Marechal et al, 1986).
1.3.7. **Residual and Recurrent Disease**

Of those squamous carcinomas that recur within the head and neck, 90% do so within two years, excluding those from the nasopharynx which may recur at up to 10 years (Harnsberger et al, 1983). Recurrence after two years may well be a second primary, rather than failure to control the primary site. The physical examination of patients who have been treated by radiation or surgery is fraught with difficulty. Scarring and induration limit even the experienced examiner's evaluation and, in some instances, recurrence at the primary site may produce small areas of local ulceration or induration but provide little other evidence of their true extent on inspection or endoscopic evaluation. This may be because some recurrent tumours spread primarily along deeper pathways (Mancuso and Hanafee, 1985, p 371).

The key to detecting residual and recurrent disease is vigilant follow-up coupled with panendoscopy, chest radiography and CAT to evaluate suspicious symptoms. Most recurrences will be diagnosed by clinical examination and biopsy alone. CAT can detect residual and recurrent disease but problems may be encountered due to obliterated fat planes and post-treatment fibrosis and scarring.
(Glazer et al, 1986b). Although it was initially thought MRI would be able to differentiate the signal of fibrotic tissue from that of tumour (Mancuso and Hanafee, 1985, p 373), subsequent studies have shown this not to be the case (Glazer et al, 1986b). However, MRI may be advantageous in those patients where CAT is indeterminate either because of anatomical distortion or suboptimal demonstration of vascular anatomy (Glazer et al, 1986b). In one series, CAT provided critical information to the clinician, either by diagnosing recurrence when no mass could be seen or felt (27%), or by demonstrating deep tissue extension of the recurrent tumour beyond that suspected from physical examination (Harnsberger et al, 1983). Some workers (Harnsberger et al, 1983; Mancuso and Hanafee, 1985) have attempted to design "ideal" recommendations for CAT follow-up of patients with treated head and neck squamous carcinoma. With this in mind, the variable of who, where, and when to scan are crucial. They suggest that major dictating factors are large primary tumours \((T_3/T_4)\), close or positive surgical margins, primary sites which include the nasopharynx, oropharynx and hypopharynx, deep seated tumours (Michael Johns, Personal Communication, 1988), and new or persistent symptoms regardless of physical examination or endoscopic findings. Harnsberger et al (1983) recommends that follow up CAT scanning should include a six week baseline scan which allows for resolution of
haemorrhage and oedema. Further follow-scans may be necessary at six monthly intervals for two years, or as needed should new symptoms arise. Nasopharyngeal tumours may need to be followed up for longer. If a follow-up scan shows a mass not seen on the baseline scan, recurrent tumour can be more accurately diagnosed. There is no doubt CAT can detect tumour beyond the limits of physical examination, and that this information may alter radiotherapy, modify surgery and be the only means of detecting recurrence so that the delay in diagnosing recurrent disease is potentially reduced (Harnsberger et al, 1983). The question arises whether such a protocol is cost-effective? Most surgeons in the United Kingdom will monitor their patients by regular clinical follow-up and only investigate those who are symptomatic with a chest radiograph, panendoscopy and then CAT as appropriate.

It has been suggested that ultrasound may be of value in the detection of residual and recurrent disease (Chodosh et al, 1980). Westhofen (1987) used ultrasound scanning to follow up patients with head and neck squamous carcinoma and claimed the technique could detect early tumour recurrence and differentiate between oedema,
inflammation, scar tissue and tumour. Obviously further trials are necessary but these early results suggest ultrasound may be a useful adjunct to clinical examination in the detection of residual and recurrent disease.

The concept of using scintigraphy to detect microscopic residual and recurrent disease is an attractive one. Both Ga$^{67}$ and monoclonal antibodies (In$^{III}$-EGFRI) have been used (Kashima et al, 1974; Soo et al, 1987), and Ga$^{67}$ has been further assessed in order to evaluate the susceptibility of tumours to irradiation and chemotherapy before treatment, and to monitor the effects of therapy (Higashi et al, 1977a; Teates et al, 1980; Poublon, 1982). Tc$^{99m}$ (v) DMSA has been reported to be of value in the detection of recurrent disease (Ohta et al, 1985a). However, uptake was only reported in one patient and this radiopharmaceutical requires further evaluation. Despite these encouraging reports, the inevitable sequel of inflammation which follows head and neck surgery and/or irradiation results in an unacceptable high incidence of false-positive results and, as such, scintigraphy plays no role at present in the routine evaluation of residual and recurrent disease, nor in the assessment of any therapy effects. It will not be discussed further.
Patients with a primary site recurrence within the oral cavity and oropharynx will almost always complain of pain. Referred pain to the ear occurs with recurrent tongue lesions and trismus is a late sign indicating infratemporal fossa extension. Physical examination may be difficult and is hampered by scarring and induration. The CAT changes in the oropharynx following radiotherapy have been described (Mancuso and Hanafee, 1985, p 189). CAT is of proven value in the detection of recurrent cancer of the oral cavity and oropharynx (Som et al, 1982; Harnsberger et al, 1983; Mancuso and Hanafee, 1983). When recurrent tumour has been detected by CAT, patients have always been symptomatic and there are no prospective studies that demonstrate that CAT can detect asymptomatic recurrence. Some patients may have symptoms without CAT evidence of recurrence. Follow up CAT studies may reveal tumour recurrence suggesting the original symptoms were, in part, due to perineural spread (Mancuso and Hanafee, 1985, p 371). Some patients with pain have persistent negative CAT studies, and the cause of the pain remains unknown. Possible reasons include microscopic disease with microscopic spread, soft tissue necrosis, low grade osteonecrosis and pterygoid muscle scarring with temporomandibular joint dysfunction (Mancuso and Hanafee, 1985, p 373).
When radiotherapy to squamous cell carcinoma of the nasopharynx is successful, the tissue planes usually return to normal although there may be some asymmetric thickening due to post-treatment scarring. The amount of residual tumour-free fibrosis is unpredictable. Symptoms such as pain or cranial nerve dysfunction often precede changes in the gross nasopharyngeal anatomy probably due to perineural spread (Mancuso and Hanafee, 1985, p 440). Both CAT and MRI have the potential to detect asymptomatic recurrence (Mancuso and Hanafee, 1985, p 440), but this has not been proven in a controlled, prospective study.

The response to radiotherapy within the hypopharynx and larynx has been described (Mancuso and Hanafee, 1985). The most visually dramatic CAT changes occur when more than 7000 rads are delivered. Not only are the responses dose dependent, but they vary from patient to patient and are affected by the patient's general health, previous chemotherapy or radiotherapy and smoking and drinking habits. Focal areas of increased density on a CAT scan within the deep tissue planes of an irradiated larynx may be sterilised areas of reactive fibrosis in an active tumour bed, focal inflammation (perichondritis or chondronecrosis), or tumour or nests of tumour within a predominantly fibrotic mass (Mancuso and Hanafee, 1985, p 266). This raises
the most difficult clinical issue in the irradiated larynx. Which one of the preceding symptoms explains a mass, persistent oedema or symptoms in a treated patient? CAT can certainly show focal masses but it cannot be tissue specific. MRI may be of value in the future if it could only distinguish the signals from tumour, oedema and fibrosis. The CAT findings of chondronecrosis of the larynx (to include the epiglottis and arytenoids) have been described (Mancuso and Hanafee, 1985, p 267). However, CAT is non-specific and it can never reliably exclude persistent tumour and its main value in this situation is to show the larynx is beyond salvage and should then be removed even though tumour cannot be confirmed at biopsy.

Using CAT, the larynx has been evaluated following supraglottic, vertical and total laryngectomy (Di Santis et al, 1984a-b, Mancuso and Hanafee, 1985; Niemeyer et al, 1987). Following total laryngectomy, it is relatively easy to evaluate the neck with CAT, MRI and contrast barium studies. The larynx is absent and the continuation of the hypopharynx with the oesophagus is all that remains in the visceral compartment of the neck above the tracheal stoma. CAT or MRI can detect residual and recurrent tumour involving the neo-pharynx or tracheal stoma (Harnsberger et al, 1983) and barium examination may
help distinguish benign structures from tumour recurrence (Balfe et al, 1982). Following partial laryngectomy, there will be various portions of the laryngeal skeleton missing depending on the extent and type of surgery. After supraglottic laryngectomy the remaining larynx retains some of its symmetry and can be easy to evaluate. The CAT findings following supraglottic laryngectomy have been described (Niemeyer et al, 1987). The CAT findings of tumour recurrence are soft tissue masses, obliteration of fat planes and bone destruction and CAT was 100% sensitive in detecting non-mucosal recurrence. Mucosal recurrence is best detected at endoscopy but if subsequent biopsies are negative, barium enhanced radiography may be particularly useful (Niemeyer et al, 1987).

Following vertical laryngectomy, interpretation is more difficult since symmetry is lost. The neck has been evaluated following vertical laryngectomy (Di Santis et al, 1984a; Mancuso and Hanafee, 1985, p 267). The soft tissues which are used to create the pseudocord may appear thickened and the detection of small recurrences is beyond the capacity of CAT. However, it may be useful in detecting and defining recurrent tumour, especially in the symptomatic patient whose endoscopic examination and blind biopsies are negative (Harnsberger et al, 1983).
Residual and recurrent mucosal tumour recurrence within the trachea can be diagnosed on clinical examination, with or without fibre-optic endoscopy. CAT may be helpful in evaluating the extent of the disease (Harnsberger et al, 1983). Residual and recurrent disease following treatment to the cervical oesophagus may be difficult to evaluate, particularly if there has been visceral trans- or interposition. Disease will be confirmed by contrast barium studies and endoscopy, and CAT may be of value in detecting extramucosal recurrent disease (Heiken et al, 1984).

The early diagnosis of residual and recurrent paranasal sinus squamous carcinoma may be difficult on clinical examination alone. The CAT appearances following partial and total maxillectomy, lateral rhinotomy and craniofacial resection have been described and patterns of tumour recurrence identified (Som et al, 1982; Som et al, 1986). The lining of the post-maxillectomy cavity should appear smooth on a CAT scan. Any focal nodularity probably represents tumour recurrence. Diffuse thickening of overlying cheek skin indicates either inflammation or tumour recurrence. Using CAT it is possible to detect clinically occult recurrent maxillary sinus disease,
especially after partial maxillectomy where the surgeon
cannot visualise all of the surgical defect
(Som et al, 1982). The post-operative CAT appearances
following craniofacial resection depends on several
variables. These include the craniotomy and muscular
support flaps, degree of intracranial resection, size of
the orbital and ethmoid maxillary defect and presence of
marginal granulomas (Som et al, 1986). The radiologist
should be aware of the changes each of these can cause. In
addition some features which are a possible cause of
false-positive results warrant special attention. The
irregular margins of the craniotomy flaps may mimic the
appearance of osteomyelitis, and dural enhancement at the
operation site may mimic tumour recurrence. Post­
operative frontal lobe changes may simulate a local
recurrence, as can fascial and muscular support flaps.
The margins of the ethmoid-maxillary cavity should be smooth
and any nodularity suggests recurrence. The one exception
to this rule occurs along the anterosuperior surgical skin
margins where granuloma formation can cause focal thickening
of the soft tissues. The distinction between granuloma
formation and tumour recurrence cannot be made by CAT alone
and biopsy is essential (Som et al, 1986). CAT is of proven
value in the detection of clinically occult recurrent
ethmoidal carcinoma following craniofacial resection
(Som et al, 1986).
The detection of residual and recurrent disease following surgery to the salivary glands and the temporal bone should begin with clinical examination supplemented by biopsy as appropriate. CAT may be of value in assessing the extent of tumour extension but, as for the other sites, is limited by the fact that normal anatomical landmarks are lost and it cannot reliably distinguish between infection, fibrosis and recurrence. The major salivary glands will show excessive contrast enhancement (Bronstein et al, 1987) for up to six months post-irradiation. Following this, involution with fatty replacement is not uncommon and correlates with the post-irradiation "sicca-syndrome" many patients experience (Mancuso and Hanafee, 1985, p 189).

This section has outlined the techniques available to image primary, occult and second primary, and residual and recurrent head and neck squamous carcinoma and briefly reviewed their limitations. Further improvements in CAT and MRI (to include new surface coils) can only lead to superior resolution and greater sensitivity. However, these two techniques are both anatomically finite and non-specific, and have limitations in detecting microscopic disease and residual and recurrent tumour. Image superimposition may answer some of these problems. The search is on to find
newer specific radiopharmaceuticals (either low molecular weight non-specific agents or specific "associated" monoclonal antibodies) to image not only tumour anatomy but also tumour physiology in an attempt to identify and treat occult primary and recurrent disease undetected by either clinical examination, CAT or MRI.
1.4. THE EVALUATION OF THE NECK

1.4.1. The Problem

1.4.2. Palpation

1.4.3. Imaging

1.4.4. Pathological Examination

1.4.5. Residual and Recurrent Disease

1.4.6. The No Neck
1.4.1. THE PROBLEM

All patients with a head and neck squamous carcinoma (primary, occult primary, second primary), together with those who have local and/or distant metastases and residual and recurrent disease should have a clinical examination to include both sides of the neck. The presence or absence, level and size of metastatic cervical lymphadenopathy is one of the most significant prognostic factors in head and neck squamous carcinoma (Section 1.1.5.), and its detection and evaluation are crucial to subsequent management. This is of particular importance in those patients with a clinically N₀ neck, since physical examination does not exclude sub-clinical or microscopic disease in either side of the neck. This section reviews the methods currently available to assess the neck and pays particular attention to the problem of evaluating the N₀ neck. The treatment of the N₀ neck is briefly reviewed in Section 1.5.
1.4.2. PALPATION

Clinically positive lymph nodes rarely produce symptoms until they are quite large. Therefore, the surgeon must depend largely on physical examination to detect involved nodes. Techniques for routine neck examination can be found in standard textbooks (Million et al, 1982; Million and Cassisi, 1984; Browne, 1986) and only special points will be added here.

It takes between two to five minutes to do a careful neck examination (Million et al, 1982; Million and Cassisi, 1984). Each side of the neck should be assessed. It is important to repeat the examination at every opportunity and to compare findings among several examiners. Detailed drawings complement the written report and are essential. Each clinician develops his or her own technique which he or she performs in the same systematic manner each time the examination is conducted. Like the good rugby fly-half who constantly practices his touch kicking, every head and neck surgeon should dedicate some time to perfecting and maintaining a good neck examination technique.
The triangles of the neck, and the lymph nodes they contain, are examined in turn. There is no pre-defined order. Attention should be paid to the suprasternal notch and the Space of Burns as clinically positive cricothyroid and pre-tracheal nodes may be discovered. The jugular chain of lymph nodes should be examined carefully, the thumb and index finger forming a "c" around the sternocleidomastoid muscle. The smallest node which can be palpated in the jugular chain is considered to be 1 cm (Sako et al, 1964). The sub-digastric node is the largest normal node in the neck and can be palpated in many normal people. Most clinically positive nodes occur in the upper jugular chain but the most superior jugular nodes are difficult to palpate, particularly in men, and positive lymph nodes in the lower jugular area may be difficult to feel since they are often small, deep and mobile (Feind, 1972, p 127; Million et al, 1982, p 305).

The submandibular gland and nodes, and the submental nodes should all be examined. These are all easier to feel, being superficial structures, and nodes down to 0.5 cm can usually be palpated (Sako et al, 1964). Preauricular nodes should not be forgotten.
There are a number of normal structures which can be confused with a lymph node. The lateral tips of the transverse processes of both C₁ and C₂ can simulate lymph nodes as can the parotid tail, the superior horn of the thyroid cartilage and the carotid bulb (Million et al., 1982). Irradiated and obstructed submandibular glands may also simulate lymph node enlargement (Evans and Ackerman, 1954; Million et al., 1982, p 306).

The reliability of the neck examination depends on the experience and ability of the examiner, the gross anatomy of the individual neck and whether, or not, there has been previous treatment, surgery or radiotherapy. A fat, thick or a muscular neck can make evaluation difficult as can a recent incisional biopsy or tracheostomy (Million and Cassisi, 1984, p 58).

There is a well recognised error in tumour palpation in general, with considerable interobserver variation when estimating tumour size (McNair and Dudley, 1960; Koran, 1975; Moertel and Hanley, 1976; Euhus et al., 1986). Hans Christian Anderson described a real princess as someone who could feel a pea through twenty matresses! (Ehrlich, 1986). These pitfalls of tumour measurement are particularly common in head and neck cancer. There is considerable error in palpating the neck with significant variation between
experienced observers (Lyall and Schetlin, 1952; Southwick et al, 1960; McGavran et al, 1961; Beahrs and Barber, 1962; Sako et al, 1964; Cady and Catlin, 1969; Spiro and Strong, 1973; Spiro et al, 1974a; Mendelson et al, 1976; Cachin et al, 1979; Martis et al, 1979; Friedman et al, 1984; Ali et al, 1985; Stevens et al, 1985; Feinmesser et al, 1987). The incidence of false-negative results ranged from 4-60% (mean 29%) with most workers reporting an incidence of 15-40%. The figures of 4% (McGavran et al, 1961) and 60% (Lyall and Schetlin, 1952) are isolated findings and have not been reported elsewhere. These figures do not affect the mean value. A recent retrospective review of elective neck dissection specimens showed an overall incidence of approximately 26% false-negative results (Byers et al, 1988). There is no relationship between T-stage and the incidence of false-negative neck nodes (Ali et al, 1985). There is however, a greater incidence of false-negative nodes in lesions of the oral cavity, oropharynx and hypopharynx (Sako et al, 1964; Ali et al, 1985; Byers et al, 1988) and in those tumours which are poorly differentiated or undifferentiated (Ali et al, 1985) although the reason for this is unclear. Although nodes are supposedly easier to palpate in the submandibular triangle Cady and Catlin (1969, The Memorial...
Hospital, New York) reported a false-negative rate of 56% for Level I disease compared with 31% for Level II, 40% for Level III and 30% for Level IV disease. The incidence of false-positive nodes ranges from 4-45% (mean 19%). There is no relationship between the incidence of false-positive nodes and the T-stage of the primary tumour (Ali et al, 1985).

The significance of the incidence of false-negative nodes is obvious. Failure to detect disease in the neck directly affects prognosis. The incidence of false-positive nodes is important for two reasons. Firstly, a false-positive node may result in unnecessarily aggressive treatment and secondly, a patient with a unilateral primary but bilateral palpable nodes may be condemned to inadequate treatment on the basis he or she has incurable disease.
1.4.3. IMAGING

In an attempt to increase diagnostic sensitivity and specificity, diagnostic radiology has been used to evaluate the cervical lymph nodes. CAT has been used to evaluate cervical lymph nodes since 1981 (Mancuso et al, 1981). Before that time, the radiologist could offer little help to the head and neck surgeon in the assessment of cervical metastatic disease. Lateral soft tissue views of the neck for the evaluation of retropharyngeal extension were inaccurate (Feinmesser et al, 1987). Cervical lymphangiography suffered from a low sensitivity and specificity due to a high frequency of technical failure and interpretative difficulties resulting from an inability to distinguish benign from malignant disease, and the fact normal lymphatic flow is interrupted by previous surgery and irradiation (Fisch and Sigel, 1964; Fisch, 1968; Feinmesser et al, 1987; Som, 1987). Intra-operative lymphography also met with similar limited success (McKelvie, 1976).

The accurate assessment of the cervical lymph nodes represents one of the most challenging problems that a radiologist faces today. This apparent difficulty has been attributed to the fact that imagers are unfamiliar
with head and neck anatomy and conflicting terminology is used to describe the topographical distribution of the cervical lymphatic system (Som, 1987). Once a lymph node is identified, the radiologist must decide on not only the most accurate nomenclature to describe its position but also determine the criteria as to whether the node is pathologically involved and what is the sensitivity of such criteria.

The normal and normal variant CAT anatomy of the cervical lymphatics has been described (Reede et al, 1982a; Mancuso et al, 1983a; Swartz et al, 1984; Mancuso and Hanafee, 1985; Jinkins, 1987; Som, 1987). Most classifications are based on the work of Rouvière and Taillens (Rouvière, 1932; Taillens, 1962) but no mention is made of junctional nodes (Fisch, 1968). In adults, all head and neck nodes are partially or totally embedded in fat which provides an outlining matrix to facilitate CAT and MRI to depict them relatively easily. The use of intravenous contrast increases sensitivity (Shapeero et al, 1983; Silver et al, 1983; Jinkins, 1987). Normal nodes are typically homogenous and have attenuation values of about 10-20 Hounsfield units (HU) below those of opacified blood vessels, and are often poorly visualised (Som, 1987). Their tissue density
is roughly equivalent to that of surrounding muscle. They tend to be elliptical or oblong and for larger (> 10 mm) nodes, a slightly non-homogenous pattern of increased attenuation may occasionally be present in the parenchyma. A peripheral rim of enhancement is not normally present (Mancuso et al, 1983a). The internal architecture of normal nodes is, for the most part, beyond the limits of CAT evaluation. Occasionally, fatty replacement may create eccentric areas of translucency within normal sized nodes which can be confused with central necrosis (Mancuso et al, 1983a). The size range of nodes, and the incidence of demonstrating them at the various anatomical neck sites on CAT scans has been described (Mancuso et al, 1983a). All lymph node groups can be reliably identified except the median retropharyngeal, occipital, postauricular, facial, juxtavisceral and anterior jugular nodes. Difficulty is also encountered in differentiating posterior submental nodes from submandibular nodes, and differentiating posterior submandibular nodes from jugulodigastric nodes (Mancuso et al, 1983a).

The size and location of normal lymph nodes seen on CAT scanning correlates well with the data available in the anatomical and pathological literature.
(Rouvière, 1932; Feind, 1972). Nodes in the upper neck are usually larger than those lower down. Nodes in the internal jugular chain and the submental and submandibular regions usually range from 3-10 mm. The lateral retropharyngeal nodes range from 3-7 mm while the parotid and the spinal accessory nodes from 3-5 mm (Mancuso et al, 1983a). Most necks do not normally contain nodes larger than 1.0-1.5 cm (Mancuso and Hanafee, 1985, p 184; Som, 1987).

The CAT criteria for diagnosing squamous cell carcinoma within cervical lymph nodes have been described and patterns of intranodal and extranodal spread and fixation have been identified (Mancuso et al, 1981; Reede et al, 1982b; Mancuso et al, 1983b; Friedman et al, 1984; Mancuso and Hanafee, 1985; Stevens et al, 1985; Tubman, 1986; Feinmesser et al, 1987; Jinkins, 1987; Som, 1987). The results of several clinical series with pathological correlation have suggested that a clinically positive node should be defined as being greater than 1 cm in diameter, non-enhancing, spherical rather than flat or ovoid, and harder than an uninvolved node (Sako et al, 1964; Lindberg, 1972; Friedman, 1984). Others have indicated that, especially for the submandibular and internal jugular chains, normal
nodes are usually less than 1.5 cm (McGavran et al, 1961; Mancuso et al, 1981; Mancuso et al, 1983b; Mancuso and Hanafree, 1985, p 184; Stevens et al, 1985; Tubman, 1986; Jinkins, 1987; Som, 1987) and therefore any non-enhancing node greater than 1.5 cm should be considered abnormal. Since most cervical lymph nodes are ovoid in shape and nodal size is based on the greatest nodal diameter, contiguous images may have to be examined to calculate the size of a node whose largest dimension is parallel to the craniocaudal axis (Som, 1987).

Because of the variations in cervical node topographical terminology, some radiologists have used the clinico-anatomical classification (Shah et al, 1981) which divides clinically palpable cervical nodes into five groups or levels (Table 11). It does not classify the retropharyngeal nodes. Using this system, the radiologist can specifically identify a particular node seen on a CAT (or MRI) scan (Som, 1987). Level I nodes can also be referred to as medial (submental) or lateral (submandibular). If retropharyngeal nodes are identified on a CAT (or MRI) scan, they should be referred to being located at a specific anatomical reference level, i.e. C₁ or C₂ (Som, 1987).
**TABLE 11**

THE SIMPLIFIED NODAL CLASSIFICATION WHICH DIVIDES CLINICALLY PALPABLE CERVICAL LYMPH NODES INTO FIVE GROUPS OR LEVELS

<table>
<thead>
<tr>
<th>LEVEL</th>
<th>LOCATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Submandibular and submental nodes</td>
</tr>
<tr>
<td>II</td>
<td>Internal jugular chain nodes from the skull base to the level of the carotid bifurcation</td>
</tr>
<tr>
<td>III</td>
<td>Internal jugular chain nodes from the carotid bifurcation to the level where the omohyoid muscle (cricoid cartilage) crosses the internal jugular vein</td>
</tr>
<tr>
<td>IV</td>
<td>Infra-omohyoid nodes of the internal jugular vein (below the cricoid cartilage)</td>
</tr>
<tr>
<td>V</td>
<td>Posterior triangle nodes</td>
</tr>
</tbody>
</table>

Shah et al, 1981; Som, 1987
Choosing a size criterion to distinguish between normal and abnormal nodes introduces an inevitable source of error (Sako et al, 1964; Mancuso et al, 1981; Reede et al, 1982a-b; Mancuso et al, 1983a; Mancuso and Hanafee, 1985, p 184). False positives occur due to benign reactive nodes larger than 1.5 cm and false negatives occur due to microscopic or small macroscopic deposits in normal sized nodes. At present these are unavoidable.

On the basis of the above studies, the most reasonable size criteria to use when evaluating cervical lymph nodes in the submandibular (Level I) and jugulodigastric regions of the internal jugular chain (low Level II, high Level III) are that any node in these regions larger than 1.5 cm must be considered abnormal (Som, 1987). For the remaining nodes in the neck, a diameter of more than 1 cm should be considered abnormal (Sako et al, 1964; Friedman et al, 1984; Feinmesser et al, 1987; Som, 1987). Using these criteria approximately 80% of enlarged nodes are truly metastatic and 20% are enlarged due to benign hyperplasia, i.e. 20% false-negative and false-positive rate (Som et al, 1987). Som states (1987) that these figures are similar to palpation (Sako et al, 1964; Stevens et al, 1985). However, a larger literature review (this section) reveals
a similar false-positive rate (19%) but a high false-negative rate (29%). The larger the size by which a node is considered abnormal, the fewer the false-positive results. However, as the number of false-positives decreases, so the number of false-negatives will increase. In some large cancer centres with a biased population, the smaller 1 cm diameter criterion is used for all nodes to raise diagnostic sensitivity (Som, 1987). This is usually only done with the proviso that before a node is called metastatic, it must be spherical rather than ovoid (Som, 1987).

CAT can be used to detect nodal necrosis, even in non-enlarged nodes. This further increases the sensitivity of nodal evaluation, for now both necrosis and size can be used as criteria. Any node with central necrosis, regardless of size, must be regarded pathological (Som, 1987). At CAT, this necrosis usually appears as a central area of low attenuation (10-18 HU) with a surrounding irregular nodular wall (Mancuso et al, 1981; Mancuso et al, 1983b; Som, 1987). False positives can occur due to fatty nodal replacement following irradiation or inflammation (Mancuso et al, 1983b) although this is usually peripheral or non-central (Som, 1987).
There are some further CAT findings which can aid the radiologist in distinguishing benign from malignant disease. The presence of a thick, irregular zone of enhancement around a necrotic central area indicates an inflammatory process (abscess or abscessed node). A thin, enhancing rim with one or more focal areas of nodularity indicates a malignant lymph node with capsular invasion (Som, 1987). Intranodal tumour is a well defined mass with a distinct interface between it and surrounding fat. Extranodal tumour has an ill defined margin without clear distinction between it and surrounding fat and there is often evidence of oedema or thickening of surrounding fat and muscle (Mancuso and Hanafee, 1985, p 184), although similar findings can occur with prior surgery, irradiation or recent infection (Som, 1987). The presence of an enhancing node indicates inflammation and a mixture of enhancing and non-enhancing nodes indicates a granulomatous process (Som, 1987). Fixation is indicated by a combination of extranodal characteristics and a loss of plane definition between the nodal mass and the structure in question (Mancuso and Hanafee, 1985, p 184). Three or more contiguous well defined nodes measuring 8-15 mm in diameter are a possible source of false-positive results. Contiguous ill defined nodes usually indicate malignancy (Tubman, 1986).
Although false-positive and false-negative results are inevitable using the above criteria, the CAT findings taken in conjunction with the history and physical examination will allow an accurate diagnosis to be made in the majority of patients. However, there will be some where both the clinician and radiologist cannot distinguish between benign and malignant disease. In these, the pathologist has the final word.

CAT has been claimed to be of value also in the staging of neck nodes (Mancuso et al, 1983b; Friedman et al, 1984; Mancuso and Hanafee, 1985; Stevens et al, 1985; Som, 1987). Some workers state it is more accurate than clinical evaluation (Mancuso et al, 1983b; Friedman et al, 1984; Stevens et al, 1985), that clinically normal necks with abnormal CAT findings should be staged N positive (Friedman et al, 1984) and that its inclusion should be mandatory in any tumour staging system (Som, 1987). It has been stated that in the untreated neck, CAT can be expected to increase the stage of the disease from $N_0$ to $N_1$ in approximately 5% of patients (Mancuso et al, 1983b). However, in subsequent series, the clinically $N_0$ neck was correctly upstaged in 28% of patients (Friedman et al, 1984) while in another (Stevens et al, 1985) upstaging
occurred in 23% of patients ($N_0$ to $N_1$, 3%; $N_0$ to $N_2$, 10%;
(31% of clinically $N_0$ necks); $N_1$ to $N_2$, 10%).

The arguments for the use of CAT when assessing the neck are that it increases the detection rate of occult metastases in the $N_0$ neck, it can predict extranodal and contralateral disease and can show multiple nodes in patients with $N_1$ necks. In Friedman's series (Friedman et al, 1984), 39% of patients necks (50 patients) were falsely negative on clinical examination. CAT reduced this figure to 11%. The size criterion for malignancy was greater than 1.0 cm. A larger study on 79 patients (100 neck dissections, Feinmesser et al, 1987) showed similar sensitivity rates for both clinical examination (62%) and CAT (60%) in predicting cervical metastases and slight superiority in the positive predictive value of clinical examination (91%) compared with CAT (82%). The size criterion for malignancy was greater than 1.5 cm. Twenty of the 100 necks were clinically $N_0$ but had pathological evidence of disease. In 16 of these 20 necks, CAT scanning was negative for malignant disease; in four CAT was positive. Upstaging occurred in 20% of clinically $N_0$ necks. Furthermore, in these patients with occult disease, the mean size of nodal involvement was 14.1 mm and, overall, morphological changes of malignancy
were reported in only 3% of cases, all of whom had palpable disease. Although this series showed CAT offered little advantage over clinical examination, the results must be interpreted in the light that the clinical examinations were performed by expert clinicians and a third generation CAT scanner was used. Fourth generation scanners are now available which could increase the sensitivity in favour of CAT. Most workers agree (Stevens and Harnsberger, 1988, p 1041) that better results are possible with CAT than those demonstrated by Feinmesser et al (1987).

An important issue is why Friedman et al (1984) reduced the incidence of undetected occult disease from 39% on clinical examination to 11% with CAT but Feinmesser et al (1987) found CAT of little value although 20% of clinically N₀ necks were correctly upstaged. Both used third generation scanners. Technical factors may be important. Friedman (1988, p 808) has stressed the importance of contrast bolus injection techniques, the use of 5 mm sections as opposed to 5-10 mm sections (Feinmesser et al, 1987) and a size criterion for malignancy of 1.0 cm compared to 1.5 cm (Feinmesser et al, 1987). In addition, all scans in Friedman's series (Friedman et al, 1984) were interpreted by one experienced
radiologist. In Feinmesser's (Feinmesser et al, 1987) they were not. Friedman (1988, p 808) states that in his experience, most occult positive nodes in the clinically N\textsubscript{0} neck are greater than 1.5 cm in size and should therefore be detected on CAT when the 1.5 cm malignancy size criterion is used. Friedman has since stated (Friedman, 1988, p 808) that although CAT may offer few advantages over clinical examination in a district general hospital or in teaching hospitals where all radiologists interpret scans, in specialised centres with one radiologist with an interest it has a distinct role to play in diagnosis and management. Although Friedman's data (Friedman et al, 1984) suggests CAT is superior to clinical evaluation, the data demands further attention. Of the 32 patients judged clinically to have cancer in lymph nodes, 30 (94%) had CAT scans which indicated this and positive pathological confirmation. One patient with a clinically abnormal node had a normal scan and negative pathology. Another with a clinically abnormal node had an abnormal scan but negative pathology. Would the authors not operate on a patient with palpable cervical lymph nodes and a normal CAT scan? Of the eighteen patients who were judged clinically to have no cancer within the cervical lymph nodes, seven had abnormal CAT scans and two had negative pathology. Two others had normal CAT scans but positive pathology. Surgery based on CAT indications
alone would have resulted in two unnecessary operations and two cases of metastatic disease being missed.

Computed tomographic scans are not a substitute for physical examination but a valuable source of additional information. It has been estimated that applying CAT scanning to all 50,000 new cases of head and neck cancer in the USA would add 20 million dollars to annual health care expenditure (Cantrell, 1984). CAT scans can only be justified if they drastically alter the treatment plan, that is from radical treatment to none at all, or from radiotherapy to surgery (Stell, 1987). It appears reasonable that CAT scanning of the neck should only be performed in those patients at high risk of occult ipsilateral and/or contralateral disease (when often the scan will be required anyway to evaluate the primary) and also to assess invasion of the carotid, jugular or deep cervical musculature. It would also seem unwise not to perform a neck dissection in the presence of unilateral significant palpable cervical nodes, even with a normal CAT scan.

MRI has been used to evaluate cervical lymphadenopathy. The normal and normal variant in-vivo MRI (and surface coil) anatomy of cervical lymph nodes has been described.
(Stark et al., 1984a; Mancuso and Hanafee, 1985) and patterns of malignant involvement identified (Dooms et al., 1984; Stark et al., 1984a-b; Glazer et al., 1986a-b). Normal cervical lymph nodes are rarely seen with in-vivo MRI. They have long $T_1$ and $T_2$ relaxation times and increased intensity relative to muscle at long pulse repetition intervals and echo delay (Stark et al., 1984a). Measurements of relaxation times for lymph nodes less than 10 mm by CAT criteria are not currently possible. In-vitro spectroscopy of normal lymph nodes shows long $T_1$ and $T_2$ relaxation times similar to in-vivo studies (Stark et al., 1984a; Dooms et al., 1985).

In-vivo MRI has been compared to CAT, but not to palpation, in the detection of malignant cervical lymphadenopathy. Similar size criteria to CAT have been applied and any node with central necrosis is considered abnormal. The imaging of any structure in the human body is based on two main factors: spatial resolution and contrast resolution. For imaging cervical lymph nodes, CAT has better spatial resolution but MRI has superior contrast resolution (Dooms et al., 1984). CAT is superior to MRI for imaging neck nodes less than 13 mm, and for nodes greater than 15 mm the two techniques are comparable in sensitivity, although MRI demonstrates these nodes better
because of superior contrast resolution (Dooms et al, 1985). MRI can clearly differentiate abnormal lymph nodes from normal fat, muscle, vessels, primary tumour and the thyroid (Dooms et al, 1985). Nodal tumour signal is more intense than skeletal muscle but less than fat, and to demonstrate contrast between these requires both $T_1$- and $T_2$-weighted spin echo images (Mancuso and Hanafee, 1985, p 185). Necrotic foci in nodes usually show low signal on $T_1$-weighted and high signal on $T_2$-weighted spin echo images. However, both in-vivo and in-vitro normal, inflammatory and tumour nodal signals overlap so much that it is impossible to distinguish benign from malignant disease with confidence (Dooms et al, 1984; Stark et al, 1984b; Dooms et al, 1985). At the present time, MRI suffers from the same limitations as CAT to a greater or lesser degree. Its only advantages are that it does not require intravenous contrast, has superior contrast resolution and is able to differentiate enlarged nodes and muscles from tortuous vessels in the neck and thoracic inlet (Stark et al, 1984b). There is a great need to improve sensitivity by identifying morphological criteria on both CAT and MRI which will distinguish tumour from non-tumour in normal sized nodes.
Ultrasound was first used to evaluate cervical lymphadenopathy in squamous cell carcinoma in 1975 (Wiley et al). Since then, there have been a number of reports comparing it with palpation and assessing its value in the detection of cervical lymph nodes (Bruneton et al, 1984; Hajek et al, 1986; Yonetsu and Ikemura, 1987; Baatenburg de Jong et al, 1988; Rothstein et al, 1988). Ultrasound can delineate all the major lymph node chains in the neck as well as structures such as the carotid artery and internal jugular vein. Normal lymph nodes have been described as having a maximum diameter of 5 mm (Hajek et al, 1986). Nodes greater than 5 mm are considered malignant by Hajek et al (1986) whereas Bruneton et al (1984) consider any node (except the jugulodigastric) greater than 8 mm malignant. Others make no comment (Baatenburg de Jong et al, 1988). Precise assessment of the size of a lymph node requires delineation in both longitudinal and transverse directions. This can readily be performed with ultrasound. Malignant nodes may be heterogenous with a mixed cystic/solid pattern (Bruneton et al, 1984).

Ultrasound is said to be more sensitive than clinical examination in detecting cervical lymph nodes (Bruneton et al, 1984; Hajek et al, 1986;
Baatenburg et al, 1988) and has been used to modify clinical staging (Bruneton et al, 1984). It can detect the size, position and volume of a lymph node and may be of value in those patients who have difficult necks to examine (Bruneton et al, 1984). It may also be of value in detecting thrombosis of the internal jugular vein and is more accurate than CAT in assessing lymph node adherence and invasion of the carotid artery (Hajek et al, 1986; Yonetsu and Ikemura, 1987; Rothstein et al, 1988). Its major limitation is it cannot distinguish benign from malignant disease (Hajek et al, 1986).

Provided the ultrasound examination is conducted in real time using a high frequency transducer, it is of proven value in the evaluation of cervical lymphadenopathy. It can detect subclinical nodes and evaluate size, number and volume. The question of nodal size is important. Although Hajek states that nodes are larger than 5mm on ultrasound in both inflammatory and malignant conditions, all malignant nodes examined histologically were 8 mm or greater. It seems reasonable to use the 8 mm as a malignant size criterion (anything less should be regarded as normal) and this agrees with the work of others (Bruneton et al, 1984) and is nearer current CAT criteria.
Using such criterion results in a low incidence of false-positive results. Bruneton et al (1984) evaluated 100 patients with cervical lymphadenopathy using both clinical examination and ultrasound. The sensitivity for clinical examination was 78% compared with 93% for ultrasound. Upstaging occurred in at least 12% of patients. There were only three false-positive results.

There is no reason why ultrasound should not be used as an adjunct to palpation and CAT, when evaluating patients with head and neck cancer. It may be particularly useful in assessing patients with difficult necks to examine, those with a "high-risk" N\text{0} neck, and in the evaluation of large neck masses when either carotid artery invasion or internal jugular vein thrombosis are distinct possibilities. Since it cannot distinguish benign from malignant disease, all nodes should be further evaluated by ultrasound guided fine needle aspiration biopsy which, in expert hands, is 85% accurate (Baatenburg de Jong et al, 1988).

Radionuclide imaging has been used to evaluate cervical lymphadenopathy due to metastatic squamous cell carcinoma. Planar imaging using Co$^{57}$-Bleomycin and Ga$^{67}$-Citrate has been assessed (Kashima et al, 1974; Kornblut et al, 1974;
Silberstein et al, 1974; Smith et al, 1975;
Higashi et al, 1977a; Sawas-Dimopoulou et al, 1978;
Woolfenden et al, 1979; Teates et al, 1980;
Cummings et al, 1981). Neither agent is taken up by normal
cervical lymphoid tissue so normal anatomy has not been
defined. Both agents suffer from a low sensitivity
and specificity, considerable cost and prolonged blood
clearance and are unable to demonstrate and differentiate
between cervical lymph nodes less than 2 cm by which time
they are clinically palpable (Cummings et al, 1981).

Lymphoscintigraphy has been used to assess cervical
lymph nodes using Tc $^{99m}$ sulphur colloid. Attempts have
been made to study normal anatomy (Thommesen et al, 1981)
but these have failed since it is probably not possible
to differentiate between small groups of lymph nodes and
solitary nodes on the gamma camera image, and there is a
pronounced variation between each side of the neck.
This may be due to either anatomical or physiological factors.
Although initial studies suggested the technique may be
able to predict lymph node metastases
(Parell et al, 1981), further work (Blakeslee et al, 1985)
has shown it is possible only to identify nodal groups
and not individual nodes and there is an unacceptable high
incidence of false-positive and false-negative results.
Therefore, the technique has no role in the assessment of cervical lymphadenopathy from squamous cell carcinoma.

There is a need for newer non-specific and specific agents which will localise in metastatic cervical lymphadenopathy. Tc $^{99m}$ (v) DMSA uptake has been reported in malignant cervical nodes but, using planar imaging, it is not possible to detect nodes less than 2 cm in size (Ohta et al, 1985a; Ohta et al, 1988). The use of SPECT may increase diagnostic sensitivity and specificity and further studies are necessary to evaluate this radiopharmaceutical.
1.4.4. PATHOLOGICAL EXAMINATION

There is a well recognised error in the pathological examination of neck dissection specimens and some workers have reported an incidence of 30% false-negative results when sectioning lymph nodes (Wilkinson and Hause, 1974). All specimens should be orientated by the surgeon, and the pathologist must be aware of any previous treatment such as radiotherapy.

Lymph nodes should be recorded according to level, size, histological characteristics and the presence or absence of extracapsular spread and any tumour cell emboli in the accompanying lymphatics should be noted (McKelvie, 1976; Van der Waal and Delemarre, 1982; Rhys Evans et al, 1987). Clearing techniques are neither feasible nor necessary. Techniques for sectioning lymph nodes depending on size are available in the literature and false-negative results are related to the size of the lymph node, the size of the primary lesion, the number of nodal sections obtained and the location of tumour within the node (Wilkinson and Hause, 1974). All results should be drawn on a Rouvière diagram and placed in the patient's notes.
1.4.5. RESIDUAL AND RECURRENT DISEASE

Detecting residual or recurrent disease in the neck following surgery and/or irradiation is not easy. Physical examination is difficult due to post-radiation fibrosis, post-surgical scar tissue and the presence of bulky flaps, and it may be impossible to differentiate scar tissue from residual and recurrent tumour.

CAT has been shown to be more sensitive than physical examination in detecting residual and recurrent tumour in the post-treatment neck (Harnsberger et al, 1983) particularly since there is a greater incidence of involvement of retropharyngeal nodes in these patients. The changes which occur in the irradiated neck have been described and are dose dependent (Mancuso and Hanafee, 1985, p 188). The factors which affect the CAT (and MRI) appearance of the post-treatment neck include the size and site of the primary tumour, the extent of cervical metastatic disease, the total amount of radiation given, any post-operative or post-irradiation complications and the use of myocutaneous or free flaps (Mancuso and Hanafee, 1985). When both the primary and cervical nodes have been involved with tumour, the post-irradiation changes may vary. In general, primary lesions should disappear
following radiotherapy although fibrosis may occur when bulky lesions are treated. Fibrotic change is much more likely when nodes are treated, particularly if there was extranodal spread. Surgery alters the anatomy much more than radiotherapy but the remaining anatomy has a remarkable ability to return to near-normal appearances. Uncomplicated neck dissection (functional or radical) leaves very little residual scar tissue. Post-operative wound infection greatly increases the amount of scar tissue in the deep tissue planes. The CAT appearances of muscle flaps are unmistakable (Som and Biller, 1983). They are seen as large fatty dense masses with strands of residual atrophic muscle and some peripheral scar tissue. With CAT, there is no way to distinguish tumour, scar tissue (or both) within a residual mass from a single study (Mancuso and Hanafee, 1985, p 189). Baseline scans are essential. Follow-up scans may be necessary in those patients at risk of residual or recurrent neck disease, i.e. those with Stage III or IV disease, extranodal spread, close surgical margins and either persistent pain or post-irradiation oedema. The use of MRI to detect residual and recurrent disease in the neck suffers from the same limitations as CAT.
Ultrasound has been used to evaluate residual and recurrent disease in the neck. It can be an adjunct to physical examination in those patients whose tissues are thickened following radiotherapy (Bruneton et al, 1984). It is more sensitive than CAT in detecting recurrent 5-10 mm nodes (Hajek et al, 1986; Westhofen, 1987) and may be useful in measuring the size of lymph nodes following radiotherapy or chemotherapy (Baatenburg de Jong et al, 1988).

Radionuclide scintigraphy has been used to evaluate tumour response to therapy and to detect residual and recurrent disease following treatment. Higashi et al (1977a) suggested that high tumour uptake with Ga$^{67}$ was associated with a good subsequent response to irradiation and chemotherapy, that post-irradiation and chemotherapy scans usually showed less activity and a subsequent negative scan indicated tumour control. However, further studies have shown that there is an unacceptably high number of false-positive results following treatment with either radiotherapy, surgery or chemotherapy and it is not possible to either predict tumour response or monitor its progress (Kashima et al, 1974; Kornblut et al, 1974; Smith et al, 1975; Teates et al, 1980). Monoclonal antibodies using In$^{III}$-EGFRI have also been evaluated with similar results (Soo et al, 1987).
Radionuclide scintigraphy, and especially monoclonal antibodies, offer the ideal solution to detecting residual and recurrent disease in the neck. At present, the use of non-specific agents such as Ga$^{67}$ is hampered by uptake into inflammatory tissue which results in a low sensitivity and specificity and there are distinct practical problems associated with using monoclonal antibodies. There is a need for more specific agents to detect residual and recurrent disease following surgery and irradiation (Watkinson and Maisey, 1988).
1.4.6. THE N\textsubscript{O} NECK

This section has outlined the problems with clinical examination of the neck together with the limitations of the adjunctive investigations aimed to increase diagnostic sensitivity and specificity. One of the main problems facing the head and neck surgeon is the detection of subclinical disease in the neck (the N\textsubscript{O} neck) together with residual and recurrent disease following surgery and irradiation. Failure to detect the former increases the incidence of the latter.

At the moment there is no substitute for vigilant clinical examination, and in those necks where there is a high risk of subclinical disease or examination is equivocal, CAT is the investigation of choice. In those necks which go to surgery, methodical pathological examination is crucial. In the future, ultrasound may prove an important adjunct to clinical examination and CAT, and current research may provide new tumour-imaging radiopharmaceuticals.

Tumour biology has taught us that subclinical disease does not mean early cancer. It takes a tumour aggregate
of at least $10^8$ cells to be detected by palpation or imaging techniques. A tumour of four log ranks higher denotes incurable cancer, and two log ranks lower, microscopic disease (Goepfert, 1988). As Goepfert (1988) says one of the challenges of the next decade is to find the means to identify and measure subclinical cancer. The search is on. Proper evaluation of the neck can only lead to correct treatment in the majority of patients. The treatment of the $N_0$ neck is briefly reviewed in the next section (1.5.).
1.5. THE TREATMENT OF THE NO. NECK
1.5. THE TREATMENT OF THE $N_0$ NECK

The evaluation of the $N_0$ neck is one of the greatest problems in head and neck surgery and its treatment remains controversial. Rudyard Kipling (1902) wrote a poem about six honest serving men which gives an admirable summary of the way we acquire most of our knowledge (Asher, 1972, p 54).

'I keep six honest serving men,
They taught me all I knew,
Their names are what, and why, and when,
And how, and where, and who'.

In this section, these six honest serving men are used as headings to discuss the treatment of the $N_0$ neck.

WHAT

What is the problem? This can be defined as whether, or not, to treat the neck in a patient who has a primary head and neck squamous carcinoma, and who has no demonstrable significant lymphadenopathy on clinical examination (the $N_0$ neck).
WHY

The reason why elective treatment to the N₀ neck has been proposed is that, on retrospective evidence from elective radical neck dissection specimens, there is a high incidence of subclinical disease in the neck, particularly for squamous carcinoma of the nasopharynx, oral cavity and oropharynx (Section 1.4.2.).

WHEN AND HOW

There is much controversy regarding whether or not the N₀ neck should be treated, when it should be treated, and what is the best form of treatment. The high incidence of occult metastatic disease has fueled the arguments for, and against, elective treatment. Once a decision has been made to treat the neck, treatment may be with either surgery or radiotherapy.

It is only recently that the question of the benefits of an elective neck dissection has been resolved. The case for, and against, elective surgery to the neck rests on the following key points (Nahum et al, 1977; Skolnick et al, 1980).
The high incidence of occult metastatic disease.

Neck dissection has a low morbidity and mortality.

If the neck has to be entered to remove the primary lesion, it is better to perform an incontinuity resection at that time.

It is impossible to provide the clinical follow-up necessary to detect the earliest conversion of a neck from N₀ to N₁.

Allowing neck metastases to develop increases the incidence of distant metastases.

The cure rate for neck dissection is decreased if gland enlargement occurs or multiple nodes appear.
Against

1. Cure rates are no lower if the surgeons waits for the neck to convert from \( N_0 \) to \( N_1 \).

2. Careful clinical follow-up will allow detection of the earliest conversion from \( N_0 \) to \( N_1 \).

3. Radiation is as effective as neck dissection.

4. Elective neck dissection results in a large number of unnecessary surgical procedures and is associated with inevitable morbidity.

5. Elective neck dissection removes a barrier to the spread of disease and also has a detrimental immunological effect.

Until 1980, all the evidence for, and against, elective surgery was retrospective. The consensus of opinion was that there was no place for elective surgery (Stell and Green, 1976; Nahum et al, 1977; Skolnick et al, 1980). There has been one controlled prospective trial which confirmed no benefit from elective surgery to the clinically \( N_0 \) neck (Vandenbrouck et al, 1980). Surgery probably has no role to
play unless radiotherapy is not available, regular follow-up is not possible or, a neck dissection is performed during the surgical procedure to provide access for reconstruction.

Which operation should be performed? The retrospective and prospective evidence evaluating the value of surgery is based on the classical radical neck dissection. This operation was described by Crile (1906), and popularised by Hayes Martin (1941) who is regarded as the father of head and neck surgery as we know it today (Figure 13). Indeed Hayes Martin himself states (1941) that there is no theoretical or statistical evidence that a prophylactic radical neck dissection has any value in the treatment of the $N_0$ neck in patients with oral and pharyngeal squamous cell carcinoma.

Recent advances in surgical technique have popularised techniques of various types of modified (less than radical) neck dissection. There are many options available with regard to elective neck dissection (Bocca et al, 1984; Suen and Goepfert, 1987). Details of which elective neck dissection to perform depending on the site and T stage of the primary lesion are available in the literature (Byers et al, 1988).
Dr. Hayes Martin 1892-1977 (by courtesy of Mr. H. J. Shaw)
A functional neck dissection removes the lymph nodes and fat from the lateral neck, and spares the spinal accessory nerve, internal jugular vein, sternocleidomastoid muscle and the submandibular salivary gland. The morbidity of the operation is less than either a radical or modified radical neck dissection, it is oncologically sound, can be performed bilaterally, and is as effective as the former two operations in preventing neck recurrence (Petroff and Fee, 1986). Anything less than this operation is not a proper cancer operation since low level three lymph nodes (UICC, 1985) are not removed and these may be involved as first echelon stations, particularly in intra-oral squamous cell carcinoma (Chu and Strawitz, 1978; Byers et al, 1988). Less than functional (supraomohyoid, suprahyoid, anterior, etc) neck dissections can only be justified as part of access providing procedures when they can assist in staging (Byers et al, 1988), although negative pathology does not exclude positive nodes lower in the neck (Chu and Strawitz, 1978).

The discovery of subsequent nodal disease following elective neck dissection should be treated on its merits.
Around the time that controversy existed with regard to the elective surgical treatment of the $N_0$ neck, it became apparent that elective neck irradiation could eradicate more than 90% of subclinical disease in the neck (Fletcher, 1972). These results were based on retrospective data and have since been confirmed by other workers (Cummings, 1986). There have been no published controlled prospective trials evaluating the value of elective neck irradiation although the results of two series evaluating its role in the treatment of patients with squamous carcinoma of the oral cavity and tongue (Royal Marsden Hospital and the Manchester Hospital, U.K; Dearnaley et al, 1988; Michael Henk, 1988, Personal Communication) show that elective neck irradiation may prolong survival by reducing subsequent local metastatic disease.

On the basis of these retrospective and prospective studies, it seems reasonable to suggest that elective neck irradiation should be offered to those patients with a greater than 25% chance of subclinical neck disease (nasopharynx, oral cavity and pharynx; Lindberg, 1972), to those where vigilant follow-up is not possible, or where clinical evaluation of the neck proves difficult.
WHERE AND WHO

Optimum treatment for patients with head and neck squamous carcinoma can only be provided by those institutions dedicated to the management of the disease and who emphasise a multidisciplinary approach. The co-operation of the head and neck surgeon with the radiotherapist, maxillofacial and plastic surgeon, speech therapist and social worker is crucial (Shaw, 1986, p 351). The concept of the multidisciplinary approach is not new. Sir Stanford Cade said in 1929 "Patients are entitled to expect the selection and use of the most effective form of treatment for their special needs" (Gerald Westbury, Personal Communication, 1988).

The question is often asked - who should be doing head and neck surgery. Should it be the general, otolaryngological, maxillofacial or plastic surgeon? Comprehensive training and suitable qualifications are essential (MacComb, 1969; Polk and Griffen, 1986, p 475; Loré, 1987) and, at the end of the day, only surgeons with adequate training should perform head and neck surgery. As Professor Harrison says "Down with the dabblers" (Harrison, 1988, p 809).
1.6. THE HISTORY OF TECHNETIUM-99m (v) DIMERCAPTOSUCCINIC ACID

1.6.1. Introduction

1.6.2. Human Studies

1.6.3. Animal Studies

1.6.4. Chemistry
1.6.1. INTRODUCTION

Technetium-99m (v) Dimercaptosuccinic Acid is a new tumour-imaging agent, the uptake of which has been reported in head and neck tumours and, in particular, medullary carcinoma of the thyroid and squamous cell carcinoma. This section outlines the history of its use and briefly reviews its \textit{in-vitro} and \textit{in-vivo} chemistry.
1.6.2. **HUMAN STUDIES**

Technetium-99m (Tc $^{99m}$) (v) Dimercaptosuccinic Acid (Pentavalent DMSA) is a new tumour-imaging agent first developed and evaluated in Japan (Yokoyama and Saji, 1980; Yokoyama et al, 1981; Hata et al, 1983). Dimercaptosuccinic acid is a low molecular weight organic acid (Figure 14) and Tc $^{99m}$ (v) DMSA is based on the same ligand as the well recognised kidney imaging agent Tc $^{99m}$ (III) DMSA, but its method of preparation at a lower tin to DMSA ratio, and at an alkaline pH, results in an altered biodistribution.

The uptake of Tc $^{99m}$ (v) DMSA in patients with medullary carcinoma of the thyroid was first described approximately six years ago by workers in Japan (Endo et al, 1983; Ohta et al, 1984b). They reported significant tracer accumulation, not only in the primary tumour and its metastases (Figure 15), but also in residual and recurrent disease following surgery (Figure 16). Since then other workers have confirmed significant uptake of Tc $^{99m}$ (v) DMSA in patients with medullary carcinoma of the thyroid (Clarke et al, 1987a-b; Clarke et al, 1988) such that it now has a distinct role to play in the management of patients with this disease. In 1984, Ohta et al evaluated 58
Figure 14

HOOC — CH — CH — COOH

\*SH \*SH

Molecular weight, 182

Dimercaptosuccinic Acid with two sulphydryl* groups
On the left is an anterior Tc$^{99m}$-TcO$_4^-$ thyroid image in a patient with a palpable solitary nodule in the right lobe of the thyroid gland. The "cold" areas of reduced pertechnetate uptake are arrowed. On the right is a right lateral planar Tc$^{99m}$ (v) DMSA head and neck image in the same patient. Note the accumulation of Tc$^{99m}$ (v) DMSA not only in the previous "cold" areas on the Tc$^{99m}$-TcO$_4^-$ image (A) but also in a metastatic cervical lymph node (B).
On the left is a right lateral planar Tc $^{99m}$ (v) DMSA image following total thyroidectomy and neck dissection (same patient as Figure 15). Note the accumulation of Tc $^{99m}$ (v) DMSA at the site of residual and recurrent disease in the thyroid bed (A) and two metastatic cervical lymph nodes (B). On the right is another patient with metastatic medullary carcinoma of the thyroid. The anterior planar thoraco-abdominal image shows positive accumulation of Tc $^{99m}$ (v) DMSA at the sites of known pulmonary, bone and liver metastases.
patients with a variety of soft tissue tumours using both Tc $^{99m}$ (v) DMSA and Ga $^{67}$-Citrate. The imaging accuracy was 78% for pentavalent DMSA and 71% for Ga $^{67}$ (Ohta et al, 1984a). Ohta concluded that although Tc $^{99m}$ (v) DMSA was taken up into some benign soft tissue tumours, there was reduced accumulation in inflammatory lesions compared with Ga $^{67}$ and, as such, pentavalent DMSA could be of use in both the localisation and evaluation of extension of soft tissue tumours.

Ohta et al (1985b) then compared the uptake of Tc $^{99m}$ (v) DMSA with Tc $^{99m}$ (III) DMSA in three patients with malignant tumours, one of whom had a recurrence of a pharyngeal carcinoma following radiotherapy. Uptake was confirmed in the recurrent mass with Tc $^{99m}$ (v) DMSA but not Tc $^{99m}$ (III) DMSA. Ohta then evaluated 78 patients with primary head and neck tumours using pentavalent DMSA and, of these, 32 also had Single Photon Emission Computerised Tomography (Ohta et al, 1985a). Patients with tumours of either the thyroid gland or central nervous system were excluded. The sensitivity for pentavalent DMSA was 75% and the detection rate was greatly influenced by the location and histology of the tumours. Visualisation was satisfactory in the region of the maxillary sinuses, mandible and pharynx and, histologically, Tc $^{99m}$ (v) DMSA appeared to be taken up by squamous cell carcinoma. An increased sensitivity was observed using SPECT.
Endo et al (1985) compared the uptake of Tc $^{99m}$ (v) DMSA and Ga $^{67}$-Citrate in another series of 36 malignant primary head and neck tumours, 27 of whom had squamous carcinoma. The sensitivity and specificity for Tc $^{99m}$ (v) DMSA was 78% and 87% while for Ga $^{67}$ it was 85% and 51% respectively. An increase in sensitivity for pentavalent DMSA was observed using SPECT.

Some head and neck tumours are treated primarily by radiotherapy and the uptake of Ga $^{67}$ has been reported in the salivary glands following radiotherapy (Bekerman and Hoffer, 1976). Endo reported no significant uptake of Tc $^{99m}$ (v) DMSA in the salivary glands, either before, or following radiotherapy.

Aw et al (1986) imaged 18 patients with proven nasopharyngeal squamous carcinoma using Tc $^{99m}$ (v) DMSA and reported a sensitivity of 28%. Aw concluded that the poor sensitivity may be related to histology since the false-negatives were either poorly differentiated or undifferentiated squamous carcinomas. However, Aw also reported uptake in the paranasal sinuses and included this in the normal biodistribution of Tc $^{99m}$ (v) DMSA. The nasal mucosa and paranasal sinuses are included in the normal biodistribution of many radiopharmaceuticals including Ga $^{67}$ and Tc $^{99m}$-MDP and this is related to the high
blood flow the area receives (Kashima et al, 1974). The poor sensitivity observed by Aw et al (1986) is much more likely to be due to the well recognised problem of differentiating normal blood pool biodistribution from positive uptake due to squamous carcinoma in the nasopharynx (Kornblut et al, 1974; Silberstein et al, 1974) rather than the histological characteristics of the tumour itself.

Ohta et al (1988) published results of a larger series of 112 patients (32 benign, 80 malignant) with head and neck tumours. There was no analysis given of the different histological subgroups but one assumes that most of the malignant tumours were squamous carcinomas. None of the tumours were staged for malignancy. The sensitivity and specificity for pentavalent DMSA was 80% compared with 89% sensitivity for Ga$^{67}$ (29% specificity). For Tc$^{99m}$ (v) DMSA, sensitivity was affected by size, location and histology, being greatest for squamous carcinomas measuring greater than 2 cm in size which were located near the mandible and maxillary sinus and not near the tongue or the nasopharynx. False-negatives commonly occurred in those patients with tumours measuring less than 2 cm of the tongue, floor of mouth, gingiva and larynx. There were eight false-positives and, of these, six were due to inflammation.
Ohta concluded that for the detection of head and neck tumours, the sensitivity of Ga $^{67}$ was superior to Tc $^{99m}$ (v) DMSA. However, the accumulation of Tc $^{99m}$ (v) DMSA in inflammatory lesions following radiotherapy was much less than that observed with Ga $^{67}$ and, as such, Tc $^{99m}$ (v) DMSA may have a role in the detection of recurrent disease following radiotherapy, particularly since it is thought not to localise in the salivary glands following treatment (Endo et al, 1985).

There is no published quantitative data on the optimal imaging time for Tc $^{99m}$ (v) DMSA in humans. Ohta in his original article (Ohta et al, 1984b) imaged patients with medullary carcinoma of the thyroid two hours post-injection but gave no reasons for choosing such an imaging time. He and others have since followed a similar protocol (Ohta et al, 1984a; Endo et al, 1985; Ohta et al, 1985a-c; Clarke et al, 1987a-b). Indeed Ohta states (Ohta et al, 1988) that although good quality pictures can be obtained as early as 30 minutes post-injection, images are best acquired at one to two hours post-injection although, again, this is based on qualitative, rather than quantitative data. Aw et al (1986) imaged two patients with nasopharyngeal squamous carcinoma at 1, 2, 3, 4 and 24 hours post-injection and stated all the images were essentially similar. Recent reports
(Guerra et al, 1989) suggest an imaging time of between three to six hours for patients with medullary carcinoma of the thyroid although no reasons are given for choosing such times.

Tc $^{99m}$Tc (v) DMSA is not 100% sensitive or specific for primary head and neck squamous carcinoma. Uptake has been reported not only in other head and neck tumours such as medullary carcinoma and rhabdomyosarcoma (Ohta et al, 1984a-b), but also in melanoma, lymphoma, adenocarcinoma and primary bone tumours (Endo et al, 1985). Positive uptake has also been reported in primary and metastatic carcinoma of the breast (Clarke et al, 1987b), lung metastases from osteogenic sarcomata (Ohta et al, 1985c), and in bony metastases from prostatic carcinoma (Jeghers et al, 1986). Accumulation has also been noted in benign head and neck tumours including parotid pleomorphic adenomas, neurofibromas and haemangiolyphomas, as well as benign lesions such as inflammatory neck masses, submucosal fibrosis and recent operation scars (Ohta et al, 1985a).

There is little published data on the pharmacokinetics of Tc $^{99m}$Tc (v) DMSA in humans. Clarke et al published results from 10 patients showing a bi-exponential blood clearance with mean half-times of 11 and 260 minutes, and with a mean cumulative urine excretion half-time of 264 minutes which was markedly different to
Tc $^{99m}$ (III) DMSA. However, this was a mixed group which contained both non-tumour patients and tumour patients with medullary carcinoma and squamous cell carcinoma (Clarke et al, 1987b; Watkinson, 1987). Watkinson emphasised that pharmacokinetic data may be different between non-tumour and tumour groups if significant tumour uptake of Tc $^{99m}$ (v) DMSA does occur.

In non-tumour patients, the normal pattern of biodistribution of Tc $^{99m}$ (v) DMSA at two hours has been defined with uptake of tracer visualised in the blood pool, bone marrow, nasal mucosa, lacrimal glands, breast tissue, testes, kidneys and bladder (Clarke et al, 1987b). Others have confirmed tracer visualisation in the breast, nasal mucosa and kidney and also noted localisation in the liver and skin (Aw et al, 1986).

Very little data has been published on the in-vivo tumour biodistribution of Tc $^{99m}$ (v) DMSA in humans. Endo et al (1985) studied two patients with medullary carcinoma who had been operated on. The mean Tc $^{99m}$ (v) DMSA tumour: normal thyroid ratio two hours post-injection was 36.2:1; the mean tumour: blood ratio was 15.3:1 and the tumour: muscle ratio was 25:1. Watkinson studied one patient operated on for medullary carcinoma
Approximately two hours post-injection, the tumour:normal thyroid ratio was 18.3:1; the metastatic nodal tumour:normal thyroid ratio was 21.7:1; the tumour:blood ratio was 6.6:1 and the tumour:muscle ratio was 10.5:1. It is difficult to explain the discrepancies between the two sets of data, particularly since so few patients were studied. However, they both confirm Tc $^{99m}$ (v) DMSA is actively accumulated by medullary carcinoma of the thyroid. The difference in the tumour:normal thyroid ratios may be explained by the varying amounts of normal thyroid which was present within the thyroid lobe involved with medullary carcinoma. This theory is supported by the fact that the metastatic nodal tumour:normal thyroid ratio was 21.7:1 when the node contained predominantly metastatic medullary carcinoma and very little normal lymphatic tissue. Watkinson also studied tumour biodistribution in two patients with head and neck squamous carcinoma (Watkinson, 1987). In one patient with hypopharyngeal carcinoma, the tumour:blood ratio was 4.1:1 four hours post-injection. In another with maxillary sinus carcinoma, the tumour:blood ratio was only 1.5:1 two hours post-injection, although this patient had had a total maxillectomy and the activity was counted from the whole specimen which included normal tissue and bone.
The dosimetry of Tc $^{99m}$ (v) DMSA has been calculated. Using human pharmacokinetic data together with the bladder and whole body as source organs, the effective dose equivalent has been estimated to be 8.2 uSv/MBq (Clarke et al, 1987b). Watkinson combined the same pharmacokinetic data with rabbit biodistribution data and using the kidney, bladder and whole body as source organs calculated the effective dose equivalent to be 14 uSv/MBq (Watkinson, 1987). Both these figures are acceptable patient radiation doses, and compare favourably with doses received from other technetium labelled compounds (Tc $^{99m}$ (III) DMSA, 12.5 uSv/MBq; Tc $^{99m}$ Diethylenetriamine pentaacetic acid (DTPA), 10 uSv/MBq; DHSS, 1988).

The mechanism by which medullary carcinoma of the thyroid and squamous carcinoma accumulates Tc $^{99m}$ (v) DMSA is poorly understood. It has been suggested that in medullary carcinoma it is due, in part, to the similarity of the TcO$_4^{3-}$ pentavalent core to the phosphate molecule which is avidly taken up by some tumour cells (Endo et al, 1985). This, however, cannot be the only mode of uptake since bony accumulation would be more prominent than is seen with Tc $^{99m}$ (v) DMSA, although high bone uptake has been demonstrated in both rodents and
and rabbits, species characterised by incomplete bone maturation (Yokoyama et al, 1985; Watkinson, 1987). At present there is no postulated mechanism to explain the accumulation of Tc $^{99m}$ (v) DMSA at sites of head and neck squamous carcinoma. However, it may be due, as for medullary tumours, to the similarity of the pentavalent core to the phosphate molecule. Although some publications have suggested the mechanism of tumour uptake of Ga $^{67}$ is, in part, transferrin dependent (Alan and Robert, 1977; Tsan and Scheffel, 1986) preliminary in-vitro and in-vivo studies suggest that the mechanism of uptake of Tc $^{99m}$ (v) DMSA into tumours is transferrin independent (Endo et al, 1985; Yokoyama et al, 1985).
1.6.3. ANIMAL STUDIES

In their original work, Yokoyama and Saji (1980) studied the in-vitro uptake by Ehrlich ascites carcinoma cells of Ga\(^{67}\)-Citrate and Tc\(^{99m}\)-TcO\(_4^-\) compared with Tc\(^{99m}\) complexes using ligands such as citric acid, dimercaptosuccinic acid and pyrophosphoric acid. These compounds were chosen since their chemical structure favoured the formation of polymeric complexes. The tumour uptake for Ga\(^{67}\) was 17.7% compared to 4.2% for Tc\(^{99m}\)-TcO\(_4^-\) and 14.4% for Tc\(^{99m}\) (III) DMSA (labelled at pH 6).

However, for Tc\(^{99m}\)-Citrate (pH 6), pyrophosphate (pH 5) and dimercaptosuccinate complexes (pH 9), uptake was 30.2%, 28.7% and 25.3% respectively. Subsequent in-vivo work carried out with Ehrlich-tumour-bearing mice reversed the above order. The results showed that Tc\(^{99m}\) DMSA (labelled at pH 8 with stannous chloride; Tc\(^{99m}\) (v) DMSA) was the better ligand with tumour:blood ratios of 1.02:1 at one hour; 2:1 at two hours and 6.5:1 at 24 hours compared with 0.86:1 at one hour; 1.31:1 at two hours and 4.36:1 at 24 hours for Tc\(^{99m}\) (III) DMSA and 0.38:1 at one hour; 0.63:1 at two hours and 2.47:1 at 24 hours for Ga\(^{67}\) (Yokoyama and Saji, 1980; Yokoyama et al, 1981; Hata et al, 1983;
Yokoyama et al. (1985) also showed that Tc-99m (v) DMSA had a bi-exponential blood clearance in Ehrlich-tumour-bearing mice with a major organ biodistribution that included bone, kidney, blood and tumour. Further work in rats (Westera et al., 1985; Ramamoorthy, 1987) has confirmed a fast bi-exponential blood clearance and major biodistribution in bone, kidneys and blood. Watkinson used a rabbit animal tumour model (Vx-2 squamous carcinoma) to evaluate the pharmacokinetics, biodistribution and dosimetry of Tc-99m (v) DMSA (Watkinson, 1987). He showed Tc-99m (v) DMSA had a fast bi-exponential blood clearance when compared to Tc-99m (III) DMSA and Tc-99m-TcO$_4^-$ (Figure 17). Watkinson postulated this fast blood clearance may be due to the DMSA sulphydryl groups of separate molecules binding to each other. This effectively shields them from the trace-element binding protein metallothionein in the proximal tubules of the kidney and consequently the complex is probably excreted via glomerular filtration in a similar manner to a Tc-99m-DTPA molecule.

Watkinson then compared blood clearance between non-tumour and tumour rabbits. The bi-exponential mean half-times were 42 and 155 minutes in non-tumour rabbits and 70 and 200 minutes in tumour rabbits. However, only two rabbits in each group were studied and, as mean data was
Figure 17

BLOOD CLEARANCE OF Tc$^{99m}$ (v) DMSA COMPARED WITH Tc$^{99m}$ (III) DMSA AND Tc$^{99m}$-TcO$_4^-$ IN NON-TUMOUR NZW RABBITS

Mean data from two rabbits


Time after injection (Hours)

Minutes
presented, Watkinson concluded further work was necessary to evaluate whether any difference between the two groups was real or apparent since variations may be due to metabolic disturbances in the tumour rabbits consequent upon dehydration and the parathormone-like substances which the tumour is known to secrete.

Watkinson also evaluated the biodistribution of Tc $^{99m}$ (v) DMSA in non-tumour and tumour rabbits. By performing serial dissections at 2, 4, 6 and 24 hours, he demonstrated a biodistribution which included bone, kidneys, bladder, the blood pool and squamous carcinoma. The tumour:blood ratios were 0.6:1 at two hours; 1:1 at four hours; 2:1 at six hours and 3.8:1 at 24 hours. These values are approximately half those obtained for Tc $^{99m}$ (v) DMSA and Ehrlich adenocarcinoma tumour cells (Yokoyama et al, 1985). Such discrepancies may be explained by histological differences between the two tumours and the relative avascularity of squamous cell carcinoma. In addition, only one rabbit was dissected for each data point and further work is necessary to confirm or refute this biodistribution data.

When any new radiopharmaceutical is evaluated, one should always question whether scintigraphic visualisation
is due to blood pool radioactivity. The rabbit squamous carcinoma data suggests no evidence of active tumour accumulation with tumour:blood ratios of 0.6:1 at two hours and 1:1 at four hours. Watkinson addressed this problem by performing simultaneous injections of Tc$^{99m}$ (v) DMSA and Iodine-125 (I$^{125}$) human serum albumin (HSA, the blood pool imaging agent) in tumour-bearing rabbits (Watkinson, 1987). He then calculated the Tc$^{99m}$ (v) DMSA:I$^{125}$ HSA ratios at two, four, six and 24 hours in the blood pool, muscle, whole tumour, outside living tumour and inside necrotic tumour (Figure 18). Watkinson demonstrated an early tumour blood pool effect at two hours with no evidence of active tumour uptake. However, there was selective retention of Tc$^{99m}$ (v) DMSA by the tumour and, when compared to the blood pool, the inside necrotic tumour appeared to wash out slower than the outside living tumour.

Yokoyama used the rabbit Vx-2 squamous carcinoma model to obtain good quality in-vivo images with Tc$^{99m}$ (v) DMSA at 30, 60 and 120 minutes post-injection (Yokoyama et al, 1985). Watkinson sequentially imaged one rabbit with four transplanted Vx-2 tumours to six hours post-injection with pentavalent DMSA (Watkinson, 1987).
Figure 18

$^{99m}\text{Tc}\,\text{V} \,\text{DMSA} : \,^{125}\text{I-HSA}$ ratios in TUMOUR-BEARING NZW RABBITS

Data from 10 Tumours

At two hours, three out of four tumours were visualised and at four and six hours, all tumours were clearly seen. Watkinson suggested the optimal imaging time in tumour rabbits was probably between four and six hours. Further work is necessary to substantiate the exact optimal imaging time for Tc $^{99m}$ (v) DMSA in rabbits with transplanted Vx-2 tumours.
1.6.4. CHEMISTRY

Technetium has the electronic configuration (Kr) 4d^6 5s^1. Its chemical characteristics are extremely complicated with seven oxidation states and hydrolysed polymeric states similar to those of manganese and rhenium (Parker et al, 1984). The outer two valency shells contain seven electrons and technetium compounds are known with valency states ranging from +7 to -1. In aqueous solution, the most stable oxidation state is the pertechnetate ion (TcO_4^- (+7)) and its saline solution can be obtained in carrier-free form from a Mo^{99} Tc^{99m} generator. Tc^{99m} TcO_4^- is, in itself, a useful radiopharmaceutical. In addition, it is the same size as the iodine molecule and as such is trapped but not organified by the thyroid gland. However, most modern day radiopharmaceuticals use Tc^{99m} complexes (eg Tc^{99m} (III) DMSA) and this requires the reduction of Tc^{99m} TcO_4^- from the +7 unreactive state to a lower more reactive oxidation state using a reducing agent such as stannous chloride (SnCl_2). Most radiopharmaceuticals are prepared using a radionuclide and the relevant kit but there is no kit currently available in this country to prepare pentavalent DMSA and its method of production is described elsewhere (Chapter 3). Dimercaptosuccinic acid
is a low molecular weight organic acid which forms the ligand for the well recognised static renal imaging agent, Tc $^{99m}$ (III) DMSA. Under alkaline conditions with a low stannous chloride:DMSA ratio, DMSA forms polymeric complexes with Tc $^{99m}$ to form a pentavalent core (Yokoyama et al, 1985). In-vitro analysis using both paper electrophoresis and thin layer chromatography (TLC) shows Tc $^{99m}$ (v) DMSA to be a distinct individual complex ($R_f$ 0.4-0.6) with different biological characteristics to Tc $^{99m}$-TcO$_4^-$ ($R_f$ 0.7-0.8) and Tc $^{99m}$ (III) DMSA ($R_f$, 0), and which is stable in-vitro to six hours (Westera et al, 1985; Yokoyama et al, 1985; Sampson, 1987; Watkinson, 1987). Further work is currently under way investigating simpler preparation methods for Tc $^{99m}$ (v) DMSA (Colin Lazarus, 1988, Personal Communication) since some workers have shown that, whereas an alkaline pH is essential for the production of pentavalent DMSA, the presence of SnCl$_2$ is not (Westera et al, 1985).

There have been very few in-vivo studies performed on Tc $^{99m}$ (v) DMSA. Watkinson studied its in-vivo stability by performing TLC studies on plasma and urine samples obtained from a tumour-bearing rabbit taken 15 minutes and then at two and four hours post-injection (Watkinson, 1987).
He showed that pentavalent DMSA is probably stable to four hours post-injection but that there may be some in-vivo conversion to Tc $^{99m}$ (III) DMSA which would explain the kidney scintigraphic uptake and uptake at necropsy of Tc $^{99m}$ (v) DMSA. Hesslewood et al (1988) studied the plasma protein binding of Tc $^{99m}$ (v) DMSA compared to Tc $^{99m}$ (III) DMSA two hours post-injection using equilibrium analysis. Approximately 94% of trivalent DMSA was protein bound whereas only 59.5% of pentavalent DMSA was specifically bound to protein. Qualitative studies suggested that both complexes bound predominantly to albumin. The lower level of protein binding of Tc $^{99m}$ (v) DMSA may partially explain its more rapid kidney clearance when compared to Tc $^{99m}$ (III) DMSA.

Further studies are necessary, both in animal and man, to evaluate not only the in-vitro and in-vivo stability and binding characteristics of Tc $^{99m}$ (v) DMSA, but also its pharmacokinetics, biodistribution and optimal imaging characteristics.
1.7. PHARMACOKINETICS AND BIODISTRIBUTION

1.7.1. Introduction

1.7.2. The Principles of Localisation

1.7.3. Protein Binding

1.7.4. Lipid Solubility

1.7.5. Determinants of Blood Clearance and Retention

1.7.6. Determinants of Renal Excretion

1.7.7. Metabolism and Subcellular Localisation

1.7.8. Principles of Tumour Localisation
1.7.1. INTRODUCTION

There are a number of basic factors involved in the choice of any radiopharmaceutical. These include its biological behaviour, radionuclide characteristics, general availability and pharmaceutical quality. With regard to biological behaviour of a radiopharmaceutical, to image any organ it is necessary to selectively localise the agent in that organ. The important determinants of localisation include molecular size and shape, degree and strength of protein binding in the blood and tissues, lipid solubility and specific cellular transport mechanisms. The route of administration of the radiopharmaceutical is also important together with the patient's general condition and current medication (Sampson, 1988). There are many factors which control the pharmacokinetics and biodistribution of any radiopharmaceutical, but the most important are the protein binding and lipid solubility of the agent, together with the determinants of blood clearance and renal excretion. This section outlines each of these factors in turn and, briefly, reviews the mechanisms of metabolism and subcellular localisation together with those of tumour localisation.
1.7.2. **THE PRINCIPLES OF LOCALISATION**

The factors which influence the biological localisation of radioactive drugs or compounds are more numerous and complex than those for simple radioactive elements. Most radiopharmaceuticals injected intravenously mix with the blood pool and then leave the vascular compartment to enter various organs and tissues. The localisation of radiopharmaceutical agents is greatly influenced by certain characteristics of the body organs themselves (McAfee and Subramanian, 1975). These include the fraction of the cardiac output the organ receives, the type of capillary endothelium within that organ, the capillary density, and the presence or absence of those receptor sites which have a specific biochemical affinity for the extraction and concentration of the agent in question. Organs with a high blood flow, such as the liver and kidney, tend to extract larger amounts of radioactivity than those structures with a low blood flow, such as tendons, fat and cartilage. Some of the mechanisms currently available to achieve the desired **in-vivo** distribution of radiopharmaceuticals are shown in Table 12.
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<td>Other</td>
<td>Tumours</td>
<td>Metal chelates</td>
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1.7.3. PROTEIN BINDING

Protein binding is important for several reasons. Drug excretion may be delayed by prevention of glomerular filtration and/or tubular secretion and both tissue distribution and subcellular localisation can be modified. In addition, binding may be altered in disease states due to changes in the nature or amount of protein, dehydration, alteration in the pH and the presence of other substances as in combination drug therapy (Sampson, 1988). There is often a marked species variation in the organ localisation, total body retention and excretion of both stable drugs and radiopharmaceuticals. Such differences can often be explained by variations in plasma protein binding, sometimes by a factor of 10, between two different species. The factors which control the binding of drugs and radiopharmaceuticals in plasma proteins have been described elsewhere (Klotz, 1957, p 91; Davidson, 1971). Serum albumin has a greater affinity for most drugs than other plasma proteins. This is contrary to theoretical expectations, since most drugs and radiopharmaceuticals are anionic and serum albumin has a net of 12 negative charges at pH 7.4, although it has about 100 negative and 100 positive charges at its iso-electric point at pH 5 (McAfee and Subramanian, 1975, p 34). This ability is
due to its low content of hydroxyl groups compared with the number of carboxyl and amino groups. In contrast, serum globulin has an abundance of hydroxyl amino acids, which interact internally with both amino and carboxyl groups, leaving a small number of amino groups to interact with other anions. Nevertheless, many compounds bind selectively to globulin and these include thyroxine, iron and other metallic ions such as gallium and indium.
1.7.4. LIPID SOLUBILITY

The key to understanding the distribution of stable or radioactive drugs is diffusion through capillary, cellular and other lipid membranes. Lipid-soluble compounds tend to distribute rapidly throughout the body tissues by rapidly diffusing through cell membranes. It has been well established that there is an excellent correlation between membrane diffusion in-vivo and in-vitro measurement of oil-water partition coefficients (Brodie, 1964). Many drugs that are lipid-soluble readily bind to plasma proteins and these two factors influence the relative distribution of the drug in question between the plasma and extravascular fluid in-vivo. Most radiopharmaceuticals have partition coefficients within a narrow range of 0.017 and 0.072 (McAfee and Subramanian, 1975, p 36). Cell membranes are approximately 100 Å thick and consist of phospholipid bilayers with protein carrier molecules, surface mucopolysaccharides and cations like calcium. Proteins responsible for the active transport of specific substances extend either partially or completely through the lipid layers. Most drugs are weak organic electrolytes. Ionised molecules do not penetrate the lipid barriers, whereas non-ionised molecules can. Therefore, membrane penetration depends on lipid solubility which is, in turn, dependent on the proportion of the non-ionised drug,
i.e. the dissociation constant or pK value. The pK value of most radiopharmaceuticals changes with the pH of different body fluids. Only that fraction of a radiopharmaceutical that is not ionised at pH 7.4 and which is not protein-bound penetrates the cell membrane (Brodie, 1964).

Various membrane transport mechanisms exist. These include simple diffusion, filtration through membrane pores, active transport mechanisms and pinocytosis. The amount of filtration which occurs through pores varies markedly from one membrane barrier to another, and is dependent on protein binding and molecular size (McAfee and Subramanian, 1975).
1.7.5. DETERMINANTS OF BLOOD CLEARANCE AND RETENTION

The factors which influence the rate of blood clearance are closely related to those which determine renal excretion, i.e. the degree and strength of red cell and plasma protein binding, the volume of distribution and renal clearance. There are two basic types of plasma disappearance (McAfee and Subramanian, 1975). Zero-order, or constant rate, disappearance tends to occur with larger concentrations of radiopharmaceuticals selectively secreted by renal tubular cells, when the maximum tubular capacity for excretion is exceeded. First-order, or exponential, rates of disappearance applies to drugs in low concentrations such as radiopharmaceuticals. In these cases, plasma disappearance is usually quantitated in a series of exponential components, the slower components being attributed to either protein binding or slower diffusion into a larger volume of distribution.
There are a number of important factors which influence the rate of renal excretion of a radiopharmaceutical. Active transport of the agent through the renal tubular cells is important together with its molecular size and resultant influence of glomerular filtration rate. Protein binding and its influence on glomerular filtration is important as is lipid solubility, its effect on tubular reabsorption together with the volume of distribution of the agent within the body fluids.

Passive filtration through the glomerular membrane depends upon the molecular size of drugs which do not bind to plasma proteins. Molecules with molecular weights of 5,000 or less have the same glomerular filtration rate as Inulin; those with molecular weights of approximately 17,000 have a filtration rate approximately half that of Inulin, and those above a molecular weight of 40,000 remain unfiltered.

Agents which are predominantly protein-bound in plasma are usually not filtered by the glomeruli but can be extracted by the renal tubular cells. These tubular cells function as a lipid barrier and lipid soluble drugs filtered into the tubular lumen by the glomerulus are
promptly reabsorbed, whereas ionised drugs reaching the
tubular lumen do not penetrate this barrier and are
consequently excreted in the urine (Brodie, 1964).
1.7.7. METABOLISM AND SUBCELLULAR LOCALISATION

There is very little written in the literature concerning the intracellular compartmentalisation of drugs. With regards to metabolism, the majority of drugs are metabolised in the liver and the enzymes responsible for these biotransformations can occur in the soluble mitochondrial or the microsomal fractions of the liver. The pathways of liver drug metabolism have been reviewed elsewhere (Lazarus, 1974). The major pathways of liver drug biotransformations can be divided into phases I and II. Phase I biotransformations can be further subdivided into oxidation, reduction and hydrolysis reactions. Generally phase I biotransformations provide a new functional group on the drug which then allows phase II, or conjugation, to proceed. Conjugation reactions involve the binding of endogenous molecules to form more water soluble compounds such as glucuronides, sulphates, mercapturates and methylated products. Conjugation with glucuronic acid is one of the more common routes of metabolism for many drugs. A number of different types of compounds undergo conjugation with glucuronic acid and these include those with hydroxyl, carboxyl, amino and sulfhydryl groups. Glucuronides are frequently excreted by the kidney via tubular secretion. However, some may also be partly or wholly excreted by glomerular filtration and some may also be eliminated in the bile.
Biological membranes are closed surfaces which circumscribe the cell and organise its contents into functional compartments, the organelles. Using subcellular fractionation techniques, it is possible to isolate these membranes and organelles into individual fractions of plasma membranes, nuclei, mitochondria, microsomes, soluble proteins, lysosomes, golgi apparatus and cytosol (Steck, 1972). Regarding the specific subcellular localisation of drugs, polymyxins and amphotericin bind to intracellular membranes (Goodman and Gilman, 1985). Steroids bind to intracellular receptors and the combination then moves into the nucleus (Mitchell, 1987). Tri-iodothyronine probably does the same. Chloramphenicol binds to the 50s ribosomal subunit as does erythromycin. Tetracyclines bind to 30s and 40s ribosomal subunits and aminoglycosides bind to a specific protein in the 30s ribosomal subunit (Goodman and Gilman, 1985). Actinomycin, bleomycin and mitomycin all bind to specific bases in the DNA helix and chloroquine interacts strongly with DNA (Parker and Irvin, 1952; Goodman and Gilman, 1985).
1.7.8. PRINCIPLES OF TUMOUR LOCALISATION

The specific accumulation of radioactive iodine (I) in metastatic thyroid cancer was reported over 40 years ago (Keston et al., 1942). Since then many tumour localising radiopharmaceuticals have been evaluated and those which are of use in current clinical practice can be divided into specific and non-specific agents. Tumour-specific agents localise only within one specific tumour, or follow one specific metabolic pathway and include I$^{131}$ for differentiated thyroid cancer (Maisey, 1981). Although, in theory, monoclonal antibodies should be regarded as tumour-specific, this is not strictly so. They are more tumour-associated than tumour-specific and, as such, exhibit non-specific uptake in organs such as the liver and spleen. Examples include Indium-III monoclonal antibodies for head and neck squamous cell carcinoma (Soo et al., 1987).

Tumour non-specific agents localise not only in a number of histologically different malignant tumours, but also in benign tumours and inflammatory lesions. Examples within the head and neck include Gallium-67 citrate for lymphoma (Turner et al., 1978) and Thallium-201 chloride for differentiated thyroid cancer (Hoefnagel et al., 1986). The mechanism of localisation of I$^{131}$ in thyroid malignancy is because of the iodine pathway although not all differentiated thyroid cancer takes up I$^{131}$. 
The methods of localisation of tumour non-specific radiopharmaceuticals are multifactorial. Ga\textsuperscript{67}-Citrate is the agent which has been studied the most but the mechanisms for its uptake are still poorly understood (Tsan and Scheffel, 1986). The factors thought to be important include an adequate blood supply, an increase in capillary permeability and the presence of the Ga\textsuperscript{67} binding proteins, transferrin, lactoferrin and ferritin. Other factors which are important in the non-specific tumour localisation of radiopharmaceuticals include an increase in interstitial fluid (Tator et al 1965) and neoplastic cell uptake. The latter includes the localisation of antibody-antigen complexes on the cell membrane (Soo et al, 1987), uptake into the cell of amino acids (Tator, 1968), nucleotides (Rotenberg et al, 1962) and fatty acids (Tator et al, 1966) as growth substitutes, together with the incorporation of glucose analogues into tumours as energy substitutes (Tator et al 1969).
1.8. ANIMAL SQUAMOUS CELL CARCINOMA MODELS AND
RADIOPHARMACEUTICAL TUMOUR IMAGING

1.8.1. Introduction

1.8.2. History

1.8.3. Methods of Transplantation

1.8.4. The Vx-2 Rabbit Squamous Cell Carcinoma

1.8.5. Experimental Radiopharmaceutical Tumour Imaging
1.8.1. INTRODUCTION

There exists an obvious need for carefully controlled laboratory studies using animal models in research on tumour-localising agents. There are many animal tumour models presented in the literature, many of which vary greatly in terms of identity, source of origin and specific characteristics. Some of these tumours have arisen spontaneously whilst others were induced using viruses, X-rays, carcinogenic chemicals or other means. Most of the tumours are transplantable and repeated transplantation aids in the selection of cells possessing the greatest resistance to the host-defence mechanism and the subsequent development of a more virulent tumour (Ketcham et al, 1966). No-one can defend the hypothesis that transplantable tumours in animals behave in a similar manner to their spontaneous counterparts in man. Although it is now possible to perform in-vivo studies on human head and neck squamous cell carcinoma transplanted into animal models (Braakhuis et al, 1984) as well as in-vitro studies on tissue-culture models (Edwards et al, 1980; Easty et al, 1981a-b; Heinerman et al, 1985; Hellquist et al, 1985; Wang et al, 1986, Wennerberg et al, 1988), studies using transplantable animal tumour models can provide important clinical and biophysical data unobtainable by other means.
This section outlines the history and current status of animal models with squamous cell carcinoma together with their methods of transplantation and requirements for experimental radiopharmaceutical tumour imaging. It provides a discussion of the Vx-2 rabbit squamous cell carcinoma, the animal model used for the experimental work in this thesis.
1.8.2. HISTORY

Over two hundred years ago in 1773, Peyrilhe performed the first experiments on the transplantation of tumours. He injected material from human breast cancer under the skin of a dog. Five days after the injection, "the whole skin, from the head to the tail, was completely emphysematous; - A little ichorous, blackish matter flowed from the wound - The eyes of the animal were vivid, and he seemed to have a great thirst; in this state the poor creature was perpetually howling ............... At length, my maid, disgusted by the stench of the ulcer, and softened by the cries of the animal, put an end to his life, and thus prevented my observing the ultimate effects of this disease" (Peyrilhe, 1777). Following this, many other attempts were made to transplant tumours from man to animals but they consistently failed (Leidy, 1851). A long period elapsed between these early experiments on heterologous transplantation and the first successful attempts in 1943 to transplant human head and neck squamous cell carcinoma into laboratory animals (Greene, 1952). Peyrilhe would have taken heart from his unsuccessful attempt to transplant human cancer had he foreseen the problems his successors were to encounter.
Early tumour transplantation studies in laboratory animals were carried out using tumours arising spontaneously. Hanau (1889) transplanted a squamous cell carcinoma from the vulval region of an old rat into other rats of the same stock for two transplant generations. However, such studies were difficult to duplicate due to the erratic nature of the spontaneous sources. Clunet was the first person to induce tumours experimentally in animals when he transplanted a sarcoma which he had induced in a rat following exposure to X-rays (Clunet, 1910). In 1914, Yamagiwa and Ichikawa applied tar to the skin of rabbits and mice and successfully induced squamous cell carcinomas and these were transplanted a few years later (Fibiger and Bang, 1920). Since these early experiments, many induced tumours have been found to be transplantable and their behaviour appears similar to that of transplanted spontaneous tumours. The most commonly induced tumours to be studied in transplantation are squamous cell carcinomas of the skin and sarcomas of different or mixed types arising in the subcutaneous region following application of carcinogens (Stewart et al, 1959).

The commonest squamous cell carcinoma models which have been used to investigate head and neck malignancy are transplantable rabbit squamous cell carcinomas and induced intra-oral models in the hamster and rat.
The Brown-Pearce carcinoma, which has been widely accepted as one of the most useful of the transplantable rabbit tumours, was observed to arise from a scrotal chancre of an experimental rabbit (Brown and Pearce, 1923a). Despite subsequent extensive discussion on the subject (Brown and Pearce, 1923b-g; Brown and Pearce, 1924) the exact origin of the antecedent tissue from which the carcinoma originated is still in doubt. Brown and Pearce believed the tumour originated in the skin of the scrotum whilst others believed that two primary neoplasms arose simultaneously in the scrotal skin and the testicle (Stewart et al, 1959). Brown and Pearce also reported a 20-25% success rate when transplanting to subcutaneous sites. Because of the possible histological ambiguity and the low transplant success rate, the Brown-Pearce carcinoma has not achieved popularity in current head and neck research.

The Vx-2 rabbit squamous cell carcinoma was derived from a papilloma of a domestic rabbit inoculated with a Shope papilloma virus in 1937 during the course of a study on the malignant transformation of such papillomas (Shope, 1933; Kidd and Rous, 1940). At first the tumour was not particularly vigorous and grew in only a small proportion of rabbits. However, by the fifth generation
it had developed the aggressive growth pattern which it now shows (Stewart et al, 1959). Haynie et al (1976) reviewed the experimental animal tumour models available for the evaluation of radioactive tumour-localising agents. Included within this review was the Vx-2 tumour which is now a well recognised model not only for radiopharmaceutical research (Okuyama et al, 1974; Okuyama et al, 1978; Abe et al, 1985; Watkinson, 1987) and head and neck squamous cell carcinoma (Jefferis, 1984) but for cancer research in general (Wood et al, 1967; Favre et al, 1982; Sugawara et al, 1983; Phelps et al, 1985). Other transplantable rabbit squamous cell carcinomas such as the Vx-7 carcinoma (Weisbroth, 1974, p 360) are available although they have not been used in the evaluation of tumour-localising agents despite being similar models to the Vx-2.

The oral mucosa in animals was considered more resistant to chemical carcinogens than skin and early attempts to produce experimental malignant oral tumours were unsuccessful. In 1925, Bonne noticed that some mice whose skin had been painted with coal tar later developed papillomas in their mouths and stomachs, presumably as a consequence of licking themselves (Woglum, 1926).
Two years later, Bonne reported three squamous cell carcinomas which developed on the palates of 50 mice which had been treated with coal tar for over a year. Roffo described leukoplakia in the palate of rabbits exposed to cigarette smoke (Roffo, 1930) and Oyoma produced lingual carcinomas in rabbits by injecting coal tar into the tongue (Oyama, 1935). However, others failed to induce malignant or pre-malignant changes in mouse labial mucosa using the carcinogen 20-methylcholanthrene. In 1954, Salley found the essential combination of susceptible tissue and a potent carcinogen when he used polycyclic hydrocarbons to induce squamous cell carcinomas in the hamster cheek pouch.

Hamster Cheek Pouch

Early attempts to produce tumours at this site proved unsuccessful (Wantland, 1954). Later that year, Salley successfully produced tumours by painting the pouch with the powerful polycyclic hydrocarbon carcinogens 9, 10 dimethyl-1, 2-benzanthracene (DMBA), 20-methylcholanthrene (20 MC) and 3, 4-benzpyrene (3, 4 BP) dissolved in either acetone or benzene. The pouch mucosa subsequently passed through four histologically distinct stages; hyperplasia,
papilloma, carcinoma-in-situ and squamous cell carcinoma, with or without metastases. DMBA in acetone was the most effective carcinogen. In his original work Salley reported frequent cervical lymph node metastases (Salley, 1954). However, he omitted to mention their occurrence in later publications (Salley, 1957; Salley, 1961) and most subsequent workers have failed to demonstrate regional metastases (Eveson, 1981). Despite this, some have shown cervical metastases occur when the tumour has been present for a long time (Craig, 1977).

The hamster cheek pouch model has been standardised (Morris, 1961; Morris and Reiskin, 1966) and details are available in the literature (Eveson, 1981).

Hamster

The oral mucosa proper of the hamster is considerably more resistant to the action of chemical carcinogens than the cheek pouch and attempts to induce tumours have been largely unsuccessful (Salley, 1954; Salley and Kreshover, 1959; Al Ani and Shklar, 1966; Mesrobian and Shklar, 1969). Dachi (1967, p 1480) induced lingual carcinomas using DMBA in DMSO but was frustrated by the development of larger and more anaplastic tumours in the
adjacent skin and oral mucosa which invariably led to the early demise of the animal. A more successful lingual carcinoma model was established by Fujita et al using DMBA (Fujita et al, 1973) and this has been popularised by later workers (Marefat and Shklar, 1977; Eveson, 1979).

Rat

Initial attempts to induce oral tumours in rats met with similar limited success (Wallenius, 1966; Giunta and Shklar, 1972). However, Wallenius and Lekholm (1973) induced palatal carcinoma in all animals by seven months using the water-soluble carcinogen 4-nitroquinoline-N-oxide (4 NQO). 75% of the animals also developed carcinomas on the dorsum of the tongue and 20% showed tumours of the gingiva or stomach. Following this work the rat palate model using either DMBA in combination with antiasialogogues or 4NQO in propylene glycol has been increasingly popular in studies of intraoral carcinogenesis (Heydon, 1974; Lekholm et al, 1975; Eveson, 1981). Yamamura et al attempted to prolong the action of chemical carcinogens by implanting them into surgically created pouches in the rat lip. A number of neoplasms were induced including squamous cell papillomas,
carcinomas, neurofibromas, haemangiomas and
haemangiosarcomas (Yamamura et al, 1975). Although this
was an interesting observation, the uncertainty surrounding
the ultimate tumour histology makes the model of limited
value in the investigation of intra-oral carcinogenesis
and head and neck squamous cell carcinoma.

Miscellaneous

The production of oral tumours in mice has proved
difficult (Van Prohaska et al, 1939; Goldhaber, 1957;
there is no current satisfactory mouse model of intra­
oral carcinogenesis. Similarly primates appear to be
extremely resistant to the actions of most known chemical
carcinogens at most sites (Kent, 1960) and to date there
exists no simian model of intra-oral cancer.
1.8.3. METHODS OF TRANSPLANTATION

The most commonly used method of transplantation is similar to that used by Peyrilhe over two hundred years ago (Peyrilhe, 1777). Solid tumours are cut into fragments less than 1 mm in dimension and transplanted using a trocar and cannula. Homogenates and minces can similarly be prepared and injected using a needle and syringe. Standardised sterile techniques are mandatory when performing these procedures and details are available in the literature (Stewart et al, 1959; Farr and Konikowski, 1964).

Tumour cell suspensions can also be prepared by digesting tumour segments with Trypsin. This has several advantages. Cell suspensions can be diluted and stored in liquid nitrogen. A known numbers of cells can be used in the transplant and their viability assessed and intravenous injections can be performed (Frank et al, 1987). It is relatively easy to inoculate large numbers of animals and the transplantation experiments can be standardised. Lastly it is possible to transplant single cells, a technique which has been used in tumour transplantation (Ishibashi, 1950; Hauschka, 1953).
1.8.4. THE Vx-2 RABBIT SQUAMOUS CELL CARCINOMA

The Vx-2 carcinoma (Vx-2, V-2, V2, V₂) is a poorly differentiated squamous cell carcinoma which developed as a result of malignant alteration in the cells of a virus-induced papilloma using the shope papilloma virus (SPV). Dr. Richard Shope who first described papillomatosis of cotton tail rabbits did so with naturally injected rabbits from Iowa (Shope, 1933; Shope, 1936; Shope, 1937). The natural disease is characterised by the formation of cutaneous pigmented papillomas which project 0.5-1.0 cm above the skin line. The papilloma can be experimentally transmitted with suspensions of papilloma cells or by cell-free extracts which are scarified onto the skin of susceptible rabbits. Following a latent period of 10-12 days papillomas begin to develop. Pre-treatment of the skin (at the same or other sites) with tar, mixtures of turpentine and acetone, or 20-MC is known to enhance the susceptibility of the skin to SPV (Rous and Friedewald, 1941; Friedewald, 1944; Rous and Friedewald, 1944). The papillomas persist for varying periods of time depending on host factors. Some regress spontaneously whilst malignant transformation to squamous cell carcinoma occurs in at least 25% of those cases acquired naturally and observed for one year following injection (Syverton and Berry, 1935).
The malignant potential of the seemingly benign cutaneous papillomas was recognised by Rous and Beard who found that papillomatous fragments implanted into muscle or certain internal organs would develop into squamous cell carcinomas and acquire invasive properties (Rous and Beard, 1934). The Vx-2 carcinoma was derived from a papilloma of a domestic rabbit inoculated with SPV in 1937 (Kidd and Rous, 1940). Initially its growth was sporadic, only growing in a few rabbits, but by the fifth generation it had developed the aggressive pattern which it now shows. The 203rd transplant generation was reached in 1970.

The recommended route for homologous transplantation is by intramuscular injection (Haynie et al, 1976). However, the tumour grows well in bone (Enneking and Flynn, 1968), brain (Cochran et al, 1985), intraperitoneally (Baker et al, 1969) larynx (Jefferis, 1984) and subcutaneous sites (Watkinson, 1987). The host range of heterotransplantability includes guinea pig, mouse, rat and hamster brain, the guinea pig eye anterior chamber and the subcutaneous tissues and testes of the hamster and mouse (Greene, 1953).

The Vx-2 invades surrounding skeletal muscle and areolar connective tissue both in the primary site and its lymph node metastases. It is not encapsulated but
induces chronic inflammation in invaded tissues. It is organised histologically into sheets and nodules surrounded by reticular fibres (Figure 19). There may be some necrosis but little or no haemorrhage. Nuclei are hyperchromatic, uniform in size and shape, and giant cells are infrequent. It is relatively avascular and its histology is indistinguishable from human squamous cell carcinoma (Iain Lindsay, 1987, Personal Communication). Metastases are common to the lungs, liver and lymph nodes but rare to other sites (Wood et al, 1967; Weisbroth, 1974, p 360; Phelps et al, 1985) and this apparent barrier to systemic metastases has been investigated by several workers (Fisher and Fisher, 1966a-b; Wood et al, 1967; Engzell et al, 1968).

It has long been proposed that lymph nodes act as a barrier to the passage of particulate matter contained in lymph (Virchow, 1860). Their ability to trap inert particles (Gilchrist and David, 1938), bacteria (Drinker et al, 1934), viruses (Yoffey and Sullivan, 1939) and red blood cells (Engeset, 1962) have all been evaluated. Zeidman and Buss in 1954 noted that when Vx-2 or Brown-Pearce carcinoma cells were injected into the afferent lymphatics of rabbit popliteal lymph nodes, only 7% of rabbits studied developed pelvic metastases within
A transplanted Vx-2 squamous cell carcinoma in the anterior aspect of the tongue of a New Zealand White Rabbit.
42 days. Although this shows lymph nodes may act as an effective temporary barrier, it is not conclusive evidence that transnodal passage of tumour cells does not occur. In their classic work, Fisher and Fisher cannulated both the afferent and efferent lymphatics of the rabbit popliteal lymph nodes and collected efferent lymph following infusion of either Vx-2, Brown-Pearce or Walker tumour cells (Fisher and Fisher, 1966a). They showed that all three tumour cells appeared in efferent lymph and concluded that while tumour cells may be sequestrated in the lymph node, they also pass through it. This has since been disputed by others. Engzell et al showed that out of 16 rabbits whose popliteal nodes were perfused with viable Vx-2 cells, only one rabbit had cancer cells in the efferent lymph (Engzell et al, 1968).

The Vx-2 is also responsible for non-metastatic manifestations in the rabbit. It is known to secrete a parathormone-like substance which depresses the renal tubular reabsorption of phosphorus and causes hypophosphataemia, hypercalcaemia and dystrophic calcification of the tumour (Figure 20) and soft tissues (Wilson et al, 1961; Gertner et al, 1964; Wilson et al, 1964; Wilson et al, 1965).
In early transplant generations, the host rabbits produced antibodies to SPV but these had disappeared by the 46th generation (Weisbroth, 1974, p 360). Within the Vx-2, free genome-size viral DNA molecules do not exist, but multiple viral genomes are present in high molecular weight forms (McVay et al, 1982). Recently the physical characteristics and restriction mapping of the SPV genome have been investigated, the details of which are available in the current literature (Favre et al, 1982; Sugawara et al, 1983; Phelps et al, 1985).
1.8.5. EXPERIMENTAL RADIOPHARMACEUTICAL TUMOUR IMAGING

In an ideal world, research and diagnosis using radioactive tumour-localising agents should be done with a human model. Practically and ethically, there are many obvious problems associated with this. Surgical biopsy may prove unreliable (Haynie et al, 1976) and when the biopsy is obtained, its intrinsic characteristics may be affected by a number of clinical conditions. Alterations in cell membrane physiology, hepatic and renal function may be caused by intravenous infusions, prolonged anaesthesia and operative procedures. Biopsy samples may be small and samples of surrounding normal tissue can be contaminated with tumour. In addition, the use of post-mortem material is open to question. The process of death, particularly in a cancer patient, may be prolonged over a period of time which may lead to unusual patterns of biodistribution due to heart failure and abnormal liver and kidney function.

Animal transplantable tumours cannot be assumed to have analogous behaviour to their human counterparts. Many transplantable and human tumours have specific individual characteristics that make them unique. Some transplantable tumours, like leukaemia in mice, closely
resemble leukaemia in man. Others, such as mouse mammary tumours, bear little resemblance to those which occur within the human breast. In addition, there are strong species variations in transplanted tumours (Haynie et al, 1976) which should serve as a warning not to extrapolate animal experimental data to man without extreme caution. It is also not uncommon to observe metaplastic transformation of one morphological type to another during the serial transplantation of a tumour, an example of which is a carcinoma becoming a sarcoma (Stewart et al, 1947). Such phenomena are notable by their absence in man.

Gullino (1966) examined the dynamics of transplanted tumours. Using tissue isolation techniques, he made blood volume, blood flow and interstitial fluid measurements in transplanted rat and mouse tumours which were served by one artery and vein. He was able to show a marked reduction in both blood flow and the vascular space of transplanted tumours with doubling of the interstitial fluid pool. This data showed that the site of a transplanted tumour should be as near normal as possible with regard to vascularity and blood flow.
Of those animal models currently available to study head and neck squamous cell carcinoma, the most satisfactory would appear to be the hamster cheek pouch and tongue models using DMBA and the rat-palate model using 4 NQO. Although the properties of any experimental model depend largely on the information the investigator is seeking, certain fundamental characteristics are desirable. The most important of these is that the site chosen should have similar histology to the equivalent human site. Of those models in the hamster and rat the only one which satisfies this criterion is the rat-palate model (Eveson, 1981). In addition, there are other major objections to the hamster cheek pouch. Its true intra-oral origin is debatable (Kolas, 1955), it is the site of immunological privilege and the mucosa is difficult to handle (Eveson, 1981). Although these intra-oral carcinogenic models are transplantable (Meng et al, 1982) this precludes the study of pre-malignant change, information which is vital to the basic understanding of the evolution of oral cancer (Eveson, 1981).

In investigational studies of radiopharmaceutical uptake, animal tumours should be rapidly reproducible, of moderate size with histology and vascularity which is similar to naturally occurring tumours. Repeated tumour transplantation creates some of these characteristics.
The experimental model must suit the experiment in question. Radiopharmaceutical images of hamsters and rats are difficult to interpret, particularly within the head and neck, and prolonged arterial access is difficult both to achieve and maintain. The Vx-2 rabbit carcinoma has been used for radiopharmaceutical tumour studies. Although it did not originate within the head and neck, cervical metastases are uncommon (Jeffersis, 1984) and histological transformation can occur (Stewart et al, 1947), it is easily transplantable to any site and both its vascularity and histology closely resemble human squamous cell carcinoma. In addition, the rabbit is large enough to facilitate easy gamma camera imaging. As such the Vx-2 is ideally suited to radiopharmaceutical tumour imaging particularly as its role in current head and neck squamous cell carcinoma research is now well established (Jeffersis, 1984; Cochran et al, 1985; Watkinson, 1987).

For over sixty years, transplantable animal tumours have been recognised as being useful in all areas of cancer research. They have been used to investigate much of which could not be performed in humans. The use of radioactivity has added a new dimension to the study of tumour physiology using animal models.
CHAPTER 2

AIMS AND OBJECTIVES
AIMS AND OBJECTIVES

The aim of this study is to establish an animal squamous cell carcinoma tumour model, and to use it to evaluate the pharmacokinetics, biodistribution (to include subcellular localisation), optimal imaging time and optimal imaging characteristics of Technetium-99m (v) Dimercaptosuccinic acid. These results will be compared with similar parameters in patients with head and neck squamous cell carcinoma. The animal biodistribution and human pharmacokinetic results will then be combined to calculate the absorbed dose and effective dose equivalent in man.
CHAPTER 3

3 MATERIALS AND METHODS

3.1. ANIMALS

3.1.1. Source of Animals

3.1.2. The Vx-2 Squamous Cell Carcinoma

3.1.3. The Radionuclide. Technetium-99m

3.1.4. The Radiopharmaceutical. Technetium-99m (v) Dimercaptosuccinic Acid

3.1.5. Animal Preparation
3.1.1. **SOURCE OF ANIMALS**

The animals used in this study were male New Zealand White (NZW) rabbits. This rabbit was chosen because it is large enough to permit easy manipulation for both anaesthetic and imaging purposes, repeated blood and urine samples can be obtained with relative ease and transplantable rabbit tumours are available. All animals were weighed on arrival, housed in individual cages and fed on 679 special rabbit pellets with coyden (Grain Harvesters Ltd.). Male rabbits (2.5 kg) were preferred to facilitate bladder catheterisation. Eighty nine rabbits were studied (27 non-tumour; 62 tumour).
3.1.2. THE Vx-2 SQUAMOUS CELL CARCINOMA

Source

The Vx-2 squamous cell carcinoma cells used in this study came from the Department of Experimental Pathology, St. Mary's Hospital, London. The cells were transported in liquid nitrogen in 2 ml stoppered ampoules (Sterilin) and stored in liquid nitrogen until required.

Preparation of Cells

The technique used for cell preparation was that described by Jefferis (1984). The frozen cells were thawed rapidly by constantly stirring the sealed tubes containing the tumour in a water bath at 37°C. The contents were decanted into a 10 ml test tube and 10% foetal calf serum (FCS, Flow Laboratories) in Rothwell Park Memorial Institute (RPMI) 1640 medium (Flow Laboratories) at 37°C was added, drop by drop, with constant agitation to prevent osmotic disruption of the cell membrane. When approximately 10 ml of medium had been added, the cell suspension was centrifuged at 60 g (700 rpm) for 10 minutes (Hettich Universal). The supernatant was decanted into a
10 ml test tube and resuspended in 10 mls of 10% FCS in RPMI 1640 at 37°C. This suspension was centrifuged again at 60G for 10 minutes and the supernatant decanted to leave approximately 1 ml of tumour cells. These were resuspended in 2 mls of 10% FCS in RPMI 1640 and a total cell and viability count performed. The cell suspension was stored in ice until required.

**Total Cell Count**

0.1 ml of the cell suspension was diluted with 0.9 mls of 3% Acetic Acid solution and a drop of the resulting mixture was placed in a counting chamber (improved neuebauer) and the cells counted in 0.1 ml³. From this the total number of cells could be calculated.

**Viability Count**

This was performed using an ultraviolet Fluorescence Microscope (Olympus BH-2) to count cells containing either ethidium bromide or acridine orange. Cells which are viable show up green under ultraviolet light whereas dead cells show up orange (Figure 21). The green staining is due to the living cells hydrolysing the acridine orange.
Vx-2 squamous cell carcinoma cells seen with an ultraviolet fluorescence microscope. Viable cells hydrolyse acridine orange and show up green (A) while dead cells appear orange (B) due to the binding of ethidium bromide into the DNA of the dead cells (magnification x 170; Exposure time, 32 seconds).
The orange staining is caused by the binding of the ethidium bromide into the DNA of the dead cells which is prevented in the living cells by the integrity of the plasma membrane.

Four milligrammes of ethidium bromide (Sigma Chemical Co. Ltd.,) was dissolved in 20 mls of phosphate buffered saline, pH 7.4. 1 mg of acridine orange (Raymond Lamb) was dissolved in 10 mls of the same buffer solution and the two solutions were then combined (1:1) and diluted (1:10) with phosphate buffered saline. One drop of the ethidium bromide/acridine orange mixture was placed on a microscope slide together with one drop of cell suspension. The resultant mixture was covered with a microscope slide and the viable and dead cells counted.

Tumour Cell Bank

Using a 23 French Gauge (FG) needle, 0.2 ml of the tumour cell suspension was injected into the thigh muscle of an anaesthetised rabbit. The number of viable cells in the 0.2 ml aliquot was approximately $3 \times 10^4$. The tumour was allowed to grow up in the thigh until it was palpable and the animal was then killed with an intravenous bolus overdose of veterinary pentobarbitone (100 mg/kg) (Euthasate).
The tumour was removed and chopped into 1 cm cubes. A sample sent for histology and the remainder placed in 10 mls of RPMI 1640 with Gentamicin and added mycostatin (0.2 mg/ml, Squibb). The tumour was chopped into 0.6 mm cubes using a McIlwain tissue chopper and washed in 10 mls of Hanks Basic Salt Solution (BSS) (magnesium/calcium deficient, GIBCO) and centrifuged at 150 G (1000 rpm) for 30 seconds (Jovan CR 411).

The supernatant was removed and an equal volume of Hanks BSS:tumour was added. Cell digestion was commenced by adding 1 ml of Hanks BSS solution with 1% collagenase (Sigma) to the mixture, which was then incubated at 37°C for 15 minutes. 3 mls of 0.25% Trypsin (Porcine Pancreas, Sigma) was added and the resultant mixture incubated for a further 15 minutes at 37°C. The liberated cells and supernatant was removed, Soya bean trypsin inhibitor (Sigma) with Deoxyribonuclease (Bovine pancreas, Sigma) added, and the mixture centrifuged at 150G (1000 rpm) for seven minutes (Jovan CR 411). The supernatant was removed and the cells suspended in RPMI 1640 with 10% FCS at 4°C. Two further trypsin digestions were performed on the remaining tumour, the resultant cell suspensions combined and the subsequent suspension strained through a
140 um gauze. A total cell count was then performed (10^6 cells per ml in 20 mls). The cells were spun down at 150G (1000 rpm) for seven minutes (Jovan CR 411) and resuspended in 10 mls of freezing mixture containing 9 mls of 10% FCS with RPMI 1640 and 1 ml of Dimethyl Sulphoxide (DMSO B.D.H., Analar grade). 1 ml aliquots of the resultant mixture containing 2 x 10^6 cells per ml were pipetted into 2 ml sterilin ampoules, placed in a -70°C fridge for two hours and transferred to storage racks in liquid nitrogen until required.

**Tumour Transplantation**

Sequential tumour transplantation was achieved using the method of passage (Stewart et al, 1959). Frozen cells were prepared for injection as previously described and 1.5 x 10^6 viable cells were injected into the thigh of an anaesthetised rabbit. Three weeks later, the animal was sacrificed, the tumour removed, chopped into 1 cm cubes and placed into 10 mls of RPMI 1640 at 4°C. A sample of tumour was retained for histological examination. The tumour was then chopped in 0.6 mm cubes using a McIlwain Tissue Chopper and resuspended in 10 mls of RPMI 1640 at 4°C. Using a 1 ml syringe with a 15 FG metal cannula, 0.2 mls of the tumour
suspension was injected subcutaneously into the flanks of four anaesthetised non-tumour rabbits. This technique was then repeated, at regular monthly intervals, using the flanks and/or shoulders of rabbits as subcutaneous sites. This ensured a constant supply of rabbits with tumours of different sizes. Most experiments were conducted two weeks following transplantation, by which time the tumours had grown to approximately 2 cm in size. Using this method a transplant success rate greater than 90% was achieved.
3.1.3. **THE RADIONUCLIDE. TECHNETIUM-99m**

The pertechnetate used in this study was obtained from an Amertec-II-Technetium-99m sterile generator produced by Amersham International. This generator contains Molybdenum-99 absorbed onto alumina in a sterilised plastic column surrounded by depleted uranium shielding. The column is eluted using an evacuated vial which draws sterile physiological saline from a reservoir through the column. Provided the eluate is used within eight hours, its radiochemical and radionuclide purity fulfill specifications for sodium pertechnetate injection prescribed by the British Pharmacopoeia. Less than 2 ug/ml of aluminium/ml is present in any eluate while the maximum permitted breakthrough of Mo$^{99}$ is 0.1% of total eluate activity.
3.1.4. **THE RADIOPHARMACEUTICAL, TECHNETIUM - 99m (v) DIMERCAPTOSUCCINIC ACID**

Tc$^{99m}$ (v) DMSA was prepared using the method described by Sampson (1987). Under laminar flow a standard Amersham DMSA vial was opened (containing 1.0 mg DMSA, 0.42 mg stannous chloride dihydrate and stabilisers). Four milligrammes DMSA (Sigma), 120 mg dextrose B.P. and 5 mg sodium bicarbonate B.P. were then added to the kit together with 0.4 mls of 7% sodium bicarbonate solution to form the 'DMSA' solution. Technetium-99m pertechnetate injection (600 MBq) was transferred to a mixing vial and made up to 2.5 mls with sodium chloride injection (0.9%). 0.1 ml of the 'DMSA' solution was also added and the solution sterilised using a 0.22 um filter and air-bleed needle into a final container. The volume and activity were both checked and the final solution was stored at 2-8°C, protected from light and used within two hours. Chromatograms of the Tc$^{99m}$ (v) DMSA injection were scanned using a Berthold Radiochromatogram Scanner (LB 2722-2) to check for radiochemical purity. Tc$^{99m}$ (III) DMSA was prepared in the conventional manner using a standard Amersham DMSA kit and Tc$^{99m}$-TcO$_4^-$.
3.1.5. **ANIMAL PREPARATION**

**Anaesthetic**

All rabbits were weighed and then given 0.1 ml/kg Fentanyl Fluanisone (Janssen) intramuscularly into the thigh as premedication. Thirty minutes later, general anaesthesia was induced with 7 mg/kg intravenous (i.v.) ketamine hydrochloride (Parke-Davis) and 2 mg/kg Xylazine (Bayer). Anaesthesia was maintained with further boluses of ketamine (every 15 minutes) and xylazine (every 30 minutes). The animals were allowed to breathe spontaneously and using this technique repeated arterial and urine sampling, sequential imaging and tumour implantation were performed.

**Pharmacokinetic Studies**

Fourteen rabbits were studied (five non-tumour; nine tumour). Following premedication, a 23 FG heparinised butterfly needle was inserted into the marginal ear vein. Under general anaesthesia, a 20 FG venous cannula was inserted into the central ear artery of the opposite ear and connected to a three-way tap kept patent with heparinised saline. A lubricated 8 FG neonatal feeding tube was inserted via the urethra into the bladder.
Background blood and urine samples were obtained and Tc $^{99m}$ (v) DMSA administered i.v. (100-200 MBq) via the heparinised butterfly needle using a pre-weighed 1 ml syringe. Following injection, the syringe was re-weighed and any residual activity measured. Using a 2 ml syringe, repeated 1 ml arterial blood samples were taken in non-tumour and tumour-bearing rabbits at 5, 15, 30, 45 and 60 minutes and then at 1½, 2, 3 and 4 hours post-injection. All animals were then allowed to recover. Following premedication a further arterial sample was obtained at six hours using a 2 ml syringe and a 23 FG needle. Urine samples were taken at 1, 2, 3, 4 and 6 hours after injection. At 24 hours all rabbits were premedicated and sacrificed. Final blood (cardiac) and urine samples were obtained. In addition, tumour-bearing rabbits were examined for metastases and all tumours removed, weighed and a sample sent for histological examination. During general anaesthesia, hydration was maintained by the administration of 10 mls of Dextrose/saline B.P. given hourly, subcutaneously, into the nape of the neck. All blood samples were placed in pre-weighed test tubes and then weighed using a balance (Sartorius) accurate to four decimal places to obtain the exact sample weight. The urine samples were placed in pre-weighed universal containers and then re-weighed to obtain an accurate sample weight.
A 1 ml aliquot was drawn up, transferred to a pre-weighed plastic test tube and re-weighed. Standards from the injection were made up in identical test tubes from 1:10 to 1:10^5 dilution. All whole blood, urine and dilutions of the standard samples were counted for 10 seconds using an automatic gamma sample counter (LKB-Wallac 80000). 

The percentage of the injected dose per gram of both blood and urine was calculated using the formula below:-

\[
\% \text{ injected dose/g} = \frac{\text{sample counts} \times \text{volume of standard (ml)}}{\text{sample wt (g)} \times \text{injection wt (g)} \times \text{standard dilution} \times \text{standard counts}} \times 100\% 
\]

The blood clearance half-time (t½) values for each rabbit were calculated using a computer (Amstrad PC 1640) with a spread sheet facility (Supercalc 4). Using linear regression, a best-fit straight line was plotted to blood data samples obtained at, and after, 120 minutes. Each data sample obtained before 120 minutes was subtracted from this best-fit line to obtain a second best-fit straight line. A correlation coefficient for each best-fit line
was calculated and from these two lines, the $t_{1/2}$ for each rabbit was calculated. The $t_{1/2}$'s for each group were compared using parametric statistics (mean $t_{1/2}$'s, student $t$-test).

The percentage of the injected dose per gram of urine was plotted graphically against time and the mean cumulative half-time urine values calculated from the curve.

**Biodistribution**

Forty rabbits were studied (20 non-tumour; 20 tumour). Following premedication, a 23 FG heparinised butterfly needle was inserted into the marginal ear vein. The radiopharmaceutical $^{99m}$Tc DMSA was administered i.v. (100-120MBq) using a pre-weighed 1 ml syringe which, following injection, was re-weighed and any residual activity counted. All animals were allowed to recover and at 2, 4, 6 and 24 hours after injection, both non-tumour and tumour-bearing rabbits were sacrificed. The body organs, together with samples of bone, muscle, tongue, nasal mucosa, marrow and small and large bowel were removed, washed and blotted dry. All organs and samples, except the liver, lung, kidneys and stomach were weighed. The liver, lung, kidneys and stomach were sectioned and representative samples were weighed. In addition, the liver, lungs and tumour regional lymph nodes were examined
macroscopically for metastases. All tumours were removed together with a sample of surrounding inflammatory tissue. The tumour and inflammatory tissue samples were washed, blotted dry and weighed. The tumours were cut in half and one half counted whole. The necrotic non-viable centre was removed from the other half and the necrotic non-viable centre and outside viable living tumour were counted separately. Samples of all tumours, systemic metastases and inflammatory tissue were examined histologically. All samples, together with dilutions of the standards (1:10 to 1:10^5) of the injection were counted on the automatic gamma sample counter. The percentage of the injected dose per gram of tissue was then calculated and the mean organ and tissue biodistribution in the non-tumour and tumour-bearing groups was compared statistically using a student t-test.

**Optimal Imaging Time**

Seven rabbits were studied (one non-tumour; six tumour). Following premedication, a 23 FG butterfly needle was inserted into the marginal ear vein and under general anaesthetic, the radiopharmaceutical Tc⁹⁹m (v) DMSA (200-240 MBq) was injected intravenously into the rabbit. Rabbits were positioned prone on the gamma camera crystal
with their limbs splayed to exclude epiphyseal hot spots as much as possible from the field of view. The position of each tumour was checked with a technetium marker syringe to ensure it was within the field of view. Sequential thoraco-abdominal planar hard copy images were acquired \((10^6\) counts) at 30 minutes and at \(1, 1\frac{1}{2}, 2, 3, 4, 5\) and 6 hours post-injection using an Ohio-Nuclear Series 120 mobile gamma camera linked to an Ohio-Nuclear data-logger (Figure 22). Prior to each image acquisition the bladder was first emptied using a neonatal feeding tube and then shielded using conventional 2 mm lead shielding. Hydration was maintained by the administration of 10 mls of Dextrose/saline given hourly subcutaneously into the nape of the neck. The data was then transferred from magnetic tape to floppy disc for analysis using a nuclear medicine dedicated computer (DEC PDP 11/73, Nuclear Diagnostics). All rabbits were sacrificed within 24 hours of imaging so tumours could be removed, measured in three dimensions using callipers, and a sample sent for histological examination. Two methods were used to estimate the optimal imaging time.

1. **Qualitative Assessment**

Image hard copies were reported blind by a physician (Dr. Susan Clarke) with an interest in tumour imaging. The
A New Zealand White Rabbit being imaged on an Ohio-Nuclear Series 120 mobile gamma camera.
sensitivity and specificity for tumour scintigraphic
detection could then be determined for each consecutive
imaging time.

2. Quantitative Assessment

Tumour:background (soft tissue) ratios at each
consecutive imaging time were calculated for four tumours.
Using the computer, area of interest were drawn within the
tumour and around an area of normal tissue. Tumour:
background ratios were calculated using the formula below:-

\[
\text{Ratio} = \frac{\text{counts per pixel (tumour-background)}}{\text{counts per pixel in background}}
\]

Optimal Imaging Characteristics and Palpation

Fifteen rabbits were studied (one non-tumour; 14 tumour).
Under general anaesthetic all animals studied were shaved
in four sites (both shoulders and both loins). Tumours
were then induced randomly in three out of four sites and
animals examined at either 2, 3 or 4 weeks so that tumours
of different sizes could be studied. The shoulders and loins were chosen as injection sites as this provided good tumour/background ratios and all tumours were visible on one field of view for comparison. At either 2, 3 or 4 weeks under general anaesthetic, the radiopharmaceutical \( \text{Tc}^{99m} \text{DMSA} \) (200-240 MBq) was injected intravenously and each rabbit then examined in each of the four sites by six independent observers (four otolaryngologists, a surgical houseman and a pre-clinical medical student). Each observer was asked whether tumour was palpable or not, and if so, to estimate the size of the tumour. The rabbits were subsequently imaged at the optimal imaging time (10^6 counts) using the method previously described, and were then sacrificed. All tumours were removed, together with the thymus. The liver, lungs and regional tumour lymph nodes were examined macroscopically for metastases. All tumours were weighed, measured in three dimensions using callipers, grouped according to the classification shown in Table 13, and a sample sent for histological examination.

Hard copy images were reported by Dr. Susan Clarke. The overall sensitivity and specificity for scintigraphy and palpation could then be calculated. In addition,
<table>
<thead>
<tr>
<th>Class</th>
<th>Size Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class 1</td>
<td>Less than 1 cm</td>
</tr>
<tr>
<td>Class II</td>
<td>1-2 cm</td>
</tr>
<tr>
<td>Class III</td>
<td>2-3 cm</td>
</tr>
<tr>
<td>Class IV</td>
<td>3-4 cm</td>
</tr>
<tr>
<td>Class V</td>
<td>Greater than 4 cm</td>
</tr>
</tbody>
</table>
both the overall and specific class sensitivities for palpation for each observer could be calculated and a comparison of each observer's ability to estimate tumour size made mathematically by using random block analysis performed with the aid of a minitab statistical package and an Amstrad PCW 1640.

Subcellular Biodistribution

(1) Method Quantification

Isotonic Tromethene, 2-Amino-2-hydroxy-methyl-1,3-propanediol (TRIS) Buffer (0.25 m TRIS buffered sucrose) was made up by dissolving 42.79 gms of sucrose (0.25 molar), 3.002 gms of TRIS (0.05 molar), 0.2033 gms MgCl₂ (0.002 molar) and 0.9319 gms of KCL (0.025 molar) in ionised water, (all compounds were Analar grade B.D.H. Chemicals Ltd). The volume was then made up to 500 mls with ionised water and the pH adjusted to 7.4 using hydrochloric acid. A tumour-bearing rabbit was sacrificed and samples of tumour and macroscopically normal liver were removed and washed in TRIS buffer at 4°C, small samples of tissue being retained for histological examination. The remaining samples were then weighed, chopped into a
0.6 mm³ mince and added to TRIS buffer (2:1) at 4°C. Both samples were then homogenised using a motorised teflon-glass Potter-Elvejhem homogeniser (Jencons) rotated at 2,800 rpm. Three homogenisations were used for liver and five for tumour. The homogenates were transferred to Beckerman ultracentrifuge tubes and centrifuged at 155 G (1200 rpm) for 15 minutes (Hettich). The resultant supernatant was decanted, the pellet washed and resuspended in TRIS buffer (1:1) and centrifuged at 155 G (Hettich) for a further 15 minutes. The subsequent supernatant was removed, the two supernatants combined and placed in a Beckerman ultracentrifuge tube while the remaining 'cell membrane' pellet was retained for analysis. It is impossible to obtain a pure cell membrane fraction as the pellet contains not only cell membrane but also endoplasmic reticulum, lysosomal and nuclear cell membrane. However, the resultant "cell membrane" pellet is referred to as cell membrane fraction for the purpose of this work and similar arguments pertain to the mitochondrial, microsomal and cytosolic fractions. The supernatant was then centrifuged at 20,000 G (1500 rpm) for 15 minutes (Becknerman L8-70 ultracentrifuge). The supernatant was removed and the remaining "mitochondrial" pellet washed with TRIS and retained for analysis. The supernatant was then centrifuged at 100,000 G (50,000 rpm) for 60 minutes,
the resulting "microsomal" pellet being washed in TRIS and retained for analysis as was the "cytosol" supernatant fraction. The amount of protein in each subcellular fraction was determined by the method of Lowry et al (1951). Each fraction was assayed for its individual characteristic marker enzyme, i.e. the cell membrane and 5' Nucleotidase (Evans and Gurd, 1973); the mitochondria and Succinate Dehydrogenase (Slater and Planterose, 1960); the microsomes and NADPH-Cytochrome C Reductase (Sottocasa et al, 1967), and the cytosol and Lactate Dehydrogenase (Schwert and Winer, 1963). The concentration of each enzyme in each of the four fractions was calculated (Figures 23 and 24) and expressed as umol/min/mg protein (5' Nucleotidase and Lactate Dehydrogenase); nmol/min/mg protein (NADPH-Cytochrome C reductase) and Absorbence units/min/mg protein (Succinate Dehydrogenase).

(ii) Method

Six tumour-bearing rabbits were studied. Following premedication the radiopharmaceutical Tc $^{99m}$ (v) DMSA (150 MBq) was injected intravenously and the animal allowed to recover. Four hours later, the animal was sacrificed at the optimal imaging time and samples of macroscopically
CELLULAR SUBFRACTIONATION QUANTIFICATION: LIVER

SUCCINATE DEHYDROGENASE

LACTATE DEHYDROGENASE

5' NUCLEOTIDASE

NADPH-CYTOCHROME C REDUCTASE

Figure 23
CELLULAR SUBFRACTIONATION QUANTIFICATION: TUMOUR

Succinate Dehydrogenase

Lactate Dehydrogenase

5’ Nucleotidase

NADPH-Cytochrome C Reductase

<table>
<thead>
<tr>
<th>% total protein</th>
<th>CM</th>
<th>MT</th>
<th>MS</th>
<th>CYT</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>40</td>
<td>1.5</td>
<td>1.0</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>60</td>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>80</td>
<td>0.5</td>
<td>0.2</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>100</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

\[ \text{Absorbance units/min/mg protein} \]

\[ \text{μmol/min/mg protein} \]

CM: Cell membrane
MT: Mitochondria
MS: Microsomes
CYT: Cytosol

Figure 24
normal liver and tumour were removed and washed in TRIS buffer at 4°C, small samples of tissue being retained for histological examination. The tumour and liver samples were homogenised, subfractionated, and the relative amounts of radioactivity determined in each of the cellular fractions by counting each fraction in the automatic gamma sample counter.

**Estimation of Total Bone Mass**

A non-tumour rabbit was premedicated, weighed and sacrificed as part of one of the biodistribution studies. All the organs and main muscle bulk were removed and the carcass then fed to African flesh eating beetles ((Domesticus lardius). The skeleton was weighed dry three weeks later.
3.2. HUMANS

3.2.1. Source of Patients

3.2.2. The Radiopharmaceuticals. Technetium-99m (v)
       Dimercaptosuccinic Acid and Gallium-67 Citrate

3.2.3. Pharmacokinetics

3.2.4. Biodistribution

3.2.5. Imaging

3.2.6. Subcellular Biodistribution
3.2.1. SOURCE OF PATIENTS

All the patients (except one) studied in this Thesis came from the Department of Otolaryngology and Head and Neck Oncology at Guy's Hospital. Patients with head and neck cancer had a full history and examination and a head and neck data sheet completed. All patients were staged (either retrospectively or prospectively) from 1987 using current UICC staging criteria (UICC, 1987) and palpable cervical lymph nodes ascribed a level according to Table 11. Ethical Committee approval was obtained to use the radiopharmaceutical Tc\(^{99m}\) (v) DMSA.

Between 1st January, 1986, and 1st March, 1989, 81 patients were studied prospectively (54 male, 27 female; age range 19-82 years, mean 58; Figure 25). Seventy one patients had a history of malignant tumour (squamous carcinoma (66), embryonal rhabdomyosarcoma (2), lymphoma (1), adenocarcinoma (1) and metastatic breast adenocarcinoma (1); Group A, Figure 25) and ten patients (Group B, Figure 25) had benign lesions. Of the 66 patients with squamous carcinoma, 62 had a history of a head and neck squamous carcinoma and all were clinically examined and imaged with Tc\(^{99m}\) (v) DMSA. The remaining four were included in the
FIGURE 25

PATIENTS STUDIED IN THIS THESIS. JANUARY 1st 1986 - MARCH 1st 1989

81 patients

10 benign (B)

4 Squamous cell carcinoma (see Biodistribution and Subcellular Biodistribution studies. Sections 4.2.2. and 4.2.5.)

71 malignant

(A)

67

Others (head and neck) 5

Embryonal rhabdomyosarcoma (2)
Adenocarcinoma (1)
Lymphoma (1)
Metastatic breast adenocarcinoma (1)

Squamous carcinoma 62 (D)

Head and Neck 58 (Past or present)

Others 4 (E)
Carcinoma of the lung (1)
Occult primaries (2)
Middle third of the oesophagus

Positive 55

Second lung primaries (2)
Distant metastases (1)

Negative 3 (F) (successfully treated with radiotherapy)

Head and Neck 52 (G)

All patients (except Group (C)) were examined and then imaged with Tc$^{99m}$ (v) DMSA planar imaging
biodistribution and subcellular biodistribution studies only (sections 4.2.2. and 4.2.5. Group C, Figure 25). Of the 62 with squamous carcinoma who were imaged (Group D, Figure 25) 12 had received previous radiotherapy, eight had received previous surgery (to include tracheostomy (3)) and five patients had received previous surgery and radiotherapy. Four of the 62 patients (Group E, Figure 25) had overt disease limited to below the clavicles (occult primary (2), lung (1) and middle third of the oesophagus (1)). Of the remaining 58 patients with head and neck carcinoma three had been successfully treated with radiotherapy (Group F, Figure 25) and 55 had active disease. Within this group of 55, 52 had head and neck squamous carcinoma (Group G, Figure 25) and of these, two patients had occult primaries (presumed head and neck sites) with cervical metastases. Of the remaining three patients (Group H, Figure 25) two patients, who had been successfully treated for head and neck squamous cell carcinoma, developed second lung primaries while a third developed distant metastases in the pancreas, liver and sigmoid colon.

Of the 66 patients with squamous cell carcinoma, there were 16 well differentiated (G1), 29 moderately well differentiated (G2), seven poorly differentiated (G3), and one undifferentiated (G4) tumours. Thirteen tumours were not assessed with regard to the degree of differentiation.
Fifty two patients (Group G, Figure 25) had a primary, occult primary or locally recurrent head and neck squamous carcinoma and, of these, TNM staging was possible in 44 primary and two second primaries (overt \(T_2\), occult \(T_2\)). Of the 52 patients, there were 13 with oral cavity tumours, six with oropharyngeal tumours, four with nasopharyngeal tumours, seven with hypopharyngeal tumours, 14 with laryngeal tumours and two occult tumours (presumed head and neck). Two patients had carcinoma of the ear and one had a recurrent posterior pharyngeal wall tumour. Three patients had been treated successfully with radiotherapy to the primary site and subsequently presented with cervical metastases. Within this group of 52 patients there were 54 primary malignant lesions.

In the 67 patients with malignant disease examined and imaged using Tc\(^{99m}\) (v) DMSA scintigraphy, "T" staging was possible in 53 tumours. There were two \(T_1\), 13 \(T_2\), 16 \(T_3\) and 22 \(T_4\) tumours. In these 67 patients with malignancy, five patients also had benign lesions. There were three patients with overt periodontal disease, one patient with an occult primary and proven fibrous dysplasia of the maxilla and an inflamed biopsy site in the right axilla, while another had an occult primary and a skull metastasis with an inflamed biopsy site in the left flank.
All the 67 patients with malignancy who were imaged had a full examination to include the head and neck. Of the 53 patients with head and neck carcinoma, 24 presented with a primary tumour alone while 29 had palpable cervical lymphadenopathy (Table 14). Within this group of 29 patients, 20 had a primary tumour with palpable ipsilateral metastases, four had tumours with bilateral palpable metastases and five had palpable cervical metastases alone. There were thus 33 clinically positive necks and 101 clinically negative necks. Of those with clinically negative necks, two patients had palpable cervical lymphadenopathy which was thought to be non-malignant. Of the 67 patients with malignancy who were examined and imaged with Tc $^{99m}$ (v) DMSA, 17 had surgery alone, 13 patients had radiotherapy alone and 17 patients had combined treatment. One patient had chemotherapy followed by radiotherapy and 19 patients had no treatment. Twenty nine patients had 36 neck dissections.

A total of 77 patients had a clinical examination and planar Tc $^{99m}$ (v) DMSA scintigraphy. Thirty two patients were also imaged at four hours and of these seven were imaged at six hours. Thirty four patients had head and neck SPECT, 54 patients had CAT scans, 17 had Gallium-67 Citrate scans, six had Tc $^{99m}$-MDP bone scans and one patient had
### TABLE 14

THE STAGING AT PRESENTATION OF TWENTY NINE PATIENTS WITH HEAD AND NECK CARCINOMA WHO HAD THIRTY THREE CLINICALLY POSITIVE LATERAL NECK COMPARTMENT NECKS

<table>
<thead>
<tr>
<th>Description</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary tumour with palpable ipsilateral nodes</td>
<td>12 $N_1$; 2 $N_{2a}$; 2 $N_{2b}$; 4 $N_3$</td>
</tr>
<tr>
<td>Primary tumour with palpable bilateral nodes</td>
<td>4 $N_{2c}$ i.e. 4 $N_1$; 4 $N_{2b}$</td>
</tr>
<tr>
<td>Palpable cervical nodes alone</td>
<td>1 $N_1$; 1 $N_{2a}$; 2 $N_{2b}$; 1 $N_3$</td>
</tr>
</tbody>
</table>

**TOTAL** (33 lateral neck compartments)  
17 $N_1$; 3 $N_{2a}$; 8 $N_{2b}$; 5 $N_3$
an in-vitro labelled red blood cell scan. Two patients with squamous carcinoma had both CAT and MRI, and one of these also had neck ultrasound.

Ten of the 77 patients had benign head and neck lesions. All of these were examined and had Tc\(^{99m}\) (v) DMSA scans performed at the optimal imaging time and, of these, three also had SPECT and CAT evaluation. Two patients with glomus tumours had planar and SPECT Tc\(^{99m}\) (v) DMSA, planar and SPECT I\(^{123}\)-MIBG and CAT scans, and one also had an MRI scan and planar I\(^{131}\)-MIBG. Three of the ten patients were treated by surgery and two were treated with antibiotics. The patient with the Stage IV glomus jugulare was treated with I\(^{131}\)-MIBG (10,000 MBq) and followed up with I\(^{123}\)-MIBG SPECT and MRI and was subsequently treated with external beam radiotherapy. Four patients had no treatment.

All of the 77 patients examined and imaged initially with Tc\(^{99m}\) (v) DMSA were followed up by routine head and neck clinical evaluation (range 1-40 months, mean 14). Tc\(^{99m}\) (v) DMSA planar follow-up scintigraphy was also carried out in 36 patients. Of these 36 patients, two had three follow-up CAT scans, three had follow-up MRI scans, two had follow-up Gallium-67 scans and one had a Tc\(^{99m}\)-MDP follow-up scan. Another patient not followed up with Tc\(^{99m}\) (v) DMSA scintigraphy had a follow-up CAT scan.
On 1st March, 1989, of the 81 patients studied 33 patients had died. Forty two patients were alive and presumed disease free, while six had overt residual or recurrent disease.
3.2.2. **THE RADIOPHARMACEUTICAL**

Technetium-99m (v) Dimercaptosuccinic Acid was prepared and its stability checked using the methods described in Section 3.1.4. Gallium-67 Citrate was obtained from Amersham International or Dupont (UK).
3.2.3. PHARMACOKINETIC STUDIES

Ten patients were studied (five with no tumour; five with tumours). Non-tumour patients included those who had had a head and neck squamous cell carcinoma treated and were being followed up with Tc $^{99m}$ (v) DMSA scans and who were free of disease at the time of the investigation (and who had remained disease-free for at least 12 months). A heparinised 20 FG venous cannula and line (Jelco) was inserted into a peripheral arm vein to facilitate acquisition of sequential venous blood samples. Prior to the administration of Tc $^{99m}$ (v) DMSA, background blood and urine samples were obtained. Tc $^{99m}$ (v) DMSA (370 MBq) was injected intravenously into the opposite arm, the syringe being weighed and the activity counted before and after injection. Using a 2 ml syringe, 1 ml blood samples were obtained at 5, 15, 30, 45 and 60 minutes and then at 1½, 2, 3, 4, 6, and 24 hours post-injection. All urine was collected into numbered vessels over a similar period and the time of each collection noted. Patients were allowed to eat and drink normally. The radioactivity in each blood sample was measured and the percentage of the injected dose per gram calculated and the first and second phase t½'s obtained for each patient and analysed.
statistically in an identical manner to the method
described in Section 3.1.5. Urine samples were weighed
and the radioactivity in a 1 ml aliquot counted along with
dilutions of the standard of the injection (1:10-1:10⁵)
and the percentage of the injected dose in the urine
calculated using the formula below:-

\[
\% \text{ injected dose} = \frac{\text{sample counts} \times \text{volume of standard (ml)} \times 100\%}{\text{wt of injection (g)} \times \text{dilution of standard} \times \text{counts from standard}}
\]

The whole body retention values (%) for each time interval
were then calculated using the formula "100 - \(\sum\% \text{ injected}
dose excreted in the urine. The urine and whole body
retention values were then plotted against time for non-
tumour and tumour patients. For the urine results, the
cumulative urine excretion \(t_{50\%}\)'s were estimated graphically
for each patient and a mean value obtained. The whole
body retention half-times for each patient were calculated
using a least-squares fit (Amstrad PCW 1640) to the points
on the graph. A mean whole body retention half-time value
was then obtained. The cumulative urine and whole body
retention results were compared statistically using the
methods described in Section 3.1.5.
3.2.4. **BIODISTRIBUTION**

Six patients having a surgical resection of a primary head and neck squamous carcinoma were injected intravenously with Tc $^{99m}$ (v) DMSA (150 MBq) at the time of premedication. The syringe was weighed and the activity measured before and after injection. Following removal of the surgical specimen, samples were obtained of tumour, normal muscle and venous blood. These were placed into pre-weighed test tubes, the radioactivity counted and the percentage of the injected dose per gram calculated using the method described in Section 3.1.5. Samples of normal tissue and tumour were sent for histological examination. During the biodistribution studies, one assistant wore a thermoluminescent dose monitor on his finger to measure the absorbed radiation dose to the fingers.
3.2.5. IMAGING

Equipment

A Scintronix Digicamera 404/NCT with a rotating SPECT facility was used (Figure 26) interfaced to a Data General Nova 4X computer. A high resolution/general all purpose collimator (Scintronix) was used with Tc\textsuperscript{99m} (v) DMSA and a medium energy collimator (Scintronix) with Gallium-67 Citrate. The CAT scans were performed with intravenous contrast (Iopamidol 300) using a Phillips Tomoscan 350 third generation scanner and images of the neck obtained using 6 mm contiguous sections.

Technique

Following radiopharmaceutical injection (Tc\textsuperscript{99m} (v) DMSA; 370 MBq; Ga\textsuperscript{67}-Citrate; 1.8 MBq/kg) planar anterior left and right lateral head and neck views were obtained in all patients and nine patients also had planar images of the abdomen and/or thorax (whole body). For SPECT, elliptical orbits were performed (where possible) and the reconstructions performed using a Parzen filter 1.5. Tc\textsuperscript{99m} (v) DMSA images were reported blind by Dr. Susan Clarke as being
A patient being imaged on the Scintronix Digicamera 404/NCT. Note the rotating SPECT facility.
either strongly positive, positive, or negative and each side of the neck (lateral neck compartment) was reported separately.

A lymph node was considered positive by CAT criteria if it was greater than 1 cm in size (except for low level II and high level III nodes when nodes greater than 1.5 cm were considered positive; Section 1.4.3.). In addition any node exhibiting central necrosis and/or a thin rim of peripheral enhancement was considered malignant. Once a lymph node was considered positive on CAT, it was ascribed a level (Section 1.4.3.).

Optimal Imaging Time

(1) **Qualitative Assessment**

Six patients with proven head and neck squamous carcinomas were imaged at two, four and six hours post-injection. The sensitivity and specificity for tumour scintigraphic detection could then be determined for each consecutive imaging time.
(2) **Quantitative Assessment**

Tumour:background (soft tissue) ratios at each consecutive imaging time were calculated for the six patients using the method described in Section 3.1.5.

(3) **Primary Imaging**

Prior to treatment, 77 patients were imaged at the optimal imaging time. Sixteen patients were imaged immediately following surgery and 35 were followed up with Tc $^{99m}$ (v) DMSA scintigraphy following treatment.
3.2.6. **SUBCELLULAR BIODISTRIBUTION**

Four patients with head and neck squamous carcinomas were studied and samples of tumour were obtained in an identical manner to the biodistribution studies (Section 3.2.4.). The tumour was washed in TRIS buffer at 4°C, a small sample being retained for histological examination. The remaining tumour was weighed, chopped and added to TRIS buffer (2:1) at 4°C. The sample was homogenised and then analysed using the method described in Section 3.1.5.
3.3. STATISTICS
3.3. **STATISTICS**

**Mean**
\[ \text{Mean} = \bar{x} = \frac{1}{n} \sum_{i=1}^{n} x_i \]

**Standard deviation**
\[ \text{Standard deviation} = (SD) = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}} \]

**Standard error of the mean**
\[ \text{Standard error of the mean} = (SEM) = \frac{SD}{\sqrt{n}} \]

**Student t-test**
\[ t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\left(\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}\right)}} \]

\[ + SD^2 = \text{Variance for the two samples combined} \]

If \( n_1 = n_2 \) then \[ SD^2 = \left(\frac{SD_1}{2}\right)^2 + \left(\frac{SD_2}{2}\right)^2 \]

In practice, \( X, SD, SEM \) and \( t \) were calculated using a Casio College fx-100 scientific calculator.

* Swinscow, 1983
Linear Regression

\[ y = a_0 + a_1 x \]

The 'method of least squares' was used to find the best estimate (\( \hat{\cdot} \)) of \( a_0 \) and \( a_1 \),

\[
\hat{a}_1 = \frac{\sum_{i=1}^{n} x_i y_i - n \hat{x} \hat{y}}{\sum_{i=1}^{n} x_i^2 - n \hat{x}^2}
\]

and \( \hat{a}_0 = \bar{y} - \hat{a}_1 \bar{x} \)

(Ryan et al, 1985).

The linear regression analysis was performed on an Amstrad PCW 1640 using a spread sheet facility (supercalc 4).

Multiple Regression Analysis

The analysis of the linear regression model can be extended in a straightforward way to cover situations in which the dependent variable is affected by several controlled variables, or in which it is affected non-linearly by one controlled variable. All multiple regression analysis was done using the minitab statistics package (Ryan et al, 1985) and an Amstrad PCW 1640.
Random Block Analysis

Randomisation is a simple but effective way of eliminating systematic error in an experiment but one particular factor can contribute substantially to the uncontrolled variation. Therefore, there is a danger of introducing systematic error unless randomisation takes this into account. To avoid this occurring, tests can be divided into groups. These groups are called blocks, and if the order of tests within each block is randomised, then the experiment is called a randomised block experiment. In this study,

Total variation in estimated rabbit tumour size = Variation due to observation + variation due to absolute tumour size + variation due to random error.

The random block analysis was carried out using the minitab statistics package (Ryan et al, 1985) and an Amstrad PCW 1640.

Sensitivity = True positives/True positives + false negatives
Specificity = True negatives/True negatives + false positives
Positive predictive accuracy = True positives/True positives + false positives
Negative predictive accuracy = True negatives/True negatives + false negatives
Accuracy = True positives + true negatives/True positives + true negatives + false positives + false negatives.
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</thead>
<tbody>
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<tr>
<td>4.2.</td>
<td>Humans</td>
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</tbody>
</table>

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CHAPTER 4

4 RESULTS

4.1. Animals

4.1.1. Pharmacokinetics

4.1.2. Biodistribution

4.1.3. Optimal Imaging Time

4.1.4. Optimal Imaging Characteristics and Palpation

4.1.5. Subcellular Biodistribution

4.1.6. Estimation of Bone Mass

4.1.7. Body and Thymus Weights

4.1.8. Regional Lymph Node and Distant Metastases

4.1.9. Radiopharmaceutical Stability
Fourteen rabbits were studied (five non-tumour, nine tumour). The blood levels following intravenous injection of Tc $^{99m}$ (v) DMSA in non-tumour and tumour bearing rabbits are given in Tables 1A and 2A. The blood clearance of Tc $^{99m}$ (v) DMSA in non-tumour rabbits was bi-exponential (Figure 27). The first and second phase $t_1$'s for each rabbit were calculated (range 23-34 and 306-347 mins, Table 15), and from these mean $t_1$'s obtained (28 and 325 mins). The blood clearance of Tc $^{99m}$ (v) DMSA in tumour-bearing rabbits was bi-exponential (Figure 27). All tumours were confirmed histologically. The first and second phase $t_1$'s for each rabbit were calculated (range 7-36 and 234-723 mins, Table 15), and from these mean $t_1$'s obtained (27 and 352 mins). Using a student t-test there was no significant difference ($p > 0.05$) between the mean $t_1$'s for non-tumour and tumour-bearing rabbits and clearance appeared unaffected by tumour mass. In an attempt to ascertain if clearance was affected by tumour mass, the tumour-bearing rabbit group was subdivided further into small tumour rabbits ($< 5g/kg$ body weight) and large tumour rabbits ($> 5g/kg$ body weight). The blood clearance of Tc $^{99m}$ (v) DMSA in small tumour-bearing rabbits was bi-exponential (Figure 28). The first and second phase $t_1$'s for each rabbit were calculated (range 26-34 and 272-411 mins, Table 16), and mean $t_1$'s obtained (29 and 311 mins).
FIGURE 27

THE BLOOD CLEARANCE OF Tc-99m-V-DMSA
IN NZW RABBITS (NON-TUMOUR)

$T_1/2 = 28$ and 325 mins
Data from 5 rabbits

THE BLOOD CLEARANCE OF Tc-99m-V-DMSA
IN NZW RABBITS (TUMOUR)

Tumour weight range 1.78g-98.8g
$T_1/2 = 27$ and 352 mins
Data from 9 rabbits
TABLE 15
BI-EXPONENTIAL HALF-TIME BLOOD CLEARANCE VALUES OF $^{99m}$Tc (v) DMSA IN NON-TUMOUR BEARING RABBITS

<table>
<thead>
<tr>
<th></th>
<th>FIRST PHASE</th>
<th>R</th>
<th>SECOND PHASE</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RANGE</strong></td>
<td>23-34</td>
<td>28</td>
<td>306-347</td>
<td>325</td>
</tr>
<tr>
<td><strong>MEAN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>STANDARD ERROR OF THE MEAN</strong></td>
<td>4</td>
<td></td>
<td>49</td>
<td></td>
</tr>
</tbody>
</table>

* CLEARANCE VALUES (MINS)

+ NON-TUMOUR

$R = \text{CORRELATION COEFFICIENT}$

* $\pm 2$ STANDARD DEVIATIONS

+ DATA FROM FIVE RABBITS
TABLE 15 (CONT)

BI-EXPONENTIAL HALF-TIME BLOOD CLEARANCE VALUES OF Tc $^{99m}$ (v) DMSA IN TUMOUR-BEARING RABBITS

<table>
<thead>
<tr>
<th>TUMOUR</th>
<th>FIRST PHASE</th>
<th>R</th>
<th>SECOND PHASE</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 ± 1</td>
<td>0.99</td>
<td>346 ± 21</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>23 ± 5</td>
<td>0.98</td>
<td>232 ± 54</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>26 ± 3</td>
<td>0.99</td>
<td>287 ± 74</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>27 ± 5</td>
<td>0.99</td>
<td>272 ± 66</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>29 ± 5</td>
<td>0.99</td>
<td>411 ± 179</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>30 ± 8</td>
<td>0.97</td>
<td>288 ± 31</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>31 ± 8</td>
<td>0.97</td>
<td>314 ± 38</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>34 ± 9</td>
<td>0.97</td>
<td>295 ± 68</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>36 ± 7</td>
<td>0.98</td>
<td>723 ± 422</td>
<td>0.86</td>
<td></td>
</tr>
</tbody>
</table>

RANGE 7-36 232-723
MEAN 27 352
STANDARD ERROR OF THE MEAN 3 80

* ± 2 STANDARD DEVIATIONS
# DATA FROM NINE RABBITS
R = CORRELATION COEFFICIENT
THE BLOOD CLEARANCE OF Tc-99m-V-DMSA IN NZW RABBITS (SMALL-TUMOURS)

Mean tumour weight=5.5g (range 1.7g-9.8g)
T1/2=29 and 311 mins
Data from 5 rabbits

THE BLOOD CLEARANCE OF Tc-99m-V-DMSA IN NZW RABBITS (LARGE-TUMOURS)

Mean tumour weight=60.0g (range 24.0-98.8)
T1/2=24 and 397 mins
Data from 4 rabbits
The blood clearance of Tc $^{99m}$ (v) DMSA in large tumour-bearing rabbits was bi-exponential (Figure 28). The first and second phase $t_1$'s for each rabbit were calculated (range 7-36 and 232-723 mins, Table 16), and mean $t_1$'s obtained (24 and 397 mins). Using a student t-test there was no significant difference in the mean $t_1$ clearance times between the non-tumour and small tumour groups ($p > 0.05$), between the non-tumour and large tumour groups ($p > 0.05$), and between the small and large tumour groups ($p > 0.05$). Clearance was thus unaffected by tumour mass.

The urine levels of radioactivity following intravenous injection of Tc $^{99m}$ (v) DMSA in non-tumour and tumour-bearing rabbits are shown in Tables 3A and 4A. Cumulative urine excretion was bi-exponential in non-tumour and tumour rabbits and in small-tumour-bearing and large-tumour-bearing rabbits (Figures 29 and 30). There was no apparent difference in the mean cumulative urine excretion $t_{50\%}$'s of the four groups and their mean cumulative urine excretion $t_{50\%}$ values are shown in Table 17. It was impossible to collect all the urine voided so the results for the urine levels of radioactivity are expressed as the percentage of the injected dose per gram of urine and not percentage of the injected dose. Therefore the mean cumulative excretion $t_{50\%}$ values for the four groups have not been subjected to statistical analysis.
<table>
<thead>
<tr>
<th>TUMOUR WT/KG</th>
<th>TUMOUR WT (g)</th>
<th>TUMOUR/BODY WT %</th>
<th>T2 (mins)</th>
<th>T2 (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1.7</td>
<td>1.4</td>
<td>1.7</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>2.6 ± 3 and 287 ± 74</td>
<td>29 ± 5 and 411 ± 179</td>
<td>27 ± 5 and 272 ± 66</td>
<td>34 ± 9 and 295 ± 68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.6</td>
<td>9.8</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>0.16 ± 0.148</td>
<td>0.18 ± 0.148</td>
<td>0.24</td>
<td></td>
</tr>
</tbody>
</table>

*MEAN + 2 STANDARD DEVIATIONS

+ STANDARD ERROR OF THE MEAN
### TABLE 16 (CONT)

**TUMOUR WEIGHTS AND BI-EXPONENTIAL HALF-TIME BLOOD CLEARANCE VALUES OF Tc-99m (v) DMSA IN FOUR LARGE-TUMOUR-BEARING RABBITS**

<table>
<thead>
<tr>
<th>TUMOUR WT/KG</th>
<th>TUMOUR WT (g)</th>
<th>% TUMOUR/BODY WT</th>
<th>$T_b$ (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.7</td>
<td>24.0</td>
<td>0.57</td>
<td>36 ± 7 and 723 ± 422</td>
</tr>
<tr>
<td>19.0</td>
<td>39.8</td>
<td>1.89</td>
<td>30 ± 8 and 288 ± 31</td>
</tr>
<tr>
<td>22.2</td>
<td>77.3</td>
<td>2.22</td>
<td>23 ± 5 and 232 ± 54</td>
</tr>
<tr>
<td>*35.3</td>
<td>98.8</td>
<td>3.52</td>
<td>7 ± 1 and 346 ± 21</td>
</tr>
</tbody>
</table>

**+MEAN**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>20.6 ± 24.3</td>
<td>60.0 ± 68.4</td>
<td>2.1 ± 2.4</td>
<td>24$^#$ (6) and 397$^#$ (107)</td>
</tr>
</tbody>
</table>

* METASTASES (LIVER, LUNG AND AXILLARY LYMPH NODE)
+ ± 2 STANDARD DEVIATIONS

$^\#$ STANDARD ERROR OF THE MEAN
FIGURE 29

THE CUMULATIVE URINE EXCRETION OF Tc-99m-V-DMSA IN NZW RABBITS (NON-TUMOUR)

% Injected Dose per Gram

Mean Cumulative T<sub>50%</sub>=200 mins

Data from 5 rabbits

TIME (MINS AFTER INJECTION)

THE CUMULATIVE URINE EXCRETION OF Tc-99m-V-DMSA IN NZW RABBITS (TUMOUR)

% Injected Dose per Gram

Mean Cumulative T<sub>50%</sub>=240 mins

Data from 9 rabbits

TIME (MINS AFTER INJECTION)
FIGURE 30

THE CUMULATIVE URINE EXCRETION OF Tc-99m-V-DMSA IN NZW RABBITS (SMALL-TUMOURS)

Mean Cumulative $T_{50\%}=250$ mins
Data from 5 rabbits

THE CUMULATIVE URINE EXCRETION OF Tc-99m-V-DMSA IN NZW RABBITS (LARGE-TUMOURS)

Mean Cumulative $T_{50\%}=180$ mins
Data from 4 rabbits
### TABLE 17

**The Mean Cumulative Urine Excretion Half-Time ($T_{50\%}$)**

Values for $^{99m}$Tc (v) DMSA in the Non-Tumour, Tumour Small-Tumour and Large-Tumour Rabbit Groups.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>CUMULATIVE URINE EXCRETION HALF-TIMES ($T_{50%}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>* NON-TUMOUR</td>
<td>200 mins</td>
</tr>
<tr>
<td>+ TUMOUR</td>
<td>240 mins</td>
</tr>
<tr>
<td># SMALL-TUMOUR</td>
<td>250 mins</td>
</tr>
<tr>
<td>° LARGE-TUMOUR</td>
<td>180 mins</td>
</tr>
</tbody>
</table>

* Data from five rabbits
+ Data from nine rabbits
# Data from five rabbits
° Data from four rabbits
4.1.2. **BIODISTRIBUTION**

Forty rabbits were studied (20 non-tumour, 20 tumour). The percentage of the injected dose per gram of tissue for all the organs in non-tumour and tumour-bearing rabbits is shown in Tables 5A-12A. All tumour and inflammatory tissue samples were confirmed histologically (Figure 31). Using a student t-test there was no significant difference ($p > 0.05$) in the organ biodistribution between the two groups. The major route of excretion for Tc $^{99m}$DMSA in non-tumour and tumour-bearing rabbits was principally via the urine with some Tc $^{99m}$DMSA being excreted in the bile.

**Non-Tumour Group**

The major sequential organ biodistribution in non-tumour rabbits is shown in Table 18 with significant radioactivity over the 24 hour period observed in bone and the kidneys.

All subsequent values for $p$ in this section have been derived using a student t-test. At two hours, there was significant uptake (i.e. greater than the blood pool) in bone (0.076%/g, $p < 0.01$), kidney (0.073%/g, $p < 0.001$) and the bladder (0.064%/g, $p < 0.025$), and there was no significant difference in uptake between these three organs ($p > 0.05$). The bladder uptake was a maximum at this time.
A print from a photomicrograph showing invasive poorly differentiated Vx2 squamous cell carcinoma (A) with surrounding inflammatory tissue (B)
# TABLE 18
THE MAJOR ORGAN BIODISTRIBUTION OF Tc $^{99m}$ (v) DMSA IN 20 NON-TUMOUR BEARING NZW RABBITS

(8 INJECTED DOSE PER GRAM OF TISSUE)

<table>
<thead>
<tr>
<th></th>
<th>*2 HOURS</th>
<th></th>
<th>*4 HOURS</th>
<th></th>
<th>*6 HOURS</th>
<th></th>
<th>*24 HOURS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORGAN</td>
<td>RADIOACTIVITY</td>
<td>ORGAN</td>
<td>RADIOACTIVITY</td>
<td>ORGAN</td>
<td>RADIOACTIVITY</td>
<td>ORGAN</td>
<td>RADIOACTIVITY</td>
</tr>
<tr>
<td>BONE</td>
<td>7.60 E-2</td>
<td>BONE</td>
<td>8.86 E-2</td>
<td>BONE</td>
<td>7.76 E-2</td>
<td>BONE</td>
<td>6.15 E-2</td>
</tr>
<tr>
<td>KIDNEY</td>
<td>7.33 E-2</td>
<td>KIDNEY</td>
<td>6.61 E-2</td>
<td>KIDNEY</td>
<td>7.70 E-2</td>
<td>KIDNEY</td>
<td>4.22 E-2</td>
</tr>
<tr>
<td>BLOOD</td>
<td>2.03 E-2</td>
<td>LIVER</td>
<td>1.16 E-2</td>
<td>LIVER</td>
<td>9.98 E-3</td>
<td>BLADDER</td>
<td>8.73 E-3</td>
</tr>
<tr>
<td>LUNG</td>
<td>1.45 E-2</td>
<td>BLOOD</td>
<td>1.04 E-2</td>
<td>BLOOD</td>
<td>8.96 E-3</td>
<td>CERVICAL LYMPH NODE</td>
<td>6.69 E-3</td>
</tr>
<tr>
<td>CERVICAL LYMPH NODE</td>
<td>1.27 E-2</td>
<td>NASAL MUCOSA</td>
<td>1.02 E-2</td>
<td>LUNG</td>
<td>7.01 E-3</td>
<td>LIVER</td>
<td>5.50 E-3</td>
</tr>
<tr>
<td>NASAL MUCOSA</td>
<td>1.22 E-2</td>
<td>CERVICAL LYMPH NODE</td>
<td>9.73 E-3</td>
<td>NASAL MUCOSA</td>
<td>6.43 E-3</td>
<td>SMALL BOWEL</td>
<td>4.81 E-3</td>
</tr>
<tr>
<td>PITUITARY</td>
<td>1.07 E-2</td>
<td>LUNG</td>
<td>7.32 E-3</td>
<td>GALL BLADDER</td>
<td>6.42 E-3</td>
<td>PITUITARY</td>
<td>4.51 E-3</td>
</tr>
</tbody>
</table>

* MEAN DATA FROM FIVE RABBITS
and the blood pool level was 0.020 %/g. There was uptake in lung (0.015 %/g), cervical lymph nodes (0.013 %/g), nasal mucosa (0.012 %/g) and the pituitary (0.011 %/g), but these were not significantly different from each other (p > 0.05). The uptake in lung was not significantly less than the blood pool (p > 0.05), but the uptake in the cervical lymph nodes, nasal mucosa and pituitary was less than that observed in blood (p < 0.025).

At four hours, there was significant uptake (i.e. greater than the blood pool, p < 0.001) in bone (0.089 %/g) and the kidneys (0.066 %/g). There was uptake in the bladder (0.046 %/g) but this was not significantly greater than the blood pool (p > 0.025) and there was no significant difference in uptake between these three organs (p > 0.025). Bone uptake at this time was a maximum, but not significantly greater than bone uptake at two hours (0.076 %/g, p > 0.05). Uptake in the kidney (0.066 %/g, p > 0.05) and the bladder (0.046 %/g, p > 0.05) remained relatively constant to four hours. The blood pool level at four hours was 0.010 %/g and this had dropped significantly from that at two hours (0.020 %/g, p < 0.025). There was uptake in the liver (0.012 %/g), nasal mucosa and cervical lymph nodes (0.010 %/g) and lung (0.007 %/g), but these were not significantly different either from each other (except for the liver and lung, (p < 0.005), or from the blood pool radioactivity (p > 0.05).
At six hours, significant uptake (i.e. greater than the blood pool, \( p < 0.005 \)) was observed in bone and kidney but not the bladder. There was no significant difference between the uptake in the bone and the bladder (\( p > 0.05 \)) but there was a significant difference in uptake between the kidney and the bladder (\( p < 0.01 \)). The uptake in bone (0.078 %/g) was less than bone uptake at four hours (0.089 %/g), but the fall was not significant (\( p > 0.05 \)). Kidney radioactivity was a maximum at this time (0.077 %/g) but the increase was not significant when compared with the four hour level (0.066 %/g, \( p > 0.05 \)). Uptake in the bladder had remained constant to six hours (0.032 %/g, \( p > 0.05 \)). The blood pool level at six hours was 0.009 %/g and this was not significantly different from the four hour level (0.010 %/g, \( p > 0.05 \)). There was uptake at six hours in liver (0.010 %/g), lung (0.007 %/g), nasal mucosa and gall bladder (0.006 %/g), but none of these were significantly different either from each other or from the blood pool (\( p > 0.05 \)).

At 24 hours significant radioactivity (i.e. greater than the blood pool, \( p < 0.001 \)) remained in the bone and the kidneys and there was no significant difference in uptake between the two organs (\( p > 0.05 \)). The radioactivity in bone (0.062 %/g) had remained relatively constant to 24 hours but the radioactivity in the kidneys (0.042 %/g) at
24 hours was significantly less than that at two, four and six hours (p < 0.005). However, bladder radioactivity (0.009 %/g) was significantly less than the 24 hour uptake in the bone and kidneys (p < 0.005). There was uptake in the gall bladder (0.01 %/g), cervical lymph nodes (0.007 %/g), liver (0.006 %/g), and the pituitary (0.005 %/g). All of these (except the gall bladder) were significantly greater than the blood pool (0.001 %/g, p < 0.01).

Radioactivity per gram and total radioactivity (% injected dose) in the bone always exceeded that in the kidney which, in turn, was always greater than that in the bladder. The total percentage of the injected dose of Tc $^{99m}$ (v) DMSA in these three organs (bone, kidney and bladder) is shown in Table 19. There was uptake in the lacrimal glands, the thymus and the marrow but radioactivity never exceeded 0.01 %/g. Uptake in the lacrimals and thymus was significantly less than the blood pool at two, four and six hours (p < 0.025) but not at 24 hours (p > 0.05). The uptake in the marrow was significantly less than the blood pool at two and four hours (p < 0.025) but not at six and 24 hours (p > 0.05).

Radioactivity in the bile was a maximum at two hours (0.0079 %/g). By four hours, uptake had dropped significantly (0.0046 %/g, p < 0.025), and then continued to decrease to 0.0037 %/g at six hours and to 0.0019 %/g at 24 hours.
TABLE 19

TOTAL PERCENTAGE (+2 STANDARD DEVIATIONS) OF THE INJECTED DOSE OF Tc\(^{99m}\) (V) DMSA IN THE BONE, KIDNEY AND BLADDER

(DATA FROM 40 RABBITS; 20 TUMOUR, 20 NON-TUMOUR)

<table>
<thead>
<tr>
<th>ORGAN</th>
<th>TIME (HOURS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>BONE</td>
<td>24.5 ± 16.4</td>
</tr>
<tr>
<td>KIDNEY</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td>BLADDER</td>
<td>0.16 ± 0.18</td>
</tr>
</tbody>
</table>

* Total overall percentage of injected dose in bone in 20 non-tumour rabbits was 28%. The rabbit bone mass was 137 g/kg body weight (Section 4.1.6.).

+ Total overall percentage of injected dose in kidney was 0.92% and the mean kidney mass was 5.2 g/kg body weight (data from 20 non-tumour rabbits).
Tumour-Bearing Group

The major organ biodistribution in tumour-bearing rabbits is shown in Table 20 with significant radioactivity over the 24 hour period observed in the kidneys and bone.

At two hours there was significant uptake (i.e. greater than the blood pool, \( p < 0.01 \)) in the kidney (0.090 %/g), bladder (0.077 %/g) and bone (0.045 %/g). There was no significant difference in uptake between kidney and bladder, and bladder and bone (\( p > 0.005 \)), but kidney uptake was significantly greater than that in bone (\( p < 0.005 \)). The bladder uptake was a maximum at this time and the blood pool level was 0.041 %/g. There was maximum uptake in whole tumour (0.026 %/g) and in inflammatory tissue (0.023 %/g) at two hours. Uptake in lung (0.022 %/g) and the nasal mucosa (0.020 %/g) was also seen although they were not significantly different from each other (\( p > 0.05 \)) but were significantly less than the blood pool (\( p < 0.025 \)).

At four hours there was significant uptake (i.e. greater than the blood pool, \( p < 0.01 \)) in the kidney (0.091 %/g) and the bone (0.072 %/g), but not in the bladder (0.047 %/g, \( p > 0.05 \)). Uptake was at a maximum in kidney and bone but there was no significant difference from the two hour levels (\( p > 0.05 \)). There was no significant difference in four hour uptake between kidney, bone and bladder (\( p > 0.05 \)),
<table>
<thead>
<tr>
<th>ORGAN</th>
<th>RADIOACTIVITY</th>
<th>ORGAN</th>
<th>RADIOACTIVITY</th>
<th>ORGAN</th>
<th>RADIOACTIVITY</th>
<th>ORGAN</th>
<th>RADIOACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIDNEY</td>
<td>8.99 E-2</td>
<td>KIDNEY</td>
<td>9.08 E-2</td>
<td>BLADDER</td>
<td>5.86 E-2</td>
<td>KIDNEY</td>
<td>8.51 E-2</td>
</tr>
<tr>
<td>BLADDER</td>
<td>7.74 E-2</td>
<td>BONE</td>
<td>7.16 E-2</td>
<td>KIDNEY</td>
<td>5.81 E-2</td>
<td>BONE</td>
<td>3.34 E-2</td>
</tr>
<tr>
<td>BONE</td>
<td>4.51 E-2</td>
<td>BLADDER</td>
<td>4.71 E-2</td>
<td>BONE</td>
<td>5.16 E-2</td>
<td>PITUITARY</td>
<td>1.62 E-2</td>
</tr>
<tr>
<td>BLOOD</td>
<td>4.06 E-2</td>
<td>TUMOUR</td>
<td>1.77 E-2</td>
<td>TUMOUR</td>
<td>1.39 E-2</td>
<td>BILE</td>
<td>1.07 E-2</td>
</tr>
<tr>
<td>TUMOUR (WHOLE)</td>
<td>2.60 E-2</td>
<td>LUNG</td>
<td>1.73 E-2</td>
<td>PITUITARY</td>
<td>8.07 E-3</td>
<td>TUMOUR</td>
<td>1.15 E-2</td>
</tr>
<tr>
<td>INFLAMMATORY TISSUE</td>
<td>2.23 E-2</td>
<td>BLOOD</td>
<td>1.67 E-2</td>
<td>SPLEEN</td>
<td>6.90 E-3</td>
<td>LIVER</td>
<td>7.22 E-3</td>
</tr>
<tr>
<td>LUNG</td>
<td>2.17 E-2</td>
<td>PITUITARY</td>
<td>1.15 E-2</td>
<td>BLOOD</td>
<td>6.80 E-3</td>
<td>SPLEEN</td>
<td>5.79 E-3</td>
</tr>
<tr>
<td>NASAL MUCOSA</td>
<td>1.99 E-2</td>
<td>NASAL MUCOSA</td>
<td>1.11 E-2</td>
<td>NASAL MUCOSA</td>
<td>6.74 E-3</td>
<td>GALL BLADDER</td>
<td>4.95 E-3</td>
</tr>
</tbody>
</table>

* MEAN DATA FROM FIVE RABBITS
and the four hour bladder uptake was not significantly different from the two hour figure (0.077 %/g, p > 0.05). There was uptake in whole tumour (0.018 %/g) which was significantly different from the two hour whole tumour figure (0.026 %/g, p < 0.025). There was uptake in lung (0.017 %/g), pituitary (0.012 %/g) and nasal mucosa (0.011 %/g). There was no significant difference between them (p > 0.05), between them and whole tumour (p > 0.05), and between them, whole tumour and the blood pool (0.017 %/g, p > 0.05), except whole tumour and pituitary (p < 0.025) and whole tumour and nasal mucosa (p < 0.01).

By six hours, there was significant uptake (i.e. greater than the blood pool, p < 0.005) in the kidney (0.058 %/g), bone (0.052 %/g) and whole tumour (0.014 %/g) but not the bladder (0.059 %/g, p > 0.05). There was no significant difference (p > 0.05) in uptake between the bladder, kidney and bone, and the kidney and bone had significantly more uptake than whole tumour (p < 0.001). There was no significant difference (p > 0.05) between the four and six hour levels for either bladder, kidney, bone and whole tumour. There was uptake in pituitary (0.008 %/g), and spleen and nasal mucosa (0.007 %/g). There was no significant difference in uptake between them (p > 0.05), and between them and the blood pool (0.007 %/g, p > 0.05) but there was a significant difference between them and whole tumour (p < 0.025).
There was no significant difference ($p > 0.05$) between the six and 24 hour levels for kidney and bone, and uptake in both these organs, as well as the bladder, remained relatively constant to 24 hours ($p > 0.05$). At 24 hours, significant radioactivity (i.e. greater than the blood pool, $p < 0.005$) remained in the kidneys ($0.085 \% / g$) and bone ($0.033 \% / g$) and these two levels were significantly different ($p < 0.025$).

There was uptake in whole tumour ($0.012 \% / g$) at 24 hours but this was not significantly different from the six hour whole tumour level ($0.014 \% / g$, $p > 0.05$). There was radioactivity in the pituitary ($0.016 \% / g$) and bile ($0.011 \% / g$), but there was no significant difference between them ($p > 0.05$), between them and whole tumour ($p > 0.05$) and between them and the blood pool ($0.003 \% / g$, $p > 0.05$). However, the uptake in whole tumour was significantly greater than the blood pool ($p < 0.025$). There was uptake in the liver ($0.007 \% / g$), spleen ($0.006 \% / g$) and gall bladder ($0.005 \% / g$). Liver uptake was not significantly less than whole tumour, spleen, gall bladder and the blood pool ($p > 0.05$). There was no significant difference between spleen and gall bladder uptake ($p > 0.05$), or between the radioactivity in the spleen, gall bladder and the blood pool ($p > 0.05$). The 24 hour biodistribution in kidney, bladder, bone, blood and whole tumour is shown in Figure 32.
THE MAJOR BIODISTRIBUTION OF Tc$^{99m}$ (v) DMSA AT 2, 4, 6 AND 24 HOURS POST-INJECTION IN TUMOUR-BEARING NZW RABBITS

Mean data from 20 rabbits ± 2SD

% INJECTED DOSE PER GRAM

<table>
<thead>
<tr>
<th>ORGANS</th>
<th>2 Hrs</th>
<th>4 Hrs</th>
<th>6 Hrs</th>
<th>24 Hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIDNEY</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BLADDER</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BONE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BLOOD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TUMOUR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Radioactivity in the bile was at a maximum at 24 hours (0.01 %/g), and there was no significant difference (p > 0.05) between this value and either the two hour (0.0067 %/g), four hour (0.0082 %/g) or six hour (0.0048 %/g) levels.

There was no significant difference (p > 0.05) between the uptake of Tc $^{99m}$ (v) DMSA in kidney, bone and bladder at two, four and six hours and between kidney and bone at 24 hours. The uptake in kidney always exceeded that in bone. However, at two hours bladder uptake was between that of kidney and bone, whereas at four hours it was less than, and at six hours was greater than kidney and bone. By 24 hours bladder activity was significantly less than kidney and bone (p < 0.005).

There was uptake of Tc $^{99}$ (v) DMSA at two, four, six and 24 hours in inflammatory tissue, lacrimal, thymus, cervical lymph glands and the marrow but uptake never exceeded 0.01 %/g (except for uptake at two hours in inflammatory tissue, cervical lymph glands and the marrow; 0.022 %/g, 0.018 %/g and 0.013 %/g respectively). There was a significant difference between the uptake in all these organs and the blood pool at two, four and six hours (p < 0.025) except for the marrow (p > 0.05), and radioactivity was not significantly less than the blood pool at 24 hours for any of the five organs (p > 0.05).
The uptake of radioactivity in whole, living and necrotic tumour together with the whole tumour:blood and whole tumour:muscle ratios is shown in Table 21. There was no significant difference (p > 0.05) between the uptake of Tc $^{99m}$ (v) DMSA in blood, whole, living and necrotic tumour at two, four and six hours (except at six hours when the whole tumour uptake was significantly greater than the blood pool, p < 0.025). By 24 hours, uptake in whole and living tumour (but not necrotic tumour) was significantly greater than the blood pool (p < 0.025).

Uptake in outside living tumour remained relatively constant to six hours (0.11 %/g, p > 0.05) but by 24 hours this had decreased significantly (0.006 %/g, p < 0.025). Uptake in necrotic inside tumour remained relatively constant between two (0.025 %/g), four (0.021 %/g) and six hours (0.021 %/g, p > 0.05) but had dropped significantly by 24 hours (0.007 %/g, p < 0.025, Figure 33).

At two hours, the whole tumour:blood ratio was less than one (0.6:1), but this increased to 1.1:1 at four hours. By six hours, the ratio was 2:1 and this further increased to 5:1 at 24 hours.

The whole tumour:muscle ratio at two hours was 4.3:1 increasing to 6:1 at four hours, to 7:1 at six hours and to 15:1 at 24 hours.
TABLE 21
TUMOUR UPTAKE OF Tc $^{99m}$ (v) DMSA in 20 TUMOUR-BEARING RABBITS
(% INJECTED DOSE PER GRAM OF TISSUE)

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>*2 HOURS</th>
<th>*4 HOURS</th>
<th>*6 HOURS</th>
<th>*24 HOURS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUMOUR (WHOLE)</td>
<td>0.026</td>
<td>0.018</td>
<td>0.014</td>
<td>0.015</td>
</tr>
<tr>
<td>TUMOUR (LIVING)</td>
<td>0.026</td>
<td>0.017</td>
<td>0.011</td>
<td>0.006</td>
</tr>
<tr>
<td>TUMOUR (NECROTIC)</td>
<td>0.025</td>
<td>0.021</td>
<td>0.021</td>
<td>0.007</td>
</tr>
<tr>
<td>BLOOD</td>
<td>0.041</td>
<td>0.017</td>
<td>0.007</td>
<td>0.003</td>
</tr>
<tr>
<td>MUSCLE</td>
<td>0.006</td>
<td>0.003</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td>TUMOUR:BLOOD (whole) RATIO</td>
<td>0.6:1</td>
<td>1.1:1</td>
<td>2.0:1</td>
<td>5.0:1</td>
</tr>
<tr>
<td>TUMOUR:MUSCLE (whole) RATIO</td>
<td>4.3:1</td>
<td>6.0:1</td>
<td>7.0:1</td>
<td>15.0:1</td>
</tr>
</tbody>
</table>

* MEAN DATA FROM FIVE RABBITS
THE TUMOUR UPTAKE OF Tc$^{99m}$m(v) DMSA COMPARED WITH WHOLE BLOOD AND MUSCLE RADIOACTIVITY AT 2, 4, 6 AND 24 HOURS POST-INJECTION IN TUMOUR-BEARING NZW RABBITS

Mean data from 20 rabbits ± 2SD

% INJECTED DOSE PER GRAM

- Blood
- Whole Tumour
- Living Tumour Organs
- Necrotic Tumour
- Muscle

2 Hrs
4 Hrs
6 Hrs
24 Hrs
404

4.1.3. **OPTIMAL IMAGING TIME**

Six rabbits with 17 tumours (range 0.5-10 cms, mean 3.6 cm ± 4.3) were studied and biodistribution observed in the bone, kidneys and bladder. All rabbits with tumours were identified as having cancer (100% sensitivity) and the sensitivities and specificities for tumour detection at each imaging time are shown in Table 22. The optimal **qualitative** imaging time was four hours (92% sensitivity, 100% specificity). There were eight false negatives and, of these, five occurred at 30 minutes when the tumours ranged in size from 0.5 cm - 3.0 cm, mean 1.9 ± 1.6 cm. By three hours, only two tumours remained undetected (2.0 x 1.0 and 0.5 x 0.5 cm) and, of these, only the smaller one was not visible at five and six hours (Figure 34). There were two false positives observed at the same site (left axilla) in one rabbit at five and six hours.

The tumour:soft tissue **quantitative** background ratios are shown in Table 13A and Figure 35. Maximum quantitative uptake occurred between 1.5 and five hours. Taking into consideration both the **qualitative** and **quantitative** results, together with the pharmacokinetic and biodistribution data, the optimal imaging time was taken to be four hours.
TABLE 22
SEQUENTIAL SENSITIVITIES AND SPECIFICITIES FOR THE
SCINTIGRAPHIC DETECTION OF 17 TRANSPLANTED TUMOURS
IN SIX NZW RABBITS USING Tc \(^{99m}\) (\(\nu\)) DMSA

<table>
<thead>
<tr>
<th>TIME AFTER INJECTION (HOURS)</th>
<th>0.5</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>SENSITIVITY (%)</td>
<td>71</td>
<td>76</td>
<td>73</td>
<td>76</td>
<td>87</td>
<td>92</td>
<td>85</td>
<td>92</td>
</tr>
<tr>
<td>SPECIFICITY (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>67</td>
<td>67</td>
</tr>
</tbody>
</table>
Sequential Tc$^{99m}$ (v) DMSA thoraco-abdominal planar images in a tumour-bearing rabbit taken at approximately 2, 3, 4 and 5 hours post-injection. There are three transplanted tumours in the right shoulder (A), right loin (B) and left loin which measured 4.5 x 3.5, 3.5 x 2.5 and 0.5 x 0.5 respectively. Both the larger tumours are visualised at all times but, although there is very little difference in image quality between 2 and 4 hours, tumour visualisation is less apparent at 5 hours. The smaller left loin tumour is a false negative.
THE Tc $^{99m}$ (v) DMSA SEQUENTIAL QUANTITATIVE TUMOUR: BACKGROUND RATIOS

Data from 4 tumours

$\pm$ 2 Standard deviations

TIME (HOURS AFTER INJECTION)

RATIO

FIGURE 35
One non-tumour rabbit was imaged sequentially to six hours and normal biodistribution confirmed in the bone, kidneys and bladder (Figure 36).
An anterior thoraco-abdominal Tc $^{99m}$ (v) DMSA planar image in a non-tumour bearing rabbit taken approximately 4 hours post-injection. Note the normal biodistribution in the bone (A), kidneys (B) and bladder (C, partially shielded).
Fifteen rabbits (14 tumour, one non-tumour) with 60 shaved sites (of which 42 had transplanted tumours) were studied at the optimal imaging time. All tumours were confirmed histologically. Fifty-eight tumours were examined (range 0.4-7.1 cm, mean 1.9 ± 2.8 cm). Eighteen sites had no tumour. There were 30 sites with one tumour, eight sites with two tumours and four sites with three tumours. Of the 30 sites with one tumour, 29 were primary transplanted growths and one was a metastasis in a right axillary lymph node.

The rabbit tumour sensitivity was 93% (13 true positives, one false negative). The overall lesion scintigraphic sensitivity was 50% with a 63% specificity. The individual sensitivities for tumour detection in each class (i.e. Class I < 1 cm, Table 13) are shown in Table 23. Fifteen percent of Class I tumours were detected scintigraphically, and this increased to 50% for Class II tumours. Overall 58% of tumours less than 2 cm in size were detected (Class I and II) and this increased to 89% for Class III tumours and to 75% for Class IV and Class V tumours. There were 29 true positives and 12 true negatives (Figure 37). There were 29 false negatives. Of these, 26 were Class II or less (Figure 37), and there was one Class III, one Class IV and one Class V tumour. There were seven false positives. Of these, three
#### TABLE 23
TUMOUR SCINTIGRAPHY RESULTS*

<table>
<thead>
<tr>
<th>CLASS</th>
<th>SENSITIVITY (%)</th>
<th>SPECIFICITY (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>+I and II</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>89</td>
<td>63</td>
</tr>
<tr>
<td>IV</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>OVERALL</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

* DATA FROM 14 RABBITS WITH 58 TRANSPLANTED TUMOURS  
+ LESS THAN 2 cm
An anterior thoraco-abdominal Tc $^{99m}$ Tc DMSA planar image in a tumour-bearing rabbit with four transplanted tumours taken approximately 4 hours post-injection. Tumours in the right loin (A, 5.5 x 4.6 cm), left loin (B, 5.1 x 2.3 cm) and left shoulder (C, 3.3 x 2.1 cm) are visualised. The smallest tumour in the left loin (0.7 x 0.7 cm) is not visualised.

An anterior thoraco-abdominal Tc $^{99m}$ Tc DMSA planar image in a tumour-bearing rabbit with the transplanted tumours taken approximately 4 hours post-injection. One tumour in the left loin (A, 0.9 x 0.6 cm) is visualised and there is a false positive in the left shoulder (B). Tumours in the right loin (1.2 x 0.8 cm), left loin (0.7 x 0.6 cm) and right shoulder (1.5 x 1.3 and 0.9 x 0.7 cm) are not visualised.
were located in the left shoulder, two in the left loin, one in the right shoulder and one in the right loin (Figure 37).

The rabbit tumour palpation sensitivity was 99% (94% specificity). There were 83 true positives, 17 true negatives, one false negative and one false positive. For lesion palpation, 530 observations were made with an overall sensitivity of 81% and a 79% specificity. There were 281 true positives and 143 true negatives. There were 39 false positives (12%) and 67 false negatives (32%). When a false negative result was obtained for a site with two tumours or more, the smallest actual tumour size (s) were assumed to be the false negative. The individual and overall observer sensitivities for each tumour class are shown in Table 24. Overall 50% of Class I tumours were palpable and this increased to 86% for Class II tumours. Seventy three percent of Class I and II tumours (less than 2 cm) were palpable, and this increased to 100% for Class III tumours thereafter. The palpation results show palpation to be a more efficient method of detecting superficially transplanted tumours in NZW rabbits than Tc $^{99m}$Tc (v) DMSA scintigraphy (Table 25).

All tumours measuring 2 cm and over were detected on palpation by all observers (100% sensitivity, Table 24). For those measuring less than 2 cm, observers 2 and 1 had the
### TABLE 24

**TUMOUR PALPATION RESULTS***

<table>
<thead>
<tr>
<th>+OBSERVER NUMBER</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>ALL OBSERVERS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CLASS I</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>67</td>
<td>27</td>
<td>40</td>
<td>47</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td><strong>CLASS II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>86</td>
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<tr>
<td></td>
<td>92</td>
<td>96</td>
<td>77</td>
<td>81</td>
<td>85</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td><strong>CLASS III</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><strong>SENSITIVITY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>% (I + II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>85</td>
<td>59</td>
<td>66</td>
<td>71</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td><strong>OVERALL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>86</td>
<td>90</td>
<td>71</td>
<td>76</td>
<td>79</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td><strong>SPECIFICITY (%)</strong></td>
<td>83</td>
<td>72</td>
<td>89</td>
<td>83</td>
<td>80</td>
<td>69</td>
<td>79</td>
</tr>
</tbody>
</table>

Data from one non-tumour rabbit and 14 tumour-bearing rabbits with 58 transplanted tumours

**Experience**
- Observer 1. Consultant ENT Surgeon
- Observer 2. Senior Registrar ENT
- Observer 3. Registrar ENT
- Observer 4. Registrar ENT
- Observer 5. House Surgeon (General Surgery/ENT)
- Observer 6. Preclinical medical student

Less than 2 cm
TABLE 25
TRUE POSITIVES AND FALSE NEGATIVES FOR SCINTIGRAPHY VS PALPATION RELATED TO THE RELATIVE SIZE OF 58 SUPERFICIALLY TRANSPLANTED TUMOURS IN 14 NZW RABBITS

<table>
<thead>
<tr>
<th>PALPATION*</th>
<th>CLASS I</th>
<th>CLASS II</th>
<th>CLASS III</th>
<th>CLASS IV</th>
<th>CLASS V</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIZE (cm)</td>
<td>0-1</td>
<td>1-2</td>
<td>2-3</td>
<td>3-4</td>
<td>&gt;4</td>
</tr>
<tr>
<td>TP</td>
<td>45</td>
<td>134</td>
<td>54</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>FN</td>
<td>45</td>
<td>22</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Tc $^{99m}$ (v) DMSA SCINTIGRAPHY

<table>
<thead>
<tr>
<th>SIZE (cm)</th>
<th>0-1</th>
<th>1-2</th>
<th>2-3</th>
<th>3-4</th>
<th>&gt;4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP</td>
<td>2</td>
<td>13</td>
<td>8</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>FN</td>
<td>13</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

TP = True positive
FN = False negative
* = Data from six independent observers
highest sensitivities (85% and 80% respectively). Observer 6 had a sensitivity of 76%, and observer 5 a 71% sensitivity. Observers 4 and 3 had sensitivities of 66% and 59% respectively.

For all tumours, observers 2 and 1 had the highest inter-observer sensitivities of 90% and 86% respectively. Observer 6 had a sensitivity of 83%, observer 5 had a 79% sensitivity while observers 4 and 3 had sensitivities of 76% and 71% respectively.

Observer 3 had the highest overall inter-observer specificity (89%). Observers 1 and 4 had specificities of 83%. Observer 5 had a specificity of 80%, observer 2 had a 72% specificity while observer 6 had a 65% specificity.

A comparison was made evaluating the ability of the six independent observers to estimate tumour size (Table 14A). Figure 38 shows the error in estimation (difference) compared against actual tumour sizes for each observer. There was no significant difference (random block analysis) between observers 1, 2, 3 and 4 in their ability to estimate tumour size (Table 26, \( \alpha > 0.05 \)). Observers 1 and 2 were the most accurate for tumours less than 2 cm in size and tumour size estimation was more accurate for tumours less than 2 cm in size.
A COMPARISON OF THE ABILITY OF SIX INDEPENDENT OBSERVERS TO ESTIMATE TUMOUR SIZE

Observer 1
\[ y = -2.2195 - 0.2523x \quad R = 0.55 \]

Observer 2
\[ y = -1.5054 - 0.2808x \quad R = 0.56 \]

Observer 3
\[ y = -7.2048 + 0.0617x \quad R = 0.12 \]

Observer 4
\[ y = -3.5716 - 0.1159x \quad R = 0.22 \]

Observer 5
\[ y = -3.2675 + 0.1636x \quad R = 0.26 \]

Observer 6
\[ y = -3.4952 - 0.0066x \quad R = 0.01 \]

R = Correlation Coefficient

*Data from 14 rabbits*
### Table 26

**Random Block Analysis Comparing the Ability of Six Independent Observers to Estimate Actual Tumour Size in 14 NZW Rabbits***

<table>
<thead>
<tr>
<th>OBSERVER NUMBER</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>NS</td>
<td>-</td>
<td>NS</td>
<td>NS</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td>NS</td>
<td>NS</td>
<td>-</td>
<td>NS</td>
<td>0.025</td>
<td>NS</td>
</tr>
<tr>
<td>4</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>-</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td>0.01</td>
<td>0.01</td>
<td>0.025</td>
<td>0.01</td>
<td>-</td>
<td>0.01</td>
</tr>
<tr>
<td>6</td>
<td>0.01</td>
<td>0.01</td>
<td>NS</td>
<td>0.01</td>
<td>0.01</td>
<td>-</td>
</tr>
</tbody>
</table>

Observer Experience:
1. Consultant ENT Surgeon
2. Senior Registrar ENT
3. Registrar ENT
4. Registrar ENT
5. House Surgeon ENT/General Surgery
6. Preclinical Medical Student

Quoted values are for $\alpha$

NS = not significant ($\alpha > 0.05$)

* Data from 58 tumours
Observers 3 and 4 were less accurate. Observer 3 tended to underestimate the smaller tumours (less than 2 cm), while observer 4 tended to underestimate tumour size in a similar manner to observers 1 and 2. Observers 5 and 6 were significantly less accurate and more erratic in predicting tumour size than observers 1, 2, 3 and 4 (Figure 38 and Table 26, $\alpha < 0.05$).
4.1.5. SUBCELLULAR BIODISTRIBUTION

Six tumour-bearing rabbits were studied. The subcellular biodistribution of Tc $^{99m}$ (V) DMSA in these rabbits with normal livers and squamous cell carcinoma is shown in Table 27. All samples were confirmed histologically. There was no apparent difference between the liver and tumour subcellular biodistribution results, except the radioactivity on the liver cell membrane appeared to decrease as a function of tumour age. There was also no apparent relationship between the tumour subcellular biodistribution results and tumour age.

For the liver, 15.5-24.6% of radioactivity was located on the cell membrane (18-39 days with tumour) while 52.1-67.3% was localised non-specifically in the cytosolic fraction. There was 9.6-19.9% of radioactivity bound specifically to mitochondria, and 5.9-8.4% to microsomes.

For squamous cell carcinoma, 16.8-37.3% of radioactivity was located on the cell membrane, and this did not appear to vary as a function of tumour age. There was 57.1-79.9% of radioactivity localised non-specifically in tumour cytosol. Only 2.4-5.5% of radioactivity was bound specifically to tumour mitochondria and 0.8-4.0% to microsomes.
<table>
<thead>
<tr>
<th>ORGAN</th>
<th>ORGANELLE</th>
<th>18</th>
<th>22</th>
<th>32</th>
<th>32</th>
<th>39</th>
<th>75</th>
<th>RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CELL MEMBRANE</td>
<td>24.6</td>
<td>23.0</td>
<td>20.3</td>
<td>15.5</td>
<td>16.1</td>
<td>-</td>
<td>15.5-24.6</td>
</tr>
<tr>
<td></td>
<td>MITOCHONDRIA</td>
<td>12.1</td>
<td>12.4</td>
<td>19.9</td>
<td>9.6</td>
<td>15.1</td>
<td>-</td>
<td>9.6-19.9</td>
</tr>
<tr>
<td></td>
<td>*LIVER</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MICROSONES</td>
<td>6.7</td>
<td>8.4</td>
<td>7.7</td>
<td>7.6</td>
<td>5.9</td>
<td>-</td>
<td>5.9-8.4</td>
</tr>
<tr>
<td></td>
<td>CYTOSOL</td>
<td>56.5</td>
<td>56.1</td>
<td>52.1</td>
<td>67.3</td>
<td>62.8</td>
<td>-</td>
<td>52.1-67.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CELL MEMBRANE</td>
<td>23.1</td>
<td>16.8</td>
<td>24.0</td>
<td>37.3</td>
<td>32.7</td>
<td>17.0</td>
<td>16.8-37.3</td>
</tr>
<tr>
<td></td>
<td>MITOCHONDRIA</td>
<td>5.5</td>
<td>3.5</td>
<td>4.1</td>
<td>4.1</td>
<td>4.4</td>
<td>2.4</td>
<td>2.4-5.5</td>
</tr>
<tr>
<td></td>
<td>*TUMOUR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MICROSONES</td>
<td>2.1</td>
<td>1.7</td>
<td>4.0</td>
<td>1.6</td>
<td>2.7</td>
<td>0.8</td>
<td>0.8-4.0</td>
</tr>
<tr>
<td></td>
<td>CYTOSOL</td>
<td>69.0</td>
<td>78.1</td>
<td>67.9</td>
<td>57.1</td>
<td>60.1</td>
<td>79.9</td>
<td>57.1-79.9</td>
</tr>
</tbody>
</table>

All tissue samples removed approximately four hours post-injection

* Data from five rabbits
+ Data from six rabbits
4.1.6. ESTIMATION OF BONE MASS

The bone mass of a non-tumour rabbit was 137 g/kg (13.7% body weight).
4.1.7. **BODY AND THYMUS WEIGHTS**

There was no significant difference in mean body weight between the non-tumour group (2.75 kg ± 0.52) in this study and the mean body weight of non-tumour normal young adult NZW rabbits quoted in the literature (Kozma et al, 1974, p 56: 2.775 kg ± 0.198, p > 0.05), but there was a significant difference between these two values and the mean body weight of the tumour group in this study (3.34 kg ± 0.38, p < 0.001).

The thymus weights for non-tumour rabbits are shown in Table 15A. The mean thymus weight in this group was 1.94 ± 1.08 g/kg body weight (range 1.32-3.36 g/kg). The thymus weights for tumour-bearing rabbits are shown in Table 16A and the mean weight was 1.48 ± 0.82 g/kg body weight (range 0.97-2.40 g/kg). Using multiple regression analysis, the thymus weight in the tumour group was unaffected by either time with tumour, or tumour mass \( y = 1.69 - 0.00234x_1 - 0.00634x_2 \), i.e. \( y = a_0 + a_1x_1 + a_2x_2 \); \( y = \text{thymus wt/kg} \); \( x_1 = \text{days and} \ x_2 = \text{tumour weight (g)} \). The coefficient of variation was 26% indicating that only 26% of the variation in thymus weight was caused by the variables, days with tumour \( (x_1) \) and tumour weight \( (x_2) \). A student t-test of the null hypothesis showed p > 0.05 for \( a_1 \) and \( a_2 \).
There was a difference between the mean non-tumour rabbit thymus weight/kg and the mean non-tumour rabbit thymus weight/kg as quoted in the literature (1.45 g/kg ± 0.88, Kozma et al, 1974, p 56) but this difference was only just significant (p < 0.025). There was a significant difference between the mean non-tumour and mean tumour thymus weights/kg in this study (p < 0.005), but there was no significant difference between the mean tumour thymus weight/kg and the mean non-tumour thymus weight/kg as quoted by Kozma et al (1974, p 56).
4.1.8. REGIONAL LYMPH NODE AND DISTANT METASTASES

Seven rabbits (18%) developed macroscopic metastases. Of these, the incidence of regional lymph node metastases was 8%, and 13% for distant metastases. All metastases were identified macroscopically and confirmed histologically (Figure 39). Two rabbits had regional lymph node metastases in the left axilla, one had a regional metastasis in the right axilla with distant lung and liver metastases, and four had liver metastases only. No bony metastases were detected. There was no apparent relationship between tumour weight, tumour age, and either the site and/or incidence of regional lymph node and distant metastases (Table 28). The exception was the rabbit with the greatest tumour mass (98.8 g) which was the only animal to develop macroscopic metanchronous metastases (right axilla, liver and lung).
Sections of rabbit liver (1) and lung (2) showing metastatic Vx2 squamous carcinoma (A)
**TABLE 28**

THE RELATIONSHIP BETWEEN SOME PROPERTIES OF TUMOURS, BODY WEIGHT, AND DISTRIBUTION OF TUMOURS IN SEVEN NZW RABBITS

<table>
<thead>
<tr>
<th>BODY WEIGHT (kg)</th>
<th>TUMOUR WEIGHT (g)</th>
<th>TUMOUR AGE (days)</th>
<th>METASTATIC SITE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.80</td>
<td>98.80</td>
<td>31</td>
<td>LUNG, LIVER AND RIGHT AXILLA</td>
</tr>
<tr>
<td>2.86</td>
<td>1.20</td>
<td>6</td>
<td>LIVER</td>
</tr>
<tr>
<td>2.95</td>
<td>1.00</td>
<td>13</td>
<td>LEFT AXILLA</td>
</tr>
<tr>
<td>3.28</td>
<td>3.80</td>
<td>14</td>
<td>LIVER</td>
</tr>
<tr>
<td>3.56</td>
<td>36.2</td>
<td>29</td>
<td>LEFT AXILLA</td>
</tr>
<tr>
<td>3.58</td>
<td>10.8</td>
<td>14</td>
<td>LIVER</td>
</tr>
<tr>
<td>3.84</td>
<td>0.25</td>
<td>9</td>
<td>LIVER</td>
</tr>
</tbody>
</table>
4.1.9. **RADIOPHARMACEUTICAL STABILITY**

The purity of Tc$^{99m}$ (v) DMSA was analysed by thin layer chromatography (Merck Silica gel, developed with n-butanol/acetic acid ($\text{H}_2\text{O}$ (3:2:3)), and no free pertechnetate or other Tc$^{99m}$ derivative was detected. A radiochromatogram scan of Tc$^{99m}$ (v) DMSA, Tc$^{99m}$ (III)DMSA and Tc$^{99m}$-TcO$_4^{-}$ injections is shown in Figure 40. The $R_f$ values are 0.4-0.6; 0 and 0.7-0.8 respectively and show the scanner can adequately identify and separate Tc$^{99m}$ (v) DMSA from Tc$^{99m}$ (III) and Tc$^{99m}$-TcO$_4^{-}$.

Tc$^{99m}$ (v) DMSA was stable *in-vitro* for up to two hours following preparation.
Typical radiochromatogram scan of Tc$^{99m}$(V)DMSA, Tc$^{99m}$(III)DMSA and Tc$^{99m}$-TcO$_4^-$ on TLC Silica gel 60 (Merck) developed with n-Butanol: acetic acid: water (3:2:3 V/V).

TLC on Silica Gel 60 (MERCK)

Solvent System:

n-Butanol : Acetic Acid : Water

3 : 2 : 3 : (V/V)
4.2. HUMANS

4.2.1. Pharmacokinetics

4.2.2. Biodistribution

4.2.3. Subcellular Biodistribution

4.2.4. Optimal Imaging Time

4.2.5. Clinical Examination and Imaging

4.2.6. Dosimetry

4.2.7. Radiopharmaceutical Stability
4.2.1. PHARMACOKINETICS

Ten patients were studied (five non-tumour; five tumour). The blood levels following intravenous injection of Tc $^{99m}$ (v) DMSA in non-tumour and tumour patients are given in Tables 17A-18A. The blood clearance of Tc $^{99m}$ (v) DMSA in non-tumour patients was bi-exponential (Figure 41). The $t_\text{v}$'s for each patient were calculated (range 27-33 and 363-426 mins, Table 29) and from these, mean $t_\text{v}$'s obtained (30 and 401 mins). The blood clearance of Tc $^{99m}$ (v) DMSA in tumour patients was bi-exponential (Figure 41). All tumours were confirmed histologically. The $t_\text{v}$'s for each patient were calculated (range 25-35 and 344-548 mins, Table 29), and from these, mean $t_\text{v}$'s obtained (30 and 387 mins). Using a student t-test, there was no significant difference ($p > 0.05$) between the mean $t_\text{v}$'s for non-tumour and tumour patients.

The sequential and cumulative urine and whole body retention values following intravenous injection of Tc $^{99m}$ (v) DMSA in non-tumour and tumour patients are given in Tables 19A-20A. The cumulative urine excretion of Tc $^{99m}$ (v) DMSA was bi-exponential in non-tumour and tumour patients (Figure 42), and the $t_{50\%}$'s are shown in Table 30.
THE BLOOD CLEARANCE OF Tc-99m-V-DMSA IN NON-TUMOUR PATIENTS

Mean $T_1/2=30$ and 401 mins
Data from 5 patients

THE BLOOD CLEARANCE OF Tc-99m-V-DMSA IN TUMOUR PATIENTS

Mean $T_1/2=30$ and 387 mins
Data from 5 patients
**TABLE 29**

**BI-EXPONENTIAL HALF-TIME BLOOD CLEARANCE VALUES OF $^{99m}Tc$ (V) DMSA IN FIVE NON-TUMOUR AND FIVE TUMOUR PATIENTS**

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>FIRST PHASE</th>
<th>$^R$</th>
<th>SECOND PHASE</th>
<th>$^R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27 ± 7</td>
<td>0.97</td>
<td>403 ± 42</td>
<td>0.99</td>
</tr>
<tr>
<td>2</td>
<td>29 ± 8</td>
<td>0.96</td>
<td>418 ± 38</td>
<td>0.99</td>
</tr>
<tr>
<td>3</td>
<td>31 ± 7</td>
<td>0.97</td>
<td>363 ± 19</td>
<td>0.99</td>
</tr>
<tr>
<td>4</td>
<td>31 ± 9</td>
<td>0.96</td>
<td>426 ± 38</td>
<td>0.99</td>
</tr>
<tr>
<td>5</td>
<td>33 ± 16</td>
<td>0.91</td>
<td>399 ± 43</td>
<td>0.99</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RANGE</th>
<th>27-33</th>
<th>363-426</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN</td>
<td>30</td>
<td>401</td>
</tr>
</tbody>
</table>

| STANDARD ERROR OF THE MEAN | 10 | 37 |

* + two standard deviations  
$^R$ = Correlation coefficient
TABLE 29 (CONT)

BI-EXPONENTIAL HALF-TIME BLOOD CLEARANCE VALUES OF $^{99m}$Tc (V) DMSA IN FIVE NON-TUMOUR AND FIVE TUMOUR PATIENTS

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>FIRST PHASE</th>
<th>$^+_R$</th>
<th>SECOND PHASE</th>
<th>$^+_R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>25 ± 5</td>
<td>0.98</td>
<td>548 ± 34</td>
<td>0.99</td>
</tr>
<tr>
<td>7</td>
<td>27 ± 4</td>
<td>0.98</td>
<td>359 ± 32</td>
<td>0.99</td>
</tr>
<tr>
<td>8</td>
<td>34 ± 7</td>
<td>0.98</td>
<td>376 ± 54</td>
<td>0.98</td>
</tr>
<tr>
<td>9</td>
<td>34 ± 9</td>
<td>0.97</td>
<td>344 ± 48</td>
<td>0.98</td>
</tr>
<tr>
<td>10</td>
<td>35 ± 14</td>
<td>0.93</td>
<td>363 ± 28</td>
<td>0.99</td>
</tr>
<tr>
<td>RANGE</td>
<td>25-35</td>
<td></td>
<td>344-548</td>
<td></td>
</tr>
<tr>
<td>MEAN</td>
<td>30</td>
<td></td>
<td>387</td>
<td></td>
</tr>
</tbody>
</table>

STANDARD ERROR OF THE MEAN 9 40

* ± two standard deviations
$^+_R$ = Correlation coefficient
FIGURE 42

THE CUMULATIVE URINE EXCRETION OF Tc-99m-V-DMSA IN NON-TUMOUR PATIENTS

Mean Cumulative $T_{50\%} = 183$ mins
Data from 5 patients

THE CUMULATIVE URINE EXCRETION OF Tc-99m-V-DMSA IN TUMOUR PATIENTS

Mean Cumulative $T_{50\%} = 244$ mins
Data from 5 patients
### TABLE 30
THE CUMULATIVE URINE EXCRETION HALF-TIME ($T_{50\%}$) VALUES OF
$^{99m}$Tc DMSA IN FIVE NON-TUMOUR AND FIVE TUMOUR PATIENTS

<table>
<thead>
<tr>
<th>CUMULATIVE HALF-TIME (MINUTES)</th>
<th>PATIENT</th>
<th>NON-TUMOUR</th>
<th>PATIENT</th>
<th>TUMOUR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>125</td>
<td>6</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>70</td>
<td>7</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>270</td>
<td>8</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>250</td>
<td>9</td>
<td>350</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>10</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>RANGE</td>
<td>70-270</td>
<td>RANGE</td>
<td>200-350</td>
<td></td>
</tr>
<tr>
<td>MEAN</td>
<td>+183</td>
<td>MEAN</td>
<td>244</td>
<td></td>
</tr>
<tr>
<td>STANDARD ERROR OF THE MEAN</td>
<td>38</td>
<td>STANDARD ERROR OF THE MEAN</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

* Mean for four patients (excluding patient 2; see Whole Body Retention and Discussion) = 211 minutes (standard error, 32)
The mean cumulative urine excretion $t_{50\%}$'s in non-tumour and tumour patients were 183 and 244 minutes (range 70-270 and 200-350 minutes, Table 30) respectively and, using a student $t$-test, there was no significant difference ($p > 0.05$) between the $t_{50\%}$'s in the two groups (including and excluding patient 2; see whole body retention data and discussion) although there appeared a wider scatter of cumulative urine excretion values in the non-tumour patients.

The whole body retention $t_{1/2}$ values in non-tumour and tumour patients are shown in Figure 43 and Table 31. The mean whole body retention $t_{1/2}$ values in non-tumour and tumour patients were 778 and 375 minutes (range 278-12,144 and 163-884 minutes) and using a student $t$-test there was a significant difference between these two values ($p < 0.005$). However, the whole body retention $t_{1/2}$ in patient 2 was 12,144 minutes (202 hours, correlation coefficient 0.60) which was radically different from all the other non-tumour patients. Excluding this patient (see Discussion) from the non-tumour group, the mean whole body retention $t_{1/2}$ was 631 minutes and using a student $t$-test there was no significant difference between this value and the value for the tumour group (375 minutes, $p > 0.05$).
FIGURE 43

WHOLE BODY RETENTION OF Tc-99m-V-DMSA IN NON-TUMOUR PATIENTS.

Mean T1/2 = 778 mins
Data from 5 patients
(Data from 4 patients
Mean T1/2 = 631 mins; see discussion)

WHOLE BODY RETENTION OF Tc-99m-V-DMSA IN TUMOUR PATIENTS.

Mean T1/2 = 375 mins
Data from 5 patients
THE WHOLE BODY RETENTION HALF-TIME VALUES OF Tc $^{99m}$ (V) DMSA IN FIVE NON-TUMOUR AND FIVE TUMOUR PATIENTS

*WHOLE BODY RETENTION HALF-TIME (MINUTES)*

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>NON-TUMOUR</th>
<th>$^{+}_R$</th>
<th>PATIENT</th>
<th>TUMOUR</th>
<th>$^{+}_R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2036 ± 422</td>
<td>0.94</td>
<td>6</td>
<td>163 ± 40</td>
<td>0.98</td>
</tr>
<tr>
<td>2</td>
<td>12144 ± 14593</td>
<td>0.60</td>
<td>7</td>
<td>541 ± 60</td>
<td>0.99</td>
</tr>
<tr>
<td>3</td>
<td>1699 ± 424</td>
<td>0.96</td>
<td>8</td>
<td>884 ± 195</td>
<td>0.95</td>
</tr>
<tr>
<td>4</td>
<td>598 ± 78</td>
<td>0.98</td>
<td>9</td>
<td>361 ± 22</td>
<td>0.99</td>
</tr>
<tr>
<td>5</td>
<td>278 ± 12</td>
<td>0.99</td>
<td>10</td>
<td>691 ± 124</td>
<td>0.98</td>
</tr>
</tbody>
</table>

**RANGE**

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>NON-TUMOUR</th>
<th>$^{+}_R$</th>
<th>PATIENT</th>
<th>TUMOUR</th>
<th>$^{+}_R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>278-12144</td>
<td></td>
<td>6</td>
<td>163-884</td>
<td></td>
</tr>
</tbody>
</table>

$^\circ$ MEAN

<table>
<thead>
<tr>
<th>標準差</th>
<th>778</th>
<th>375</th>
</tr>
</thead>
</table>

STANDARD ERROR OF THE MEAN

<table>
<thead>
<tr>
<th>46</th>
<th>49</th>
</tr>
</thead>
</table>
TABLE 31 (CONT)

* ± 2 standard deviations
+ R = Correlation coefficient
# mean $t_{\frac{1}{2}} = \frac{\ln 2}{\text{mean}}$
$^o$ mean (Patients 1, 3, 4 and 5) = 631.

(Standard error = 29; (See Discussion))
4.2.2. BIODISTRIBUTION

The human biodistribution results of six patients three to five hours post-injection of Tc $^{99m}$ (v) DMSA are given in Table 32. Two had hypopharyngeal tumours, two had laryngeal tumours and the remaining two had tumours of the tongue and maxillary sinus respectively. There was a wide range observed in the tumour:blood radioactivity ratios (0.3-4.1:1) and in the tumour:muscle ratios (0.6-4.0:1). The two patients with hypopharyngeal lesions had the highest tumour:blood ratios (4.1:1 and 2.5:1). The ratios in the other four patients showed no apparent uptake in the tumour when compared with blood and muscle. With one hypopharyngeal tumour, the tumour:blood and tumour:muscle ratios were approximately the same (4.0:1 and 4.1:1), but the tumour:blood ratio (2.5:1) was greater than the tumour:muscle ratio (0.6:1) in the second patient with a hypopharyngeal tumour. With all the other tumours studied, the tumour:muscle ratios were greater than the tumour:blood ratios. There was no apparent relationship between tumour:blood and tumour:muscle ratios and tumour histology.

The finger dose to one surgeon (1st assistant) during two biodistribution studies (total operating time, nine hours)
THE HUMAN BIODISTRIBUTION RESULTS 3-5 HOURS POST-INJECTION Tc $^{99m}$ (V) DMSA

(\% INJECTED DOSE PER GRAM OF TISSUE)

<table>
<thead>
<tr>
<th>PATIENT NUMBER AND PRIMARY SITE</th>
<th>*30 HYPOPHARYNX</th>
<th>14 HYPOPHARYNX</th>
<th>*64 LARYNX</th>
<th>66 LARYNX</th>
<th>63 TONGUE</th>
<th>65 MAXILLA</th>
<th>RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>+HISTOLOGY</td>
<td>G2</td>
<td>G2</td>
<td>G3</td>
<td>G2</td>
<td>G2</td>
<td>G1</td>
<td></td>
</tr>
<tr>
<td>#TIME P.I. (MINS)</td>
<td>240</td>
<td>252</td>
<td>192</td>
<td>290</td>
<td>175</td>
<td>195</td>
<td></td>
</tr>
<tr>
<td>BLOOD</td>
<td>2.40 E-3</td>
<td>2.15 E-3</td>
<td>2.25 E-3</td>
<td>3.13 E-3</td>
<td>5.5 E-3</td>
<td>3.45 E-3</td>
<td>2.15 E-3 - 5.5 E-3</td>
</tr>
<tr>
<td>MUSCLE</td>
<td>2.50 E-3</td>
<td>9.59 E-3</td>
<td>1.06 E-3</td>
<td>7.90 E-4</td>
<td>2.1 E-3</td>
<td>1.87 E-3</td>
<td>7.90 E-4 - 9.59 E-3</td>
</tr>
<tr>
<td>TUMOUR</td>
<td>9.90 E-3</td>
<td>5.46 E-3</td>
<td>2.38 E-3</td>
<td>7.98 E-4</td>
<td>3.1 E-3</td>
<td>4.41 E-3</td>
<td>7.98 E-4 - 9.90 E-3</td>
</tr>
<tr>
<td>TUMOUR:BLOOD RATIO</td>
<td>4.1:1</td>
<td>2.5:1</td>
<td>1.1:1</td>
<td>0.3:1</td>
<td>0.6:1</td>
<td>1.3:1</td>
<td>0.3-4.1:1</td>
</tr>
<tr>
<td>TUMOUR:MUSCLE RATIO</td>
<td>4:1</td>
<td>0.6:1</td>
<td>2.3:1</td>
<td>1:1</td>
<td>1.5:1</td>
<td>2.4:1</td>
<td>0.6-4.0:1</td>
</tr>
</tbody>
</table>

* Watkinson, 1987    + UICC, 1987    # P.I. = Post-injection    ° Previous irradiation
was less than 0.3 mSv (legal limit, 12.5 mSv per month). The maximum finger exposure time for one surgeon during the biodistribution studies was 12 hours.
4.2.3.  SUBCELLULAR BIODISTRIBUTION

The human tumour subcellular biodistribution of Tc $^{99m}$ (v) DMSA in four patients with squamous cell carcinoma is shown in Table 33. All tumours were confirmed histologically.

For the four primary sites (hypopharynx, larynx, tongue and maxilla), 25-45% of radioactivity was localised on the cell membrane and 28-60% of radioactivity was located non-specifically inside the cell in the cytosol. There was 10.8-20.4% of radioactivity specifically bound inside the cell to the mitochondria and 1.1-5.8% to the microsomes.
THE SUBCELLULAR BIODISTRIBUTION OF Tc $^{99m}$ (v) DMSA IN HUMAN TUMOURS

(RESULTS EXPRESSED AS PERCENTAGE OF TOTAL RADIOACTIVITY IN THE TUMOURS)

<table>
<thead>
<tr>
<th>PATIENT NUMBER AND PRIMARY SITE</th>
<th>*HISTOLOGY</th>
<th>CELL MEMBRANE</th>
<th>MITOCHONDRIA</th>
<th>MICROSOMES</th>
<th>CYTOSOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 HYPOPHARYNX</td>
<td>G2</td>
<td>45.1</td>
<td>20.4</td>
<td>5.8</td>
<td>28.8</td>
</tr>
<tr>
<td>66 LARYNX</td>
<td>G2</td>
<td>25.2</td>
<td>10.8</td>
<td>3.5</td>
<td>60.6</td>
</tr>
<tr>
<td>63 TONGUE</td>
<td>G2</td>
<td>34.0</td>
<td>19.6</td>
<td>1.1</td>
<td>45.3</td>
</tr>
<tr>
<td>65 MAXILLA</td>
<td>G1</td>
<td>30.1</td>
<td>16.4</td>
<td>1.9</td>
<td>51.3</td>
</tr>
<tr>
<td><strong>RANGE</strong></td>
<td><strong>25.2-45.1</strong></td>
<td><strong>10.8-20.4</strong></td>
<td><strong>1.1-5.8</strong></td>
<td><strong>28.8-60.6</strong></td>
<td></td>
</tr>
</tbody>
</table>

All tumour samples were removed 3-5 hours post-injection

*UICC, 1987
Six patients with a head and neck squamous carcinoma were imaged at two, four and six hours following an injection of Tc\(^{99m}\) (v) DMSA. All tumours were confirmed histologically. Five had T\(_4\) primary growths and the sixth had an 8 cm tumour of the pinna (Figure 44). Two patients had cervical metastases.

(i) Qualitative Optimal Imaging Time

For primary lesions at two, four and six hours post-injection there were six true positives (Table 34 and Figure 44). The patient planar sensitivity for primary lesions at each consecutive imaging time was therefore 100%. For nodal metastases (12 lateral neck compartments), there were no true positives. There were 10 true negatives and two false negatives at two and four hours with no false positives (Table 34). The patient nodal planar sensitivity at these times was 0%. At six hours there was one false positive, ten true negatives and one false negative, (patient nodal planar sensitivity, 0%; 91% specificity). The overall patient (primary and nodal) sensitivity was 67% at two, four and six hours.
Sequential anterior Tc$^{99m}$ (v) DMSA planar head and neck images in a patient with an 8 cm squamous carcinoma of the right pinna taken at 2, 4 and 6 hours post-injection. The tumour is visualised at 2 (A), 4 (B) and 6 (C) hours. Image quality is best at 4 hours.
THE QUALITATIVE PLANAR IMAGING RESULTS FOR SIX PATIENTS WITH HEAD AND NECK SQUAMOUS CARCINOMA AT TWO, FOUR AND SIX HOURS FOLLOWING AN INTRAVENOUS INJECTION OF Tc\(^{99m}\) (v) DMSA

<table>
<thead>
<tr>
<th>PATIENT NUMBER</th>
<th>PRIMARY SITE AND TNM</th>
<th>TIME (HOURS)</th>
<th>Tc(^{99m}) (v) DMSA PLANAR SCAN</th>
<th>NUMBER OF NECK NODES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LATERAL NECK COMPARTMENT</td>
<td>PRIMARY</td>
<td>RIGHT</td>
<td>LEFT</td>
</tr>
<tr>
<td>+23</td>
<td>SUPRAGLOTTIS (rT_4N_0)</td>
<td>2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>TONGUE BASE (T_4N_1)</td>
<td>2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>25</td>
<td>TONSIL (T_4N_0)</td>
<td>2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>33</td>
<td>PINNA (T_xN_0)</td>
<td>2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>46</td>
<td>SUPRAGLOTTIS (T_4N_2b)</td>
<td>2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>+49</td>
<td>SUPRAGLOTTIS (rT_4N_0)</td>
<td>2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
TABLE 34 (CONT)

+ positive scan. There were no strongly positive scans
- negative scan
*C = level of certainty (UICC, 1987)
+ = previous irradiation
(ii) **Quantitative Optimal Imaging Time**

The primary tumour:background (soft tissue) ratios were calculated for each tumour at each consecutive imaging time (Table 35). In patients 24, 25, 46 and 49, maximum uptake was observed at two hours. In patients 23 and 33, maximum uptake occurred at four hours. Taking into account the qualitative and quantitative data, the pharmacokinetic and biodistribution data together with the half-life of Technetium-99m and the busy schedule of any nuclear medicine department, the human optimal imaging time was taken to be between two and four hours.

One patient (62) with post-irradiation change in the larynx following radiotherapy to a T2 tumour and who had no evidence of residual and recurrent disease was also imaged at two, four and six hours post-injection. The inflammation: background ratios in this patient were the highest observed for all the patients studied (except patient 23 at four hours) and maximal uptake in patient 62 occurred at six hours.
TABLE 35
THE PRIMARY TUMOUR: BACKGROUND (SOFT TISSUE) QUANTITATIVE RATIO AT TWO, FOUR AND SIX HOURS POST-INJECTION OF $^{99m}$Tc (V) DMSA IN SEVEN PATIENTS WITH A HISTORY OF A HEAD AND NECK SQUAMOUS CARCINOMA

<table>
<thead>
<tr>
<th>PATIENT NUMBER</th>
<th>PRIMARY SITE AND TNM</th>
<th>TIME (HOURS)</th>
<th>2</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>*23</td>
<td>SUPRAGLOTTIS $rT_4N_0$</td>
<td></td>
<td>1.46</td>
<td>1.86</td>
<td>1.73</td>
</tr>
<tr>
<td>24</td>
<td>TONGUE BASE $T_4N_1$</td>
<td></td>
<td>0.97</td>
<td>0.53</td>
<td>0.68</td>
</tr>
<tr>
<td>25</td>
<td>TONSIL $T_4N_0$</td>
<td></td>
<td>1.47</td>
<td>1.36</td>
<td>1.39</td>
</tr>
<tr>
<td>33</td>
<td>PINNA $T_xN_0$</td>
<td></td>
<td>0.81</td>
<td>0.84</td>
<td>0.68</td>
</tr>
<tr>
<td>46</td>
<td>SUPRAGLOTTIS $T_4N_{2b}$</td>
<td></td>
<td>0.68</td>
<td>0.51</td>
<td>0.61</td>
</tr>
<tr>
<td>*49</td>
<td>SUPRAGLOTTIS $rT_4N_0$</td>
<td></td>
<td>0.55</td>
<td>0.40</td>
<td>0.48</td>
</tr>
<tr>
<td>RANGE</td>
<td></td>
<td></td>
<td>0.55-1.47</td>
<td>0.40-1.86</td>
<td>0.48-1.73</td>
</tr>
</tbody>
</table>

* = previous irradiation
+ = patient with a previous $T_2N_0$ laryngeal squamous carcinoma treated with radiotherapy who presented subsequently with stridor. He was diagnosed as having post-irradiation oedema, no recurrent tumour was identified and he remains alive and well.
4.2.5. **CLINICAL EXAMINATION AND IMAGING**

The subsequent results for palpation, scintigraphy and CAT evaluation are based on histological evidence (Figure 45) from all primary tumours together with data from 29 patients who had 36 neck dissections. Data on the other patients is based on clinical examination, CAT evaluation and subsequent follow-up (although some patients had, or had had, radiotherapy to one or both sides of the neck).

**Clinical Evaluation: Overall**

77 patients (67 with malignancy; 10 with benign head and neck lesions) were all examined at presentation (Figure 1A) and then followed up until 1st March 1989 (Tables 21A-23A). In addition, 35 patients (who had a total of 81 follow-up clinical examinations) were also followed up using Tc $^{99m}$ (v) DMSA planar scintigraphy. For overall clinical examination there were 80 true positives, 68 true negatives, three false positives and seven false negatives (92% sensitivity, 96% specificity; 96% positive predictive accuracy, 91% negative predictive accuracy).
Print of a photomicrograph showing invasive moderately well differentiated squamous carcinoma of the hypopharynx (patient 30). Below is poorly differentiated Vx2 squamous carcinoma.
Clinical Evaluation: At Presentation

77 patients (67 with malignancy; 10 with benign head and neck lesions; Figure 1A) had a full head and neck examination to include both sides of the neck. The overall patient sensitivity for detecting malignancy at presentation was 94% (85% specificity; 97% positive predictive accuracy, 73% negative predictive accuracy). There were 60 true positives, 11 true negatives, two false positives and four false negatives. Of the two false positives, one patient had had irradiation to a T₂ squamous carcinoma of the glottic larynx and had presumed recurrence, while the other had had an iridium implant to a tongue squamous carcinoma and was diagnosed as having tumour recurrence but turned out to have radiation fibrosis. One false negative patient had metastatic bony head and neck disease from adenocarcinoma of the breast, one had metastatic squamous cell carcinoma to the abdominal wall undetected by clinical examination and two had occult primaries.

For detecting patients with primary head and neck tumours (63 patients) the sensitivity was 92% (67% specificity; 96% positive predictive accuracy, 44% negative predictive accuracy). There were 52 true positives, four true negatives, two false positives and five false negatives. The two false positives have previously been
described. Of the five false negatives, two had presumed occult primary head and neck sites with cervical metastases and three had nasopharyngeal carcinoma undetected by clinical examination.

The primary tumour sensitivity (63 patients, 73 lesions, Table 37) was 90% (86% specificity; 96% positive predictive accuracy, 67% negative predictive accuracy). There were 53 true positives, 12 true negatives, two false positives and six false negatives. One patient had a second lung primary detected on clinical examination (extra true positive), five areas of oral periodontal disease were correctly identified as being benign (five true negatives), one patient (false negative) had an occult second primary in the apex of the right lung and one patient had an occipital arteriovenous malformation. Two patients who had developed second lung primaries were negative on examination at the treated primary site.

Four patients had distant metastases (15 lesions; 10 malignant, five benign). No patients were identified clinically as having malignancy. There were thus four false negatives (11 false negative lesions). The two inflammatory lesions were excluded. The one true negative
occurred in the patient who had had a previous stomach pull-up repair for hypopharyngeal cancer and who had no clinical (and subsequent pathological) evidence of recurrence at the primary site.

**Cervical Metastases: Clinical Evaluation**

67 patients with malignancy were evaluated and of these 28 had clinically positive necks \(13 N_1; 3 N_{2a}; 4 N_{2b}; 4 N_{2c}, \) i.e. \(4 N_2; 4 N_{2b} \) and \(5 N_3\). Five patients had bilateral neck disease and three had palpable nodes thought to be clinically negative. There were thus 101 clinically negative lateral neck compartments. In the 31 patients with palpable nodes (36 lateral neck compartments), 41 nodes (38 presumed malignant, three presumed benign) were palpated. Based on data from 36 neck dissections, one autopsy together with clinical examination, CAT evaluation and neck ultrasound (70 lateral neck compartments) and subsequent follow-up (29 lateral neck compartments), out of the 67 patients 29 had 37N-positive lateral neck compartments \(11 N_1; 1 N_{2a}; 20 N_{2b}; 5 N_3\). There were 91 cervical masses (83 malignant nodes, eight benign). In all patients with cervical metastases, the normal patterns of lymphatic spread from the various primary sites were confirmed (Section 1.2.5.).
For the patient sensitivity in the detection of metastatic carcinoma, there were 25 true positives, 35 true negatives, four false positives and three false negatives. The sensitivity for detecting patients with metastatic neck disease was therefore 89% (90% specificity; 86% positive predictive accuracy, 92% negative predictive accuracy). The sensitivity for detecting metastatic neck carcinoma in 134 lateral neck compartments was 76% (95% specificity; 85% positive predictive accuracy, 91% negative predictive accuracy). There were 28 true positives, 92 true negatives, five false positives (15%) and nine false negatives (9%: 5 N₁; 4 N₂b).

For nodal palpation, there were 32 true positives, 92 true negatives, six false positives (16%) and 51 false negatives (36%). The sensitivity for detecting metastatic carcinomatous neck nodes was thus 39% (94% specificity; 84% positive predictive accuracy, 64% negative predictive accuracy).

{Tc⁹⁹m} (v) DMSA Planar Imaging

77 patients were studied (67 malignant; 10 benign). All were evaluated and imaged at the optimal imaging time at presentation (Figure 1A and Tables 21A-22A) and 36
patients were followed up after treatment (Table 23A).
There were no adverse reactions to Tc\(^{99m}\) (v) DMSA.

**Overall:** For patient planar imaging there were 68 true positives, 30 true negatives, 43 false positives and 17 false negatives. The **patient** planar sensitivity for detecting malignancy was therefore 80%, Table 36 (42% specificity; 61% positive predictive accuracy, 64% negative predictive accuracy). There was no difference in sensitivity for the patients scanned at both two and four hours although image quality was better at four hours. In 50 patients (51 tumours: squamous cell carcinomas (49), adenocarcinoma (1) and embryonal rhabdomyosarcoma (1)) where the histological differentiation of the tumour was available, out of the 38 true positives 11 were well differentiated, 22 were moderately well differentiated, three were poorly differentiated and two were undifferentiated tumours. Of the 13 false negatives, there were four well differentiated, six moderately well differentiated and three poorly differentiated tumours. In all patients with no evidence of malignancy, the normal biodistribution of Tc\(^{99m}\) (v) DMSA was confirmed with radioactivity observed in the lacrimal glands, nasal mucosa, blood pool, kidney and bladder.
<table>
<thead>
<tr>
<th>(%)</th>
<th>Clinical Examination</th>
<th>Scintigraphy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>92</td>
<td>80</td>
</tr>
<tr>
<td>Specificity</td>
<td>96</td>
<td>42</td>
</tr>
<tr>
<td>Positive Predictive Accuracy</td>
<td>96</td>
<td>61</td>
</tr>
<tr>
<td>Negative Predictive Accuracy</td>
<td>91</td>
<td>64</td>
</tr>
</tbody>
</table>

* Total number of studies = 158
Patient Imaging: At Presentation

For patient planar imaging of the 77 patients studied using Tc $^{99m}$ Tc DMSA scintigraphy, there were 50 true positives, eight true negatives, six false positives and 13 false negatives (79% sensitivity, 57% specificity; 89% positive predictive accuracy; 38% negative predictive accuracy) in contrast to clinical examination (94% sensitivity, 85% specificity; 97% positive predictive accuracy, 73% negative predictive accuracy).

Patient Imaging of Head and Neck Cancer: At Presentation
(59 malignant lesions; excluding distant metastases).

Sixty three patients were examined. The patient planar sensitivity for imaging primary head and neck cancer was 80% (33% specificity; 96% positive predictive accuracy, 8% negative predictive accuracy) compared with clinical examination (92% sensitivity, 67% specificity; 96% positive predictive accuracy, 44% negative predictive accuracy). There were 48 true positives (Figure 46), one true negative, two false positives and 12 false negatives. Of the 48 true positives, seven were strongly positive. The two false positive results occurred in two patients who had had radiotherapy to the larynx and who were imaged immediately after treatment (Figure 46). Of the 12 false negative
$^{99m}$Tc DMSA planar images in 3 patients with primary squamous carcinomas taken 2 hours post-injection. Positive accumulation of radioactivity is seen at the site of tumours in the right retromolar trigone (anterior, $T_4$ (A)), right lower buccal alveolus (right lateral, $T_4$ (B)) and larynx (right lateral, $T_2$ (C)). The fourth patient is a false positive. Uptake is seen in the larynx (anterior, (D)) following successful radiotherapy to a $T_3$ laryngeal tumour. Note the normal biodistribution in the lacrimal glands, nasal mucosa and the blood pool.
results, one patient had an occult primary (presumed head and neck) with an $N_{2b}$ cervical mass, another had a lymphoma of the cervical oesophagus and 10 patients had primary head and neck squamous carcinomas (1 $T_1$, 5 $T_2$, and 4 $T_3$ tumours).

**Primary Tumour Patient Imaging: At Presentation**

(63 patients).

For detecting patients with primary tumours, there were 40 true positives, four true negatives, two false positives and 17 false negatives. This gave a sensitivity of 70%, Table 37 (66% specificity; 95% positive predictive accuracy, 19% negative predictive accuracy) compared with clinical examination (90% sensitivity, 83% specificity; 96% positive predictive accuracy, 63% negative predictive accuracy). The two false positives have been described. Of the five extra false negatives, one patient had an occult primary (presumed head and neck) and the remaining four had nasopharyngeal (2 $T_3$), oropharyngeal ($T_3$) and laryngeal ($T_3$) primaries.

For detecting head and neck primary tumours using Tc $^{99m}$ (v) DMSA imaging (73 lesions; 59 malignant, 14 benign) there were 42 true positives, six true negatives, eight false positives and 17 false negatives. This gave a sensitivity of 71%, Tables 37-38 (43% specificity; 84% positive predictive accuracy, 26% negative predictive accuracy) compared with clinical examination (90%
Table 37
Tc$^{99m}$ (v) DMSA Planar Scintigraphy: Primary Site. (All tumours are squamous carcinomas unless otherwise stated).

<table>
<thead>
<tr>
<th>PRIMARY SITE</th>
<th>TRUE POSITIVE</th>
<th>TRUE NEGATIVE</th>
<th>FALSE POSITIVE</th>
<th>FALSE NEGATIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tongue and oral cavity (13)</td>
<td>$T_2(1), T_3(3), T_4(5)$</td>
<td>1</td>
<td>1</td>
<td>$T_2(4)$</td>
</tr>
<tr>
<td>Nasopharynx (4)</td>
<td>$T_2(1)$</td>
<td>1</td>
<td>-</td>
<td>$T_3(3)$</td>
</tr>
<tr>
<td>Oropharynx (6)</td>
<td>$T_3(1), T_4(3)$</td>
<td>-</td>
<td>-</td>
<td>$T_3(2)$</td>
</tr>
<tr>
<td>Hypopharynx (7)</td>
<td>$T_3(1), T_4(5)$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Larynx (14)</td>
<td>$T_1(1), T_2(1), T_3(1), T_4(6)$</td>
<td>-</td>
<td>2</td>
<td>$T_1(1), T_2(1)$, $T_3(3)$</td>
</tr>
<tr>
<td>Ear (2)</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Maxilla (1)</td>
<td>$T_3$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>* Lungs (5)</td>
<td>$T_2(3), T_3(1), T_4(1)$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oesophagus (1)</td>
<td>$T_4(1)$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Occult Primary (2) Presumed Head and Neck</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Embryonal Rhabdomyosarcoma (2)</td>
<td>$T_2(2)$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
TABLE 37 (Cont)

<table>
<thead>
<tr>
<th>PRIMARY SITE</th>
<th>TRUE POSITIVE</th>
<th>TRUE NEGATIVE</th>
<th>FALSE POSITIVE</th>
<th>FALSE NEGATIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma (1)</td>
<td>T4(1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(Oral Cavity)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma (cervical oesophagus) (1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Previous treated primary site.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No pathological evidence of recurrence (5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 (Pinna (1)</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tongue (2)</td>
<td>(Larynx)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nasopharynx (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data from 63 patients (73 lesions; 59 malignant, 14 benign).

Patient sensitivity: planar Scintigraphy, 70%; clinical examination, 90%
Tumour sensitivity: planar Scintigraphy, 71%; clinical examination, 90%

* Includes 1 second primary and 1 second occult primary.
### Table 38

Tc$^{99m}$ (v) DMSA Planar Imaging: Primary Tumours

Clinical Examination Vs Scintigraphy in 63 patients with 73 lesions (59 malignant, 14 benign)

<table>
<thead>
<tr>
<th>(%)</th>
<th>Clinical Examination</th>
<th>Scintigraphy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>90</td>
<td>71</td>
</tr>
<tr>
<td>Specificity</td>
<td>86</td>
<td>43</td>
</tr>
<tr>
<td>Positive Predictive Accuracy</td>
<td>96</td>
<td>84</td>
</tr>
<tr>
<td>Negative Predictive Accuracy</td>
<td>67</td>
<td>26</td>
</tr>
</tbody>
</table>
sensitivity, 86% specificity; 96% positive predictive accuracy, 67% negative predictive accuracy). The two extra true negatives were derived from patients with previously successfully treated head and neck sites and the six extra false positives occurred in four patients with five proven areas of periodontal disease and in one patient with an occipital arteriovenous malformation.

In those patients with a primary head and neck cancer, "T" staging was possible in 49 (51 tumours; 48 squamous cell carcinomas, two embryonal rhabdomyosarcomas and one adenocarcinoma for Tc $^{99m}$Tc (v) DMSA planar scintigraphy in this group there were 37 true positives and 14 false negatives (Table 39). Approximately 50% of T$_1$, T$_2$ and T$_3$ tumours were detected scintigraphically and this increased to 100% for T$_4$ tumours. On the basis of scintigraphy, one clinically occult tumour was detected but no other tumours were upstaged using current UICC criteria (UICC, 1987).

Cervical Metastases: Tc $^{99m}$Tc (v) DMSA Planar Imaging

67 patients were evaluated with 91 cervical lesions (83 malignant, eight benign). For patient planar imaging there were 13 true positives, 32 true negatives, seven false positives and 15 false negatives. This yielded a 46%
TABLE 39

Tc $^{99m}$ Tc (v) DMSA PLANAR SCINTIGRAPHY IN 49 PATIENTS
WITH 51 TUMOURS "T" STAGED USING CURRENT UICC CRITERIA*

<table>
<thead>
<tr>
<th>SCINTIGRAPHY</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRUE POSITIVES</td>
<td>1</td>
<td>6</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>FALSE NEGATIVES</td>
<td>1</td>
<td>5</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CLINICAL EXAMINATION</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRUE POSITIVES</td>
<td>2</td>
<td>10</td>
<td>14</td>
<td>22</td>
</tr>
<tr>
<td>FALSE NEGATIVES</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

All tumours were squamous cell carcinomas except three
(embryonal rhabdomyosarcoma (2),
adeno carcinoma (1))

*UICC, 1987
sensitivity (82% specificity; 65% positive predictive accuracy, 68% negative predictive accuracy) compared with clinical examination (89% sensitivity, 90% specificity; 86% positive predictive accuracy, 92% negative predictive accuracy). For lateral neck compartment imaging (Table 40) there were 16 true positives (3N₁; 1N₂a; 7N₂b; 5N₃), 88 true negatives, nine false positives (36%, Figure 47) and 21 false negatives (19%: 8 N₁; 13 N₂b). The sensitivity for scintigraphically detecting lateral neck compartments with metastatic carcinoma was therefore 43% (91% specificity; 64% positive predictive accuracy, 81% negative predictive accuracy) compared with clinical examination (76% sensitivity, 95% specificity; 85% positive predictive accuracy, 91% negative predictive accuracy).

For detecting malignant nodes in 134 lateral neck compartments (Table 41, 91 cervical lesions; 83 malignant, eight benign) there were 16 true positives, 89 true negatives, nine false positives (36%) and 67 false negatives (43%). The nodal sensitivity for cervical metastases was therefore 19% (91% specificity; 64% positive predictive accuracy, 57% negative predictive accuracy) in contrast to clinical examination (39% sensitivity, 94% specificity; 84% positive predictive accuracy, 64% negative predictive accuracy). Each side of the neck was reported as being
TABLE 40

TC $^{99m}$ Tc DMSA PLANAR SCINTIGRAPHY OF THE NECK:

SCINTIGRAPHY VS PALPATION IN 67 PATIENTS OF WHOM 29

(37 LATERAL NECK COMPARTMENTS) HAD CERVICAL METASTASES*

<table>
<thead>
<tr>
<th>EACH LATERAL NECK COMPARTMENT REPORTED SEPARATELY</th>
<th>SCINTIGRAPHY</th>
<th>PALPATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>SENSITIVITY (%)</td>
<td>43</td>
<td>76</td>
</tr>
<tr>
<td>SPECIFICITY (%)</td>
<td>91</td>
<td>95</td>
</tr>
<tr>
<td>POSITIVE PREDICTIVE ACCURACY (%)</td>
<td>64</td>
<td>85</td>
</tr>
<tr>
<td>NEGATIVE PREDICTIVE ACCURACY (%)</td>
<td>81</td>
<td>91</td>
</tr>
</tbody>
</table>

* There were 91 cervical lesions (83 malignant, eight benign). All metastases were squamous carcinoma except three which were adenocarcinoma. Data from 36 neck dissections (29 patients) and one post-mortem. Information on the other necks was obtained from clinical data supplemented by CAT evaluation (70 lateral neck compartments) and subsequent follow-up.
Tc $^{99m}$ (v) DMSA planar images taken 2 hours post-injection in 4 patients with palpable neck nodes. Positive accumulation of radioactivity is seen in a T$_2$ tongue (A), N$_2$ mass (B) with a necrotic centre (right lateral), T$_4$ pyriform sinus (C) N$_2$ mass (D, left lateral) and N$_3$ mass (E, left lateral) with a false negative occult primary. Note the false positive uptake in periodontal disease (F). The last patient with a T$_4$N$_0$ floor of mouth tumour (G) has false positive uptake in the contralateral neck (H).
TABLE 41

Tc^{99m} (v) DMSA PLANAR SCINTIGRAPHY OF NECK NODES:

SCINTIGRAPHY VS PALPATION IN 67 PATIENTS WITH

91 CERVICAL LESIONS* (83 MALIGNANT, 8 BENIGN)

<table>
<thead>
<tr>
<th>EACH LATERAL NECK COMPARTMENT REPORTED SEPARATELY</th>
<th>SCINTIGRAPHY</th>
<th>PALPATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>SENSITIVITY (%)</td>
<td>19</td>
<td>39</td>
</tr>
<tr>
<td>SPECIFICITY (%)</td>
<td>91</td>
<td>94</td>
</tr>
<tr>
<td>POSITIVE PREDICTIVE ACCURACY (%)</td>
<td>64</td>
<td>84</td>
</tr>
<tr>
<td>NEGATIVE PREDICTIVE ACCURACY (%)</td>
<td>57</td>
<td>64</td>
</tr>
</tbody>
</table>

* Data from 36 neck dissections (29 patients#, 59 nodes) and one post-mortem (one node). Information on the other patients was obtained from clinical data supplemented by CAT evaluation (70 lateral neck compartments) and subsequent follow-up.

# Seven patients had bilateral neck dissections
scintigraphically positive or negative. No attempt was made to ascribe a level or size to areas of increased radioactivity within the neck and it was never possible to discern more than one positive area of nodal uptake within an area of increased radioactivity on the image. Details such as normal capsular outline and extracapsular spread were never identified but in one patient with a fixed N3 cervical nodal mass with a proven necrotic centre, a central "cold" scintigraphic area compatible with central necrosis was clearly observed (Figure 47).

There were no scintigraphic features which were specific for malignancy. Of the nine nodal false positives (Table 22A), six occurred in five patients (six lateral neck compartments) with no palpable nodes while three occurred in three patients (three lateral neck compartments) with four palpable, presumed inflammatory, neck nodes. This latter uptake of radioactivity could not be distinguished in any way from the uptake of radioactivity which occurred in the region of malignant nodes.

For the detection of metastatic squamous carcinoma within the neck, the size of the nodal mass was an important factor and palpation was more efficient than scintigraphy in the detection of metastatic nodal masses (Table 42).
TABLE 42

TRUE POSITIVES AND FALSE NEGATIVES FOR SCINTIGRAPHY VS PALPATION RELATED TO THE RELATIVE SIZE OF NODE(S) OR NODAL MASSES IN 29 PATIENTS (37 LATERAL NECK COMPARTMENTS) WHO HAD CERVICAL METASTASES

<table>
<thead>
<tr>
<th>RELATIVE SIZE (cm)</th>
<th>SCINTIGRAPHY</th>
<th>PALPATION</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-3</td>
<td>3-6</td>
</tr>
<tr>
<td>TRUE POSITIVE</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>FALSE NEGATIVE</td>
<td>11</td>
<td>10</td>
</tr>
</tbody>
</table>

Information based on findings at palpation, CAT, and at pathology
Compared with information available from clinical examination, supplemented by CAT (and MRI and ultrasound in one patient) and by information from pathology, four clinically $N_0$ lateral neck compartments would have been upstaged by scintigraphy. One patient was correctly upstaged from $N_0$ to $N_1$, while two other patients (three lateral neck compartments) were upstaged from $N_0$ to $N_1$, when the true pathological neck status was $N_{2b}$. Of the last two patients who were upstaged both had bilateral neck disease. One had palpable contralateral ($N_1$) disease, the other did not.

**Distant Metastases: Tc $^{99m}$ (v) DMSA Planar Imaging**

For imaging patients with distant metastases (Table 43: four patients, 15 lesions; 10 malignant, five benign), there were two true positives (Figure 48), one false negative and one false positive. For tumour lesion scintigraphy, there were five true positives, three true negatives, two false positives and five false negatives. Of these, one false positive result was observed in a patient with an occult primary who exhibited positive uptake in an area of fibrous dysplasia of the maxilla while another was observed in a patient with an inflamed biopsy site (but no residual disease) in the right axilla following the removal of a metastases from an unknown primary (Figure 49). Of the five false
Patients with Distant Metastases who were examined and then imaged using Tc$^{99m}$ (v) DMSA planar Scintigraphy.

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Patient Details</th>
<th>Clinical Examination</th>
<th>Tc$^{99m}$ (v) DMSA planar Scintigraphy</th>
</tr>
</thead>
<tbody>
<tr>
<td>* 56</td>
<td>Distant metastases in Liver, pancreas and sigmoid colon (4 lesions)</td>
<td>+2 1 1 1</td>
<td>1 3 1 3</td>
</tr>
<tr>
<td>* 57</td>
<td>Occult primary metastatic mass left flank. Two bony metastases 3 lesions 2 malignant</td>
<td>+2 1 1 1</td>
<td></td>
</tr>
<tr>
<td>* 58</td>
<td>Occult primary metastatic mass right axilla. Also fibrous dysplasia of maxilla. 3 lesions (2 benign)</td>
<td>+2 2 1</td>
<td></td>
</tr>
<tr>
<td>71</td>
<td>Breast adenocarcinoma for bony metastases Hypophysectomy for severe bone pain. 5 lesions (1 benign, 4 malignant)</td>
<td>+4 4 1</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 43 (Cont)

15 lesions (10 malignant, 5 benign)

* Whole body scans
+ Inflammatory lesions excluded

TP = True Positive
TN = True Negative
FP = False Positive
FN = False Negative

All patients (except 71) had squamous carcinoma.
FIGURE 48

Head and neck planar images in a patient with bony metastases from a breast adenocarcinoma. Lesions in the skull, cervical spine, left clavicle and first rib are visualised with both Tc $^{99m}$ (v) DMSA (A) and Tc $^{99m}$-MDP (B).

Head and neck planar (left lateral) images in a patient with a solitary bony metastasis in the skull from an occult squamous carcinoma. The lesion is visualised with both Tc $^{99m}$ (v) DMSA (A) and Tc $^{99m}$-MDP (B).
On the left is a planar head and neck $^{131}$-MIBG image showing positive accumulation of radioactivity at the site of a functional Stage IV glomus jugulare (A). On the right is a coronal SPECT Tc $^{99m}$ (v) DMSA image in the same patient showing similar accumulation of radioactivity (B).
On the left is a posterior thoracic planar Tc $^{99m}$ (v) DMSA image in a patient with an occult squamous carcinoma. Positive accumulation of radioactivity is seen in an inflamed biopsy site (A) following complete removal of a metastasis in the right axilla. On the right is a right lateral head and neck image showing positive accumulation of Tc $^{99m}$ (v) DMSA not only in a recurrent T$_2$ laryngeal tumour (B) following radiotherapy but also in a benign occipital arteriovenous malformation (C).
negatives, three occurred in one patient with distant metastases in the liver, pancreas and sigmoid colon while the other two were undetected occult primaries.

**Benign Lesions: Tc $^{99m}$Tc (v) DMSA Planar Imaging**

In those patients with benign lesions who were clinically examined, there were no false positives (Table 44). For Tc $^{99m}$Tc (v) DMSA planar imaging there were three false positives and seven true negatives. Of the three false positives, one patient with a Stage IV glomus jugulare exhibited strongly positive Tc $^{99m}$Tc (v) DMSA uptake (planar and SPECT) and was also positive on both planar $^{131}$I-MIBG (Figure 49) and planar and SPECT $^{123}$I-MIBG imaging. The patient was subsequently treated with a therapeutic dose of $^{131}$I-MIBG. The other patient who had a glomus tympanicum (Stage I) was negative on planar and SPECT Tc $^{99m}$Tc (v) DMSA and $^{123}$I-MIBG imaging. She was subsequently treated by surgery. These patients with glomus tumours exhibited the normal CAT (both patients) and MRI (Glomus jugulare) features of glomus lesions.

Another patient who had a squamous papilloma of his oropharynx was negative on imaging the oral cavity but exhibited positive uptake in the region of the larynx which
Table 44

The patients with benign head and neck pathology who were studied using Tc$^{99m}$(v) DMSA planar imaging.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Sex (M,F) and Age (Years)</th>
<th>Pathology</th>
<th>*Tc$^{99m}$ DMSA imaging</th>
</tr>
</thead>
<tbody>
<tr>
<td>72</td>
<td>F, 60</td>
<td>+Glomus jugulare stage IV (Also had pituitary empty sella syndrome)</td>
<td>++(Planar and SPECT). Marked uptake in pituitary region</td>
</tr>
<tr>
<td>73</td>
<td>F, 32</td>
<td>+Glomus tympanicum stage I</td>
<td>-</td>
</tr>
<tr>
<td>74</td>
<td>F, 28</td>
<td>Inflammatory neck mass (4 x 2 cm)</td>
<td>-</td>
</tr>
<tr>
<td>75</td>
<td>M, 19</td>
<td>Infected Branchial cyst (5 x 3 cm)</td>
<td>-</td>
</tr>
<tr>
<td>76</td>
<td>M, 55</td>
<td>Infected Branchial cyst (3 x 2 cm)</td>
<td>- primary site + mandible (planar and SPECT).</td>
</tr>
<tr>
<td>77</td>
<td>M, 49</td>
<td>Acromegaly. Post-surgery and external beam radiotherapy</td>
<td>-</td>
</tr>
<tr>
<td>78</td>
<td>M, 39</td>
<td>Nasopharyngeal abscess (Indeterminate size)</td>
<td>-</td>
</tr>
<tr>
<td>79</td>
<td>M, 31</td>
<td>Oral cavity squamous papilloma (2 x 1 cm)</td>
<td>- primary site + larynx</td>
</tr>
<tr>
<td>80</td>
<td>F, 81</td>
<td>#Parotid pleomorphic adenoma (5 x 4 cm)</td>
<td>-</td>
</tr>
<tr>
<td>81</td>
<td>M, 58</td>
<td>Chronic laryngitis</td>
<td>-</td>
</tr>
</tbody>
</table>
TABLE 44 (Cont)

* ++ = strongly positive, + = positive, - = negative
+ also had Spect Tc$^{99m}$ DMSA and CAT
# Diagnosed on FNAB. Also had Ga$^{67}$-Citrate and Tc$^{99m}$-TcO$_4^-$ scans
was also present on the post-operative scan (see residual and recurrent disease). The last patient with a false positive result had a branchial cyst. He was negative on imaging the neck with both planar and SPECT Tc\(^{99m}\) (v) DMSA but positive for metastatic squamous cell carcinoma on CAT criteria. In addition, on both the planar and SPECT studies he exhibited positive uptake in the mandible (strongly positive on SPECT) in an area of known periodontal disease. The one patient with a pleomorphic adenoma of the parotid was negative not only with Tc\(^{99m}\) (v) DMSA but also Ga\(^{67}\)-Citrate and Tc\(^{99m}\)-TcO\(_4^-\) imaging. No patients exhibited bilateral symmetrical uptake of Tc\(^{99m}\) (v) DMSA in the salivary glands and the one patient with the Stage IV glomus jugulare and the empty pituitary sella syndrome exhibited positive uptake in the region of the pituitary gland.

In addition to the above, in those patients with malignancy, there were seven patients with a total of eight inflammatory palpable neck nodes, one patient had fibrous dysplasia of the maxilla, four patients had five proven areas of periodontal disease and three patients had inflamed operation or biopsy sites (Table 45 and Figure 49). One patient had an occipital arteriovenous malformation (Figure 49) and eight patients had no evidence of residual tumour at the primary site following initial treatment.
### TABLE 45

Tc $^{99m}$ (V) DMSA PLANAR SCINTIGRAPHY: BENIGN LESIONS (OVERALL)

<table>
<thead>
<tr>
<th>Lesion Type</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLOMUS TUMOURS</td>
<td>2</td>
</tr>
<tr>
<td>INFLAMMATORY NECK NODES OR MASSES</td>
<td>12</td>
</tr>
<tr>
<td>ACROMEGALY (POST-SURGERY AND SUBSEQUENT RADIOThERAPY)</td>
<td>1</td>
</tr>
<tr>
<td>NASOPHARYNGEAL ABSCESS</td>
<td>1</td>
</tr>
<tr>
<td>CHRONIC LARYNGITIS</td>
<td>1</td>
</tr>
<tr>
<td>SQUAMOUS PAPILLOMA OF THE ORAL CAVITY</td>
<td>1</td>
</tr>
<tr>
<td>PAROTID PLEOMORPHIC ADENOMA</td>
<td>1</td>
</tr>
<tr>
<td>FIBROUS DYSPLASIA OF THE MAXILLA</td>
<td>1</td>
</tr>
<tr>
<td>PERIODONTAL DISEASE</td>
<td>6</td>
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<tr>
<td>POST-OPERATIVE HAEMATOMA</td>
<td>1</td>
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<tr>
<td>INFLAMED BIOPSY OR OPERATION SITES</td>
<td>3</td>
</tr>
<tr>
<td>OCCIPITAL ARTERIOVENOUS MALFORMATION</td>
<td>1</td>
</tr>
</tbody>
</table>

**TRUE NEGATIVES** = 17

*FALSE POSITIVES* = 16

* One patient had two true negative sites (squamous papilloma of the oral cavity; pre and post-operation) with two false positive sites of uptake in the larynx.
Miscellaneous Imaging: At Presentation

Scintigraphy

Six patients had Tc\(^{99m}\)-MDP bone scans and one patient had an in-vitro labelled Tc\(^{99m}\) red blood cell (rbc) head and neck scan. Of those patients having Tc\(^{99m}\)-MDP scans, one had bony metastatic carcinoma from an occult primary (positive, Figure 48), while a second had bony metastatic breast adenocarcinoma (positive, Figure 48). Two had embryonal rhabdomyosarcomata (both positive), one had a T\(_2\) nasopharyngeal squamous carcinoma (negative) and one had fibrous dysplasia of the maxilla (positive). All five patients positive on Tc\(^{99m}\)-MDP scintigraphy had positive Tc\(^{99m}\) (v) DMSA scans (four strongly positive). One patient with an occult primary and metastatic neck mass (N\(_3\)) had a positive head and neck Tc\(^{99m}\) (v) DMSA scan but a negative Tc\(^{99m}\)-rbc scan.

Magnetic Resonance

Three patients had Tc\(^{99m}\) (v) DMSA planar scintigraphy, CAT and MRI at presentation and of these, two also had SPECT. One patient had a clinically T\(_3\)N\(_1\) squamous carcinoma which was negative (primary and neck) on planar scintigraphy but
positive on SPECT (neck only, N_1). CAT and MRI both
confirmed a T_3 primary mass, and CAT showed a node or nodal
mass in level 1 (4 x 2 cm, N_2a'). MRI showed three nodes in
level 1 (1.5 x 1, 1 x 1 and 1 x 1 cm respectively; N_2b',
Figure 50). Another patient had a clinically T_3N_0 squamous
carcinoma of the floor of the mouth which was T_3N_0 on CAT
and MRI but T_4 on pathology (mandibular invasion). The last
patient had a Stage IV glomus jugulare with intracranial
extension which was shown on both CAT and MRI. Positive
uptake at the primary site was seen not only with
Tc^{99m} (v) DMSA (planar and SPECT) but also I^{123}-MIBG (planar
and SPECT) and planar I^{131}-MIBG (Figure 49).

Ultrasound

The one patient with a clinically T_3N_1 (level 1)
nasopharyngeal carcinoma had neck ultrasound in addition
to Tc^{99m} (v) DMSA planar and SPECT scintigraphy, CAT and
MRI. Although negative on Tc^{99m} (v) DMSA planar
scintigraphy, SPECT clearly identified a metabolically active
lesion in the region of level 1, CAT identified a malignant
cervical lymph node (also in level 1), while MRI identified
three malignant nodes in level 1. Ultrasound of the neck
identified two separate lymph node masses in level 1
(Figure 50) which were positive for malignancy on size and
Tc $^{99m}$ DMSA SPECT images in the transaxial, coronal and sagittal planes in a patient with a clinical $T_3N_1$ squamous carcinoma of the nasopharynx. Strongly positive accumulation of radioactivity is seen at the site of known primary disease (A) and also at the site of the node or nodal mass at level 1 in the left lateral neck compartment (B).
CT (A), MRI (B) and ultrasound (C) images in the patient with a clinically T_{3}N_{1} squamous carcinoma (previous page). Although the CT scan (and SPECT Tc^{99m} (v) DMSA) demonstrated one node or nodal mass (arrowed), the MRI and ultrasound images demonstrated more than one node (arrowed) and therefore upstaged the neck to N_{2b}. 
loss of border outline. The neck was upstaged to N2b by both MRI and ultrasound. Although SPECT, CAT, MRI and ultrasound all correctly identified a node (or nodes) in level 1, there were no absolute features on any of the scans which could reliably distinguish benign from malignant disease (Figure 50).

\[ \text{Tc}^{99m} (v) \text{ DMSA: Planar VS SPECT} \]

Thirty four patients (31 with a history of head and neck malignancy and three patients with benign lesions, Table 22A and Figure 2A) were all examined clinically and then imaged at the optimal imaging time using Tc \( 99m \) (v) DMSA head and neck planar imaging and SPECT.

Overall, for detecting patients with head and neck malignancy using SPECT there were 27 true positives, two true negatives, three false positives and two false negatives (93% sensitivity, 40% specificity; 90% positive predictive accuracy, 50% negative predictive accuracy) compared with Tc \( 99m \) (v) DMSA planar imaging (22 true positives, two true negatives; two false positives and eight false negatives: 73% sensitivity, 50% specificity; 92% positive predictive accuracy, 20% negative predictive accuracy) and clinical examination and palpation (30 true positives, three true
negatives and one false positive: 100% sensitivity, 75% specificity; 97% positive predictive accuracy, 100% negative predictive accuracy). Of the three false positives using SPECT, two patients had recently received radiation treatment to squamous carcinomas of the tongue (one external beam and one iridium-192 implant) and both showed positive uptake in the tongue in the region of the previously treated tumour. One of these patients was thought to be clinically free of local recurrence while the other was thought to have local recurrence which subsequently turned out to be an area of radiation fibrosis. The former patient remains alive and well. The last false positive result occurred in a patient who had a highly vascular Stage IV glomus jugulare tumour. Of the two false negatives, one occurred in a patient with a T₂ tumour of the buccal mucosa and the other in a patient with a T₄ tumour of the retromolar trigone which had been positive on planar imaging.

For detecting primary lesions (33 patients, 37 lesions: 30 malignant, seven benign) using SPECT there were 24 true positives (22 strongly positive), two true negatives, seven false positives and four false negatives (Table 46 and Figure 51: 86% sensitivity, 22% specificity; 77% positive predictive accuracy, 33% negative predictive accuracy) compared with Tc^{99m} (v) DMSA Planar Imaging (21 true positives (five strongly positive), three true negatives, six false positives and seven false negatives:
<table>
<thead>
<tr>
<th>(%)</th>
<th>Clinical Examination</th>
<th>Planar Scintigraphy</th>
<th>SPECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>89</td>
<td>75</td>
<td>86</td>
</tr>
<tr>
<td>Specificity</td>
<td>89</td>
<td>33</td>
<td>22</td>
</tr>
<tr>
<td>Positive Predictive Accuracy</td>
<td>96</td>
<td>78</td>
<td>77</td>
</tr>
<tr>
<td>Negative Predictive Accuracy</td>
<td>73</td>
<td>30</td>
<td>33</td>
</tr>
</tbody>
</table>
Tc$^{99m}$ (v) DMSA SPECT studies in patients with primary squamous carcinomas. Strongly positive accumulation of radioactivity (arrowed) is seen at the site of maxillary (A), ear (B), hypopharyngeal (C) and laryngeal (D) tumours.
FIGURE 51 (cont)
75% sensitivity, 33% specificity; 78% positive predictive accuracy, 30% negative predictive accuracy) and clinical examination and palpation (25 true positives, eight true negatives; one false positive and three false negatives: 89% sensitivity, 89% specificity; 96% positive predictive accuracy, 73% negative predictive accuracy). Of the seven false positive results with SPECT, three have previously been mentioned. The other four occurred in three patients with four areas of proven periodontal disease. Of the four false negatives, two have been previously mentioned. The other two occurred in patients with a T3 nasopharyngeal primary and an occult primary squamous carcinoma.

In those patients with primary malignant tumours evaluated by Tc $^{99m}$ Tc DMSA planar imaging and SPECT, "T" staging was possible in 26 (24 carcinomas, two embryonal rhabdomyosarcomas). For SPECT (Table 47) there were three false negatives (one T2 (buccal mucosa), one T3 (nasopharynx) and one T4 retromolar trigone tumour (all detected clinically)) compared with planar imaging (six false negatives; three T2 (buccal mucosa, supraglottis and tongue) and three T3 (nasopharynx (2), tonsil (1)) and clinical evaluation (two false negatives; both nasopharynx (one T2 and one T3)).
TABLE 47

A COMPARISON OF CLINICAL EXAMINATION, PLANAR AND SPECT Tc $^{99m}$ (v) DMSA IMAGING IN 26 PATIENTS WITH "T" STAGEABLE PRIMARY HEAD AND NECK TUMOURS (24 CARCINOMAS, 2 EMBRYONAL RHABDOMYOSARCOMAS)

<table>
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</tr>
<tr>
<td>TP</td>
<td>1</td>
<td>5</td>
<td>7</td>
<td>11</td>
</tr>
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<td>FN</td>
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<th>T3</th>
<th>T4</th>
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</thead>
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<tr>
<td>PLANAR SCINTIGRAPHY</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>FN</td>
<td>0</td>
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<td>SPECT</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP</td>
<td>1</td>
<td>5</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>FN</td>
<td>0</td>
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<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

TP = True Positive  FN = False Negative
Based on data from 23 neck dissections, 30 CAT scans and subsequent follow up, the 34 patients (53 cervical masses: 47 malignant, seven benign; all lymphs nodes except one infected branchial cyst) were examined clinically and then imaged with planar and SPECT Tc $^{99m}$ (v) DMSA imaging.

For lateral neck compartment SPECT imaging there were 11 true positives (nine strongly positive), 45 true negatives, four false positives (Figure 52) and eight false negatives (Table 48. 58% sensitivity, 92% specificity; 73% positive predictive accuracy, 85% negative predictive accuracy) compared with Tc $^{99m}$ (v) DMSA planar imaging (seven true positives, 44 true negatives; five false positives, 12 false negatives; 37% sensitivity, 90% specificity; 58% positive predictive accuracy, 79% negative predictive accuracy) and palpation (13 true positives, 45 true negatives; four false positives and six false negatives; 68% sensitivity, 92% specificity; 76% positive predictive accuracy, 88% negative predictive accuracy). Of the four false positives, two patients had inflammatory neck nodes thought to be positive clinically and which exhibited positive Tc $^{99m}$ (v) DMSA uptake on planar imaging and which were shown subsequently to be benign. The two other false positives occurred in clinically N° necks although one of these patients subsequently received radiotherapy.
Tc $^{99m}$ Tc DMSA SPECT images in a patient with a $T_3N_1$ nasopharyngeal squamous carcinoma. Strongly positive accumulation of radioactivity is seen in the left lateral neck compartment (arrowed).

Tc $^{99m}$ Tc DMSA coronal SPECT image of a $T_3N_0$ laryngeal squamous carcinoma. Strongly positive accumulation of radioactivity is seen at the site of known primary disease (A) but also in the mid-cervical region (B) of the left lateral neck compartment.
Tc $^{99m}$ (v) DMSA SPECT images in a T$_4$ maxillary sinus carcinoma. False positive uptake of radioactivity is observed in the right lateral neck compartment (arrowed).

Transaxial Tc $^{99m}$ (v) DMSA SPECT image illustrating false positive uptake in the left lateral neck compartment (arrowed) following neck dissection and subsequent radiotherapy for metastases from a squamous carcinoma of the left pinna.
### TABLE 48

\( ^{99m} \text{Tc} \) \(^{99m} \text{Tc} \) DMSA IMAGING: CLINICAL EXAMINATION COMPARED WITH PLANAR VS SPECT SCINTIGRAPHY IN 34 PATIENTS WITH 54 CERVICAL MASSES (47 MALIGNANT, 7 BENIGN)

<table>
<thead>
<tr>
<th>Necks with metastatic carcinoma (%)</th>
<th>Clinical Examination</th>
<th>Planar Scintigraphy</th>
<th>SPECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>68</td>
<td>37</td>
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</tr>
<tr>
<td>Specificity</td>
<td>92</td>
<td>90</td>
<td>92</td>
</tr>
<tr>
<td>Positive predictive accuracy</td>
<td>76</td>
<td>58</td>
<td>73</td>
</tr>
<tr>
<td>Negative predictive accuracy</td>
<td>88</td>
<td>79</td>
<td>85</td>
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<table>
<thead>
<tr>
<th>Positive nodes within the neck (%)</th>
<th>Clinical Examination</th>
<th>Planar Scintigraphy</th>
<th>SPECT</th>
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</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>58</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Specificity</td>
<td>29</td>
<td>57</td>
<td>71</td>
</tr>
<tr>
<td>Positive predictive accuracy</td>
<td>78</td>
<td>70</td>
<td>85</td>
</tr>
<tr>
<td>Negative predictive accuracy</td>
<td>6</td>
<td>9</td>
<td>12</td>
</tr>
</tbody>
</table>

(Each side of the neck was reported separately)
to both sides of the neck and remains alive and well. Of the false negatives, there were four $N_1$, and four $N_{2b}$ necks compared with Tc$^{99m}$ (v) DMSA planar imaging (12 false negatives, five $N_1$; seven $N_{2b}$) and palpation (six false negatives, six $N_1$; Table 49).

For nodal SPECT imaging of each lateral compartment of the neck (51 lesions) there were 11 true positives, five true negatives, two false positives and 36 false negatives (Table 48. 30% sensitivity, 71% specificity; 85% positive predictive accuracy, 12% negative predictive accuracy) compared with Tc$^{99m}$ (v) DMSA planar imaging (seven true positives, four true negatives; three false positives and 40 false negatives; 15% sensitivity, 57% specificity; 70% positive predictive accuracy, 9% negative predictive accuracy) and palpation (18 true positives, two true negatives; five false positives and 29 false negatives; 38% sensitivity, 29% specificity; 78% positive predictive accuracy, 6% negative predictive accuracy). No nodes less than 1.5 cm were detected on SPECT and never more than one nodal mass within a neck was identified.

**Residual and Recurrent Disease**

Following initial staging and Tc$^{99m}$ (v) DMSA imaging (Tables 21-22A) patients were treated and the majority
**TABLE 49**

A COMPARISON OF PALPATION, PLANAR AND SPECT Tc$^{99m}$ (v) DMSA IMAGING IN 16 PATIENTS (19 LATERAL NECK COMPARTMENTS) WITH "N" STAGEABLE HEAD AND NECK CARCINOMA

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<td></td>
<td>SIZE (cm)</td>
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<td>3-6</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP</td>
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<td>3</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
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<td></td>
<td>SIZE (cm)</td>
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<td>3-6</td>
<td>&gt;6</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>2</td>
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<td>3</td>
</tr>
<tr>
<td>FN</td>
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<table>
<thead>
<tr>
<th></th>
<th>SPECT</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>SIZE (cm)</td>
<td>0-3</td>
<td>3-6</td>
<td>&gt;6</td>
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<td>TP</td>
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<td>FN</td>
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<td>3</td>
<td>0</td>
</tr>
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</table>

TP = True Positive  
FN = False Negative
followed up at regular intervals (range 0-41 months, mean 15) in the Head and Neck Oncology Clinic at Guy's Hospital (as per Table 23A). Most patients were treated by one of two head and neck surgeons (Mr. O. H. Shaheen and Mr. J. Hibbert) and there was no uniform policy as to the management of the N<sub>0</sub> neck. Within the group of 77 patients imaged with Tc<sup>99m</sup> (v) DMSA at presentation, 36 were followed up with Tc<sup>99m</sup> (v) DMSA planar scintigraphy (98 studies). Within this group, 16 patients (17 studies) were imaged immediately following surgery when it was not feasible to detect residual and recurrent disease by clinical examination. Fifteen of these patients, together with the remaining 20 patients (35 in total) were followed up after treatment to include clinical examination and Tc<sup>99m</sup> (v) DMSA planar scintigraphy (81 studies).

**Tc<sup>99m</sup> (v) DMSA Planar Scintigraphy: Immediate Post-Operative Period**

Sixteen patients were imaged (17 studies) immediately following surgery. Two patients also had Ga<sup>67</sup>-Citrate scans and of these one also had an MRI scan. One patient had both planar and SPECT Tc<sup>99m</sup> (v) DMSA scintigraphy. Of the 15 patients, six had microscopic positive margins and two had
macroscopic residual disease. All patients (except one) who had positive margins received post-operative radiotherapy.

Of those patients scanned immediately after surgery at the optimal imaging time, there were 12 positive scans (four strongly positive) and five negative scans. One patient was negative on planar scanning but positive on SPECT (Figure 53). All patients were imaged at two hours and, in addition, three patients were imaged at four hours which improved image quality since one patient was positive at two hours and strongly positive at four hours. Of those 12 patients with positive scans, eight had positive resection margins and, of these, seven had had bony manipulation or resection. Three patients had positive salivary uptake in the region of the parotid glands following radical neck dissection and, of these, two had had previous radiotherapy.

Tc$^{99m}$ (v) DMSA Scintigraphy: Residual and Recurrent Disease Following Treatment

35 patients (34 who had had malignancy; 31 squamous cell carcinomas, one adenocarcinoma, two embryonal rhabdomyosarcomas: one benign lesion (squamous papilloma)) were followed up following treatment to include clinical examination and Tc$^{99m}$ (v) DMSA scintigraphy (81 studies). Of these, 10 had had surgery alone, 16 had had surgery preceded or
Pre-operative Tc $^{99m}$ (Tc$^{99m}$) DMSA coronal SPECT image in a patient with a squamous carcinoma of the left external auditory meatus. Strongly positive accumulation of radioactivity (A) is seen at the site of the primary tumour which extended up into the zygoma, medially to the floor of the middle cranial fossa and inferiorly into the infratemporal fossa. At the time of operation, a clip was left on residual disease on middle fossa dura extending down into the infratemporal fossa. The post-operative Tc $^{99m}$ (Tc$^{99m}$) DMSA coronal SPECT image shows strongly positive accumulation of radioactivity (B) at the site of known residual disease but it is impossible to say whether it is in residual or recurrent tumour, inflammatory tissue or both.
followed by radiotherapy and eight had had radiotherapy alone (six external beam; two iridium-192 implants). One patient was treated by chemotherapy followed by radiotherapy.

For detecting patients with residual and recurrent disease using Tc $^{99m}$ (v) DMSA planar scintigraphy, there were 18 true positives (four strongly positive), 22 true negatives; 37 false positives and four false negatives (Table 50 and Figure 54. 82% sensitivity, 37% specificity; 33% positive predictive accuracy, 85% negative predictive accuracy) compared with clinical examination (20 true positives, 57 true negatives; one false positive, three false negatives: 87% sensitivity, 98% specificity; 95% positive predictive accuracy, 95% negative predictive accuracy). All images were acquired at the optimal imaging time and in addition, 21 were also acquired at four hours. This did not improve either the sensitivity or specificity of the investigation but did improve image quality (seven strongly positive scans). Of the 35 patients scanned, 19 (54%) exhibited uptake in the salivary glands following treatment (Figure 54) and, of these, 18 had received radiotherapy (17 external beam, two iridium-192 implants).

No patients developed second or third primaries. One patient subsequently developed contralateral neck disease which was treated by surgery and he later developed distant
<table>
<thead>
<tr>
<th>(%)</th>
<th>Planar Scintigraphy</th>
<th>Clinical Examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>82</td>
<td>87</td>
</tr>
<tr>
<td>Specificity</td>
<td>37</td>
<td>98</td>
</tr>
<tr>
<td>Positive Predictive Accuracy</td>
<td>33</td>
<td>95</td>
</tr>
<tr>
<td>Negative Predictive Accuracy</td>
<td>85</td>
<td>95</td>
</tr>
</tbody>
</table>
On the left is a false negative Tc $^{99m}$ (v) DMSA anterior planar image in a patient with a $T_4N_0$ squamous carcinoma of the floor of the mouth. He underwent radical surgery to include mandibular split and tracheostomy and subsequent myocutaneous flap repair. His post-operative Tc $^{99m}$ (v) DMSA planar image is on the right. Although there was microscopic residual disease left behind, the positive accumulation of radioactivity (B) far exceeds this and is still there following radiotherapy on the Tc $^{99m}$ (v) DMSA images (below) taken six months (C) and two years (D) later. The patient remains alive and well. Part of this accumulation of radioactivity is due to uptake in the salivary glands which occurred in approximately 50% of patients following radiotherapy (see overleaf).
Anterior Tc ${}^{99m}$ Tc planar image in a patient with a successfully treated T$_{1s}$ squamous carcinoma of the larynx. Two years following radiotherapy there is bilateral symmetrical accumulation of radioactivity in the submandibular salivary glands (arrowed). The patient remains alive and well.
Bony metastases. Three patients had post-mortem examinations.

Residual and Recurrent Disease: Miscellaneous Scans

For Tc $^{99m}$ (v) DMSA scintigraphy and the detection of residual and recurrent disease, of the false positive results four patients exhibited uptake in the sternoclavicular joint following neck dissection and, of these, one had uptake of Tc $^{99m}$ (v) DMSA in the region of both sternoclavicular joints and had had bilateral neck dissections (Figure 55). One of the patients was also imaged with Tc $^{99m}$-MDP. The Tc $^{99m}$ (v) DMSA scan showed positive uptake in the region of the right sternoclavicular joint and the Tc $^{99m}$-MDP scan confirmed positive uptake in the joint itself (Figure 55). The patient remains alive and well.

Of the two patients who had post-operative Ga $^{67}$ scans, both had had composite resections with pectoralis myocutaneous flap repair. One of these patients (who was diabetic) developed an inflammatory collection under the flap which was negative on Tc $^{99m}$ (v) DMSA planar scintigraphy but positive with Ga $^{67}$ (Figure 56). The other patient developed a large haematoma under the flap. This was negative on both Tc $^{99m}$ (v) DMSA and Ga $^{67}$ scintigraphy and the haematoma was clearly demonstrated on MRI (Figure 56).
Uptake of Tc $^{99m}$ (v) DMSA into the right sternoclavicular joint (A) following right radical neck dissection. The site of uptake is confirmed using Tc $^{99m}$- MDP (B).

Similar uptake of Tc $^{99m}$ (v) DMSA into both sternoclavicular joints following bilateral radical neck dissections.
Post-operative planar right lateral Tc $^{99m}$ Tc (v) DMSA and Ga $^{67}$ scans in a diabetic patient following commando procedure and pectoralis flap repair for a T$_4$N$_{2b}$ retromolar trigone lesion. An abscess under the flap is not detected with Tc $^{99m}$ (v) DMSA but can be seen (A) on the Ga $^{67}$ study.
Post-operative right lateral planar Tc $^{99m}$ (v) DMSA (A) and Ga $^{67}$ (B) scans in a patient following excision of a T$_4$N$_0$ tonsil carcinoma and pectoralis flap repair. A post-operative haematoma is not demonstrable on either scan but can clearly be visualised on MRI (C).
Of the patients followed up and imaged with Tc\textsuperscript{99m} (v) DMSA scintigraphy (three scans), two had CAT scans and two had MRI. One of the patients had both CAT and MRI while another had a follow-up CAT scan nine months later. One patient had follow-up CAT alone and one patient had follow-up Tc\textsuperscript{99m} (v) DMSA and Ga\textsuperscript{67} scintigraphy as well as an MRI scan (Figure 56). Of the three patients who had four CAT scans, there were three true positives (all detected or suspected clinically). One of these had locally recurrent embryonal rhabdomyosarcoma of the maxilla following chemotherapy (1 cm mass on CAT) which was also detected on MRI and planar Tc\textsuperscript{99m} (v) DMSA scintigraphy. He was subsequently treated with radiotherapy but developed local recurrence after six months which was suspected clinically and confirmed on CAT (3 cm mass). The other patient had recurrence in the left neck which was detected clinically and on CAT (3 x 1.5 cm mass) but not planar Tc\textsuperscript{99m} (v) DMSA scintigraphy. The last patient who had follow-up Tc\textsuperscript{99m} (v) DMSA, Ga\textsuperscript{67}-Citrate planar scintigraphy and MRI (and who developed a haematoma under a pectoralis major flap following composite resection) has been discussed above. One patient who had Tc\textsuperscript{99m} (v) DMSA scintigraphy and MRI follow-up had developed a 2 cm tongue recurrence following local resection with neck dissection and flap repair. This was detected clinically and on MRI but not on scintigraphy.
The one false positive CAT result occurred in a patient who had had a composite resection for a squamous carcinoma of the tongue. He subsequently developed progressive stridor six months later due to subglottic stenosis at the tracheostomy site and required total laryngectomy. A CAT scan at this time showed three nodes (positive on CAT for squamous cell carcinoma by size, central necrosis and peripheral enhancement criteria) but which were subsequently shown to be inflammatory. The patient remains alive and well.

"The Pituitary Sign"

In 19 patients (18 with malignancy, one with benign disease; age range 45-82 years, mean 68) positive uptake was observed in the region of the pituitary gland on planar scintigraphy and the site of localisation was confirmed on SPECT (Figure 57). In 11 of these patients who were subsequently followed-up, a positive pituitary sign was a constant finding (Figure 57). Eight of the patients were female (age range 45-82 years, mean 66) and 11 were male (age range 55-80 years, mean 69).

Of these 19 patients, 17 had no known pituitary pathology. One patient was imaged after having had a hypophysectomy for bone pain due to metastatic breast cancer, while another had the empty sella syndrome.
Uptake of Tc $^{99m}$ (v) DMSA in the region of the pituitary gland in a patient with a $T_4N_0$ floor of mouth squamous carcinoma. Positive accumulation of radioactivity is seen pre-operatively (A), immediately post-operatively (B), following radiotherapy six months later (C) and at follow-up 15 months after the operation (D).
Gallium-67 Citrate

Seventeen patients (16 with malignancy) were examined clinically and then imaged using Tc $^{99m}$ (v) DMSA and gallium-67 citrate planar head and neck scintigraphy (Figure 3A and Tables 21A and 24A). Of the 16 patients with malignancy, 15 had squamous carcinoma and one had a lymphoma of the cervical oesophagus. One patient had a pleomorphic adenoma of the parotid gland while two others with squamous carcinoma had proven periodontal disease (three lesions). The normal head and neck biodistribution of Ga $^{67}$ was observed with radioactivity seen in the lacrimal and salivary glands and the nasal mucosa (Figures 58-59).

For detecting patients with malignancy at presentation using Ga $^{67}$-Citrate, there were 15 true positives, one true negative and one false negative (94% sensitivity, 100% specificity; 100% positive predictive accuracy, 50% negative predictive accuracy) compared with clinical examination and palpation (16 true positives and one true negative: 100% sensitivity, 100% specificity; 100% positive predictive accuracy, 100% negative predictive accuracy) and Tc $^{99m}$ (v) DMSA planar scintigraphy (12 true positives (two strongly positive), four false negatives and one true negative: 75% sensitivity, 100% specificity; 100% positive predictive accuracy, 20% negative predictive accuracy).
Ga $^{67}$ and Tc $^{99m}$ (v) DMSA anterior planar scans in a patient with a $T_4 N_2 b$ squamous carcinoma of the right retromolar trigone. Positive accumulation of radioactivity at the site of known primary disease is seen on both studies. Below are two Ga $^{67}$ images showing positive accumulation of radioactivity in a patient with a $T_4 N_0$ squamous carcinoma of the pyriform sinus (A) and in a patient with a lymphoma of the cervical oesophagus (B).
Left lateral planar Ga$^{67}$ and Tc$^{99m}$ (v) DMSA images in a patient with an occult head and neck primary and a N$_2$b nodal mass in the left lateral neck compartment. The Ga$^{67}$ study shows positive accumulation of radioactivity (A) at the site of the nodal mass. There were two nodes palpable and four positive nodes demonstrated in the neck dissection specimen. The Tc$^{99m}$ (v) DMSA image (B) is a false negative study.
Including two patients followed up with both Ga$^{67}$-Citrate and Tc$^{99m}$ (v) DMSA planar scintigraphy (see residual and recurrent disease), the overall sensitivity for Ga$^{67}$ was 94% (67% specificity; 94% positive predictive accuracy, 67% negative predictive accuracy) compared with clinical examination and palpation (100% sensitivity and specificity; 100% positive and negative predictive accuracy) and Tc$^{99m}$ (v) DMSA planar scintigraphy (75% sensitivity, 100% specificity; 100% positive predictive accuracy, 43% negative predictive accuracy).

For detecting primary tumours using Ga$^{67}$-Citrate (Table 51), there were 13 true positives, four true negatives (Figure 5.8) and four false negatives. There were no false positives (76% sensitivity, 100% specificity; 100% positive predictive accuracy, 50% negative predictive accuracy) compared with examination and palpation (15 true positives, four true negatives and two false negatives: 88% sensitivity, 100% specificity; 100% positive predictive accuracy, 67% negative predictive accuracy) and Tc$^{99m}$ (v) DMSA planar scintigraphy (12 true positives (two strongly positive), one true negative, three false positives and five false negatives: 71% sensitivity, 25% specificity; 80% positive predictive accuracy, 17% negative predictive accuracy).
<table>
<thead>
<tr>
<th>PRIMARY TUMOURS</th>
<th>(%)</th>
<th>CLINICAL EXAMINATION</th>
<th>Ga $^{67}$</th>
<th>Tc $^{99m}$ (v) DMSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>SENSITIVITY</td>
<td>88</td>
<td></td>
<td>76</td>
<td>71</td>
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<tr>
<td>SPECIFICITY</td>
<td>100</td>
<td></td>
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<td>25</td>
</tr>
<tr>
<td>POSITIVE PREDICTIVE ACCURACY</td>
<td>100</td>
<td></td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>NEGATIVE PREDICTIVE ACCURACY</td>
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<td>50</td>
<td>17</td>
</tr>
<tr>
<td>(%)</td>
<td>CLINICAL EXAMINATION</td>
<td>Ga $^{67}$</td>
<td>Tc $^{99m}$ (v)</td>
<td>DMSA</td>
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</tr>
<tr>
<td>SENSITIVITY</td>
<td>81</td>
<td>50</td>
<td>38</td>
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<tr>
<td>SPECIFICITY</td>
<td>94</td>
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</tr>
<tr>
<td>POSITIVE PREDICTIVE ACCURACY</td>
<td>93</td>
<td>100</td>
<td>75</td>
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<tr>
<td>NEGATIVE PREDICTIVE ACCURACY</td>
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<td>69</td>
<td>58</td>
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</table>
Cervical Metastases: Gallium-67 Citrate

Seventeen patients were studied and each lateral neck compartment was reported separately. For identifying lateral neck compartments with metastatic squamous cell carcinoma using Ga\textsuperscript{67}, there were eight true positives (0-3cm (4), 3-6cm (3) and > 6 cm (1), Figure 59), 18 true negatives and eight false negatives (0-3cm (4), 3-6cm (4)). There were no false positives (Table 51. 50% sensitivity, 100% specificity; 100% positive predictive accuracy, 69% negative predictive accuracy) compared with examination and palpation (13 true positives (0-3cm (5), 3-6cm (6), > 6cm (1)), 17 true negatives, one false positive and three false negatives (0-3cm (3)): 81% sensitivity, 94% specificity; 93% positive predictive accuracy, 85% negative predictive accuracy) and Tc\textsuperscript{99m} (v) DMSA planar scintigraphy (six true positives (3-6cm (5), > 6cm (1)), 14 true negatives, two false positives and 10 false negatives (0-3cm (5), 3-6cm (5)): 38% sensitivity, 88% specificity; 75% positive predictive accuracy, 58% negative predictive accuracy).

Within these 34 lateral neck compartments (based on data from 12 neck dissections, 12 CAT scans and subsequent clinical follow-up) there were 38 cervical lymph node masses
(two benign, 36 malignant). For identifying individual lymph node masses using Ga$^{67}$-Citrate, there were eight true positives, two true negatives and 28 false negatives (22% sensitivity, 100% specificity; 100% positive predictive accuracy, 7% negative predictive accuracy) compared with examination and palpation (17 true positives, two false positives and 19 false negatives: 47% sensitivity, 0% specificity; 89% positive predictive accuracy; 0% negative predictive accuracy) and Tc$^{99m}$ (v) DMSA planar scintigraphy (six true positives, one true negative; one false positive and 30 false negatives: 17% sensitivity, 50% specificity; 86% positive predictive accuracy, 3% negative predictive accuracy). In none of the lateral neck compartments evaluated was Ga$^{67}$ scintigraphy able to identify more than one nodal mass (Figure 59).

**Computerised Axial Tomography**

Fifty four patients (51 with malignancy (52 tumours); three with benign lesions) were all examined clinically and then evaluated with Tc$^{99m}$ (v) DMSA planar scintigraphy and CAT (Figure 4A and Table 21A). Of the 54 patients, 52 had head and/or neck scans and, of these, one also had chest CAT. The last two patients had CAT of the chest alone.
Evaluation Overall

For the ability to detect patients with malignancy at presentation, there were 49 true positives, three true negatives and two false positives (100% sensitivity, 60% specificity; 96% positive predictive accuracy, 100% negative predictive accuracy) compared with clinical examination (49 true positives, three true negatives and two false positives: 100% sensitivity, 60% specificity; 96% positive predictive accuracy, 100% negative predictive accuracy) and $\text{Tc}^{99m}$ (v) DMSA planar scintigraphy (40 true positives, one true negative; four false positives and nine false negatives: 82% sensitivity, 20% specificity; 91% positive predictive accuracy, 10% negative predictive accuracy).

For overall detection of malignancy (including residual and recurrent disease; two patients had follow-up CAT scans), there were 51 true positives (100% sensitivity; 96% positive predictive accuracy) compared with clinical examination (50 true positives and one false negative; 98% sensitivity, 75% negative predictive accuracy) and $\text{Tc}^{99m}$ (v) DMSA planar scintigraphy (41 true positives and 10 false negatives; 80% sensitivity, 9% negative predictive accuracy).
Primary Lesions
(48 malignant primary tumours, six benign lesions).

There were 43 true positives (Figure 60), five true negatives, one false positive (Figure 60) and five false negatives. This gave a sensitivity of 90%, Table 52, (80% specificity; 96% positive predictive accuracy, 50% negative predictive accuracy) compared with clinical examination (43 true positives, five true negatives; one false positive and five false negatives: 90% sensitivity, 83% specificity; 98% positive predictive accuracy, 50% negative predictive accuracy) and Tc$^{99m}$ (v) DMSA planar scintigraphy (35 true positives, five true negatives; two false positives and 12 false negatives: 75% sensitivity, 71% specificity; 95% positive predictive accuracy, 29% negative predictive accuracy).

The CAT features of the patterns of malignant spread of all the primary tumours, as well as recurrent disease following radiotherapy, were confirmed and no previously undescribed findings were noted (Section 1.3.3.).

The one false positive result occurred in a patient with a T$_2$ glottic carcinoma treated with radiotherapy.
Transaxial CAT scans showing squamous carcinomas (arrowed) of the larynx (T\textsubscript{3}; A), hypopharynx (T\textsubscript{3}; B) and hypopharynx (T\textsubscript{4}; C). Both patients in (B) and (C) were clinically T\textsubscript{3} and pathologically T\textsubscript{4}. CAT correctly upstaged (C) by demonstrating cartilage invasion (arrowed). Study (D) is a false positive result showing thickening and irregularity and areas of central necrosis (arrowed) in the vocal cords following radiotherapy to a T\textsubscript{2} larynx.
TABLE 52

COMPUTERISED AXIAL TOMOGRAPHY: A COMPARISON OF CLINICAL EXAMINATION, Tc\textsuperscript{99m} (v) DMSA PLANAR SCINTIGRAPHY AND CAT IN 54 PRIMARY LESIONS (48 MALIGNANT PRIMARY TUMOURS, 6 BENIGN LESIONS)

<table>
<thead>
<tr>
<th>(%)</th>
<th>Clinical Examination</th>
<th>Scintigraphy</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>90</td>
<td>75</td>
<td>90</td>
</tr>
<tr>
<td>Specificity</td>
<td>83</td>
<td>71</td>
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</tr>
<tr>
<td>Positive Predictive Accuracy</td>
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<td>98</td>
</tr>
<tr>
<td>Negative Predictive Accuracy</td>
<td>50</td>
<td>29</td>
<td>50</td>
</tr>
</tbody>
</table>
Following treatment, the patient remained hoarse with an oedematous larynx and a CAT scan suggestive of recurrent disease (Figure 60). In the absence of pain, treatment was expectant and the patient remains alive and well. Of the five true negatives, two were glomus tumours and three had primary sites (nasopharynx (1) and tongue (2)) previously treated by radiotherapy. All of these, but one, were negative on clinical examination.

Of those patients with false negative CAT scans for a primary head and neck tumour, two had presumed occult head and neck primaries and the other three had $T_2$ tumours of the floor of the mouth, buccal mucosa and lateral border of the tongue that had been detected clinically. Four patients had had previous successful radiotherapy to primary sites, two had ear tumours and one patient had a lymphoma of the cervical oesophagus. In the remaining 42 patients (43 tumours) there were two $T_1$, 10 $T_2$, 13 $T_3$ and 18 $T_4$ tumours. In these 43 tumours, CAT primary tumour staging correlated with the clinical findings in 21, and with the clinical and pathological findings in 11. In the remaining 11 tumours, three patients with clinically occult nasopharyngeal primaries were staged $T_2$ (1) and $T_3$ (2). Based on subsequent pathological information four tumours were correctly
upstaged ($T_3$ to $T_4$ (larynx (2)); $T_3$ to $T_4$ (hypopharynx (2)) Figure 60). In the remaining four patients, the CAT findings were in concordance with clinical evaluation but based on subsequent information from four pathological sections, CAT had understaged the tumours ($T_3$ floor of mouth (1); $T_3$ tonsil (1); $T_3$ hypopharynx (2), Figure 60).

**Computerised Axial Tomography: Cervical Metastases**

The following information is based on clinical examination in all patients supplemented by pathological examination in 26 patients (31 neck dissections, one post-mortem). All patients were followed up as per Table 23A. Five patients had received previous radiotherapy to the neck and a further nine had a neck dissection followed by radiotherapy. Three received radiotherapy to the neck alone.

The normal CAT anatomical distribution of the lymph nodes of the head and neck was confirmed (Section 1.4.3.) and no new lymph nodes or lymph node groups were identified.

Of the 54 patients studied, 51 had a CAT scan of the neck. For identifying patients with metastatic neck cancer, there were 21 true positives, 23 true negatives; four false
positives and three false negatives. This gave a sensitivity of 88% (85% specificity; 84% positive predictive accuracy, 88% negative predictive accuracy) compared with palpation (20 true positives, 24 true negatives; three false positives and four false negatives: 83% sensitivity, 89% specificity; 87% positive predictive accuracy, 86% negative predictive accuracy) and Tc$^{99m}$ (v) DMSA planar scintigraphy (11 true positives, 22 true negatives; six false positives, 12 false negatives: 48% sensitivity, 79% specificity; 65% positive predictive accuracy, 65% negative predictive accuracy).

Each lateral compartment of the neck was reported as a separate site. For the 102 lateral neck compartments the ability of CAT to identify lateral neck compartments with metastatic carcinoma yielded an 81% sensitivity (Table 53, 94% specificity; 86% positive predictive accuracy, 92% negative predictive accuracy). There were 25 true positives, 67 true negatives; four false positives (14%) and six false negatives (8%) compared with palpation (22 true positives, 67 true negatives; four false positives (15%) and nine false negatives (12%): 71% sensitivity, 94% specificity; 85% positive predictive accuracy; 88% negative predictive accuracy) and Tc$^{99m}$ (v) DMSA scintigraphy (15 true positives, 64 true negatives; seven false positives and 16 false negatives: 48% sensitivity, 90% specificity; 68% positive predictive accuracy, 80% negative predictive accuracy).
TABLE 53

COMPUTERISED AXIAL TOMOGRAPHY: A COMPARISON OF THE ABILITY OF CLINICAL EXAMINATION, Tc^{99m} (v) DMSA PLANAR SCINTIGRAPHY AND CAT TO DETECT METASTATIC CARCINOMA IN THE NECK

<table>
<thead>
<tr>
<th>(%)</th>
<th>Clinical Examination</th>
<th>Scintigraphy</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>71</td>
<td>48</td>
<td>81</td>
</tr>
<tr>
<td>Specificity</td>
<td>94</td>
<td>90</td>
<td>94</td>
</tr>
<tr>
<td>Positive Predictive Accuracy</td>
<td>85</td>
<td>68</td>
<td>86</td>
</tr>
<tr>
<td>Negative Predictive Accuracy</td>
<td>88</td>
<td>80</td>
<td>92</td>
</tr>
</tbody>
</table>

Each lateral neck compartment reported as a separate site

Data from 51 patients
There were 71 clinically $N_0$ lateral neck compartments and of these, five (four patients) were upstaged by CAT. One patient was correctly upstaged to $N_1$ while another was correctly upstaged from $N_0$ to $N_{2c}$ (i.e. $2N_{2b}$). Both patients subsequently died of their neck disease without further treatment. The other two patients were false positives. One had a floor of mouth tumour with an obstructed submandibular gland which was thought to be clinically benign. CAT showed a mass in level two positive for malignancy by size, peripheral enhancement and central necrosis criteria. The patient subsequently had excision of the primary lesion with bilateral functional neck dissections (which were clear of disease) and free flap repair. He remains well and disease free. The other patient with a false positive CAT result had a branchial cyst which was presumed to be infected since only inflammatory cells were obtained on FNAB. CAT showed a cervical mass which was positive for metastatic squamous cell carcinoma on size, peripheral enhancement and central necrosis criteria. Treatment was expectant and the patient remains alive and well.

Of the four false positive results, two have previously been described and both these patients had palpable masses which were thought to be benign. The other two patients also had palpable cervical lymphadenopathy (clinically $N_1$)
and both had positive lymph nodes by CAT criteria (N₁ and N₂b respectively). Both subsequently had neck dissections and were found to have pathologically N₀ necks.

There were six false negative necks (four N₁; two N₂b). Of these, five were operated on immediately after CAT scanning while the sixth had a second neck dissection nine months following initial treatment to include primary resection of a hypopharyngeal T₄ lesion and left radical neck dissection without post-operative radiotherapy. In all the false negative necks no "normal" nodes were identified except in one patient who had two nodes (less than 1 cm) in level 2 on CAT and who, on subsequent pathological evaluation of the neck dissection specimen, was found to have eight nodes in level 2 (all negative) and eight in level 3 (one of which was positive).

In two patients (two lateral neck compartments) carcinoma was correctly diagnosed by CAT but based on information from subsequent pathological examinations and ultrasound evaluation, these necks had been understaged. One patient with a clinically T₃N₁ tumour of the nasopharynx was upstaged to T₃N₂a on CAT. MRI showed three lymph nodes and ultrasound of the neck clearly showed two separate lymph node masses all positive for malignancy and the patient was therefore
upstaged to $T_3N_2b$ (Figure 50). Another patient was $N_1$ by clinical and CAT criteria but subsequent pathological examination of the neck dissection specimen confirmed $N_2b$ status.

Three clinically positive necks were downstaged by CAT. One was downstaged from $N_2b$ to $N_1$ and one from $N_1$ to $N_0$. The last patient was clinically $N_2c$ (i.e. $N_2b$; $N_1$) and was downstaged to $N_0$; $N_1$ by CAT criteria. He subsequently died of his primary disease with metastatic disease in the left neck and with no clinical evidence of disease in the right neck.

Thirty eight malignant nodes were detected on CAT. No retropharyngeal, occipital, postauricular, facial, juxtavisceral or anterior jugular nodes were identified. Twenty six were positive by size, peripheral enhancement and central necrosis criteria, two were positive by peripheral enhancement and central necrosis, eight were positive by size and peripheral enhancement and two were positive by size alone (Figure 61). Of these last two, one measured 5 x 5 cm on CAT while the other patient (who had not received intravenous contrast) had a node measuring 2 x 2 cm.

There were five false positive nodes on CAT criteria. Four were falsely positive by size, central necrosis and peripheral enhancement criteria (Figure 61) while two
Transaxial CAT scans showing tomographically positive nodes for carcinoma. Nodes (arrowed) were positive on size, peripheral enhancement and central necrosis criteria (A), size and enhancement (B) and size alone (C). Scan (D) shows a branchial cyst (arrowed) falsely positive for malignancy on size, peripheral enhancement and central necrosis criteria.
measuring 1 cm each were falsely positive by central necrosis and peripheral enhancement criteria.

Metastatic Neck Carcinoma: A Clinical, Scintigraphic, Computerised Axial Tomographic and Pathological Study

Within the group of patients that had Tc $^{99m}$ (v) DMSA planar scintigraphic evaluation, there was a subgroup of 26 patients who were all examined clinically, imaged at the optimal imaging time with Tc $^{99m}$ (v) DMSA, and who then had CAT followed by formal surgery to include neck dissection (Table 25A). Of the 26 patients, 25 had had squamous carcinoma and one had adenocarcinoma. The primary tumour sites are listed in Table 54. In 18 patients, primary tumour staging was possible and there were three T$_2$, four T$_3$ and 11 T$_4$ cancers. Twenty one had unilateral neck dissections, four had simultaneous bilateral neck dissections and one patient had staged neck dissections nine months apart.

In all, 31 neck dissections were performed on 26 patients. In 15 of these lateral neck compartments nodes were palpable and of these 12 were found to be pathologically positive. In 16 lateral neck compartments nodes were not palpable and seven of these were shown to contain metastatic carcinoma.
**TABLE 54**

**PRIMARY TUMOUR SITES IN 26 PATIENTS WHO SUBSEQUENTLY UNDERWENT A TOTAL OF 31 NECK DISSECTIONS**

<table>
<thead>
<tr>
<th>Tumour Site</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasopharynx</td>
<td>1</td>
</tr>
<tr>
<td>Retromolar Trigone</td>
<td>1</td>
</tr>
<tr>
<td>Buccal Mucosa</td>
<td>2</td>
</tr>
<tr>
<td>Floor of Mouth</td>
<td>3</td>
</tr>
<tr>
<td>Tongue</td>
<td>6</td>
</tr>
<tr>
<td>Tonsil</td>
<td>1</td>
</tr>
<tr>
<td>Supraglottic Larynx</td>
<td>2</td>
</tr>
<tr>
<td>Transglottic Larynx</td>
<td>1</td>
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<tr>
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<tr>
<td>Pyriform Sinus</td>
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<tr>
<td>Ear</td>
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<tr>
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<td>3</td>
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</table>
A total of 728 lymph nodes were examined pathologically and of these 49 were malignant. Excluding three patients on whom there was insufficient information to evaluate the total number of nodes sampled, 725 nodes (range 4-62, mean 23) were obtained from 28 neck dissections.

On clinical examination the sensitivity for detecting which lateral compartments necks contained metastatic carcinoma was 63% (75% specificity; overall accuracy 68%, Figure 62). There were 12 true positives, nine true negatives; three false positives (20%) and seven false negatives (44%) compared with nodal sensitivity (31%; 15 true positives, 34 false negatives: 83% positive predictive accuracy, 21% negative predictive accuracy).

For Tc\textsuperscript{99m} (v) DMSA planar scintigraphy, the sensitivity for detecting which lateral neck compartments contained metastatic carcinoma was 42% (58% specificity; overall accuracy 48%, Figure 62). There were eight true positives (one strongly positive), seven true negatives; five false positives (38%) and 11 false negatives (61%) compared with nodal sensitivity (16%. eight true positives (one strongly positive) and 41 false negatives; 15% negative predictive accuracy).
A comparison of patient sensitivity, specificity and accuracy for detecting metastatic carcinoma in the neck using clinical examination, Tc\textsuperscript{99m}(v) DMSA Planar Scintigraphy and Computerised Axial Tomography.

Data from 31 Neck dissections
For computerised axial tomography the sensitivity for detecting which lateral neck compartments contained metastatic carcinoma was 68% (75% specificity; overall accuracy 71%, Figure 62). There were 13 true positives, nine true negatives; three false positives (19%) and six false negatives (40%) compared with nodal sensitivity 41% (20 true positives, 29 false negatives and five false positives; 64% specificity; 80% positive predictive accuracy, 24% negative predictive accuracy). Of the 20 nodes detected on CAT and subsequently proven to be histologically positive for carcinoma, 15 were positive on size, peripheral enhancement and central necrosis criteria, two were positive on size and peripheral enhancement criteria and two were positive on peripheral enhancement and central necrosis criteria. Only one node was positive on size criteria alone.

Overall, 13% of lateral neck compartments had their staging correctly changed by Tc $^{99m}$ (v) DMSA planar scintigraphy (Tables 55 and 25A. $N_0$ to $N_1$ (3); 10% (19% of clinically lateral compartment $N_0$ necks): $N_1$ to $N_0$ (1; 3%). Of these lateral $N_0$ neck compartments which were upstaged, two were understaged (actual status $N_{2b}$). Based on the number of neck nodes detected on pathology, in those five lateral neck compartments with $N_1$ palpable disease correctly identified as $N_1$ by Tc $^{99m}$ (v) DMSA scintigraphy, three had been understaged (correct stage $N_{2b}$).
<table>
<thead>
<tr>
<th></th>
<th>CLINICAL EXAMINATION VS TC (99m^t) (V) DMSA PLANAR SCINTIGRAPHY IN 26 PATIENTS</th>
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<tbody>
<tr>
<td>NUMBER OF LATERAL</td>
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<td>NECK COMPARTMENTS</td>
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<td>CLINICALLY NORMAL NECKS</td>
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* (31 LATERAL NECK COMPARTMENTS)
TABLE 55 (CONT)

+ = positive or abnormal
- = negative or normal
* Data from 31 neck dissections
In 16 clinically N₀ lateral neck compartments, Tc⁹⁹m (v) DMSA planar scintigraphy was in agreement with the clinical findings in six (38%). Although upstaging occurred in three (19%), three (19%) were incorrectly upstaged to N₁, while four (25%) had positive disease which was missed by scintigraphy.

Overall, 10% of lateral neck compartments had their staging correctly changed by CAT (Tables 56 and 25A, N₀ to N₁ (1); 3% (6% of clinically lateral compartment N₀ necks): N₁ to N₂b (1); 3%: N₁ to N₀ (1); 3%). Of these, one was correctly upstaged to N₁, one was correctly downstaged and one clinically N₀ lateral neck compartment which was CAT N₁ was subsequently shown to be pathologically N₂b*. Based on the number of nodes detected on pathology, CAT had understaged four necks (N₁. actual status N₂b (3); N₂a. actual status N₂b (1); 13%).

In 16 clinically N₀ lateral neck compartments, CAT was in agreement with the clinical findings in 50%. Although upstaging occurred in 6%, one patient (6%) was incorrectly upstaged to N₁ while CAT missed positive disease in six patients (38%).
**TABLE 56**

**CLINICAL EXAMINATION VS CAT IN 26 PATIENTS (31 LATERAL NECK COMPARTMENTS)**

<table>
<thead>
<tr>
<th>NUMBER OF LATERAL NECK COMPARTMENTS</th>
<th>CLINICAL</th>
<th>CAT</th>
<th>PATHOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CLINICALLY PALPABLE NODES</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2</td>
<td>+</td>
<td>+</td>
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<tr>
<td>1</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
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<tr>
<td>CLINICALLY NORMAL NECKS</td>
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<tr>
<td>6</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>1</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = positive or abnormal
- = negative or normal

* Data from 31 neck dissections
In two clinically N0 lateral neck compartments with proven occult disease, Tc $^{99m}$ (v) DMSA planar scintigraphy correctly upstaged the neck while CAT did not.
4.2.6. DOSIMETRY

For the dosimetric calculations a combination of both animal and human data was used. A compartmental model was constructed (Figure 5A) using the whole body (WB), kidney and bladder as source organs. Data from six patients was used. The whole body cumulative activity ($\bar{A}$) was calculated using the human whole body retention results (i.e. $WB = 100 - \sum \%$ injected dose excreted; Section 4.2.1., Figure 6A). Kidney $\bar{A}$ (as a fraction of the injected dose) was estimated using the rabbit kidney biodistribution data (Section 4.1.2., Figure 6A) correcting for bone uptake and kidney mass in rabbits (assuming minimal bone uptake in humans). The rabbit bone mass was 137 g/kg body weight (Section 4.1.6., Figure 5A). Estimation of kidney uptake assumes all radioactivity detected was due to uptake with no washout occurring during the study. Bladder $\bar{A}$ was calculated from the area under a plot of the radioactivity in the bladder (expressed as a fraction of the injected dose) against time (Figure 6A).

The cumulative activities for whole body, kidney and bladder are shown in Figure 6A. Using the "S" values for Tc $^{99m}$ for whole body, kidney and bladder from the MIRD (Medical Internal Radiation Dose; Synder et al, 1975) tables,
the absorbed dose ($D$ (i.e. $\tilde{A}$S)) for each of the organs in Table 26A was calculated (Figure 7A).

The effective dose equivalent was then calculated (Figure 7A) from the sum of the products of the weighting factors ($W_T$) for each target organ and the absorbed dose ($\tilde{A}$S) to that target organ from each of the three source organs, i.e.

$$\text{effective dose equivalent} =$$

$$\left( \sum W_{\text{target organ}} \times S_{\text{WB} \rightarrow \text{target organ}} \right) \tilde{A}_{\text{WB}}$$

$$+ \left( \sum W_{\text{target organ}} \times S_{\text{bladder} \rightarrow \text{target organ}} \right) \tilde{A}_{\text{bladder}}$$

$$+ \left( \sum W_{\text{target organ}} \times S_{\text{kidney} \rightarrow \text{target organ}} \right) \tilde{A}_{\text{kidney}}$$

**Effective dose equivalent** = 5.1 uSv/MBq (mean from six patients)
4.2.7. RADIOPHARMACEUTICAL STABILITY

The purity and stability of the Tc\textsuperscript{99m} (v) DMSA preparations injected into humans were confirmed in a similar manner to the method described in Section 4.1.9.
DISCUSSION

Pharmacokinetics

Tc $^{99m}$ (v) DMSA has a fast bi-exponential blood clearance and cumulative urine excretion in both rabbits and humans, and these are faster than those observed with Tc $^{99m}$ (III) DMSA or Tc $^{99m}$-TcO$_4^-$ (Watkinson, 1987). Blood clearance is dependant upon the amount of red cell and plasma protein binding, the volume of distribution and on the mechanisms of renal clearance. Tc $^{99m}$ (v) DMSA has a large blood pool component in rabbits and humans, with the majority of activity probably loosely bound to plasma proteins with minimal activity in, or on, red blood cells (Avtar Bharij, Personal Communication, 1989). Renal clearance is dependent, not only on the volume of distribution, but also on molecular size, lipid solubility and plasma protein binding. Tc $^{99m}$ (v) DMSA is a larger molecule than either Tc $^{99m}$ (III) DMSA or Tc $^{99m}$-TcO$_4^-$. It has a low lipid solubility, a negative charge and a pentavalent core in which the sulfhydryl groups of separate dimercaptosuccinic acid molecules possibly bind to each other (Avtar Bharij, Personal Communication, 1989). This prevents them binding to the metallothionein binding protein in the proximal
tubules of the kidney, and consequently the larger non-lipid soluble complex is probably excreted by glomerular filtration in a similar manner to a Tc $^{99m}$ Diethylenetriaminepentaacetic acid molecule. A radiopharmaceutical with a fast blood clearance offers distinct imaging advantages, particularly with a short-lived radionuclide such as Tc $^{99m}$, i.e. a shorter optimal imaging time and increased tumour:background ratios.

The finding that there was no significant difference between the first and second phase mean $t_k$ blood clearance times in the non-tumour, small tumour-bearing, large tumour and combined tumour groups, and that blood clearance appeared unaffected by tumour mass, is to be expected since no evidence of any tumour uptake has been demonstrated in rabbits with tumours. Therefore, the organ biodistribution was essentially the same in non-tumour and tumour groups. There was very little inter-rabbit variation in the first phase (range 11 minutes), and second phase (range 41 minutes) blood clearance times in the non-tumour group. The wider range observed in the first phase (29 minutes) and second phase (491 minutes) blood clearance times in the rabbits with tumours could have been due to tumour uptake, particularly since the rabbit with the greatest tumour mass (98.8 gms, 3.52% body weight) had the fastest first phase blood clearance
(7 minutes ± 1). However, in the absence of any demonstrable tumour uptake, these inter-rabbit differences in the tumour group are more likely to be due to variations in the animals physical and physiological condition. The rabbits with tumours were metabolically unstable and sometimes dehydrated, the tumour secreting a parathormone-like substance which has a known effect on renal metabolism. Only nine rabbits with tumours were studied (five with small tumours and four with large tumours), and further studies are necessary on a larger number of rabbits with increasing tumour sizes to confirm whether or not blood clearance is affected by tumour mass.

There was no significant difference between the first and second phase mean $t_1$ blood clearance times in the patients with no tumour and patients with tumour groups. Although this is almost certainly due to the fact that the tumours were relatively small in relation to body weight (in contrast to rabbit tumours) and so would have very little effect on pharmacokinetic data, it may also be further evidence of absent tumour uptake of Tc $^{99m}$ (v) DMSA. As in the rabbits, there was little inter-human variation in the first phase (range six minutes) and second phase (range 63 minutes) clearance results in patients with no tumour.
There was no significant difference \( (p > 0.05) \) in the mean blood \( t_\frac{1}{2} \) clearance times between the rabbit and human groups with no tumour. In the absence of any tumour uptake, the wider range observed in first phase (range 10 minutes) and second phase (range 204 minutes) blood clearance times in humans with tumour is likely to be due to variations in the patients clinical condition which could affect renal function. For example, non-metastatic manifestations of bronchial squamous carcinoma are well recognised and hypercalcaemia due to the secretion of a parathormone-like substance and other substances such as Vitamin D (or its metabolites) or prostaglandins have been described (Tashjian, 1974). There was no significant difference \( (p > 0.05) \) in the mean blood \( t_\frac{1}{2} \) clearance times between the rabbit and human groups with tumours suggesting, in conjunction with the results from the groups with no tumour, mechanisms of clearance may be similar in rabbits and humans.

The pharmacokinetic results from the rabbits with no tumours and rabbits with tumour groups, and the humans with tumour group are different from those quoted in earlier studies (Section 1.6., and Watkinson, 1987). However, in this earlier work, the rabbit results were calculated as mean values for each group from only two rabbits and the clearance
half times were calculated graphically. In this work 14 rabbits were studied, and a first and second phase blood $t_\frac{1}{2}$ clearance value calculated for each rabbit thereby providing more accurate mean values. The previous human results were calculated in a similar manner using results from both patients with no tumour and patients with tumour (squamous cell carcinoma, medullary carcinoma of the thyroid). Results in this thesis are more accurate since it divides patients into groups containing those with no tumour and those with tumour and calculates the first and second phase $t_\frac{1}{2}$ blood clearances for each individual patient.

The rabbit mean cumulative urine excretion half-times were not analysed statistically. This is because it was impossible to collect all the urine passed in the first four to six hours post-injection as metabolic cages were not available, and the turbid rabbit urine often blocked the narrow neonatal feeding tube. Therefore the results presented are percentage injected dose/g of urine, and not percentage injected dose over the period of the study. However, the results show Tc $^{99m}$ (v) DMSA has a fast bi-exponential cumulative urine excretion with approximately 50% of radioactivity being excreted within the first four hours post-injection. There was very little apparent difference in the mean cumulative urine excretion half-times between the rabbits with no tumour group and those rabbit groups with
small tumours, large tumours and combined tumours. In the absence of tumour uptake, such apparent small differences are probably due to inter-rabbit variation and the fact that the rabbits with tumours were metabolically unstable.

For the human studies it was possible to collect all the urine passed in the 24 hours following radiopharmaceutical injection (in contrast to the rabbit results) so that the mean cumulative urine excretion half-times could be analysed statistically. Cumulative urine excretion was bi-exponential and, (as for rabbits) approximately 50% of radioactivity was excreted within four hours post-injection. The finding that there was no significant difference in mean cumulative urine excretion between the patients with no tumour and patients with tumour groups is to be expected since there was no significant difference in blood clearance between the two groups.

There was quite an unexpectedly large range in the cumulative urine excretion half-times in both the human group with no tumour (range 200 minutes) and the human group with tumour (range 150 minutes). One would anticipate a narrower range in the former compared with the latter. However, as some urine samples were collected at home by patients, inevitable errors in collection could possibly account for variations in results leading to the unexpected large range.
The human whole body retention values in patients with no tumours and patients with tumour were calculated assuming whole body retention (%) was given by the expression "100-urinary excretion (%)". This may not be absolutely true since traces of radioactivity in rabbit bile did occur (but were insignificant when compared with the urinary route) and similar mechanisms may operate in man. Therefore errors which occurred in the urinary excretion results are reflected in the whole body retention results. Initially, there appeared a significant difference in whole body retention between the two groups. However, one of the patients (patient2) with no tumour who had collected his own urine overnight had the shortest cumulative urine accumulation excretion half-time (70 minutes) and a whole body retention value which was radically different from any of the other patients. Excluding this whole body retention value from the results, there was no significant difference between the two groups. This is to be expected, based on the absence of tumour uptake together with previous blood and urine data.

The human cumulative urine excretion and whole body retention results in this thesis are different to those quoted previously (Watkinson, 1987). However, in this
earlier work, the mean urine cumulative half-time values were obtained graphically with urine collected only up to six hours, and no distinction was made between patients with and without tumours. Therefore the work in this thesis may be considered more accurate.

Biodistribution

In rabbits with no tumours, the predominant biodistribution of Tc $^{99m}$ (v) DMSA in bone over 24 hours, with maximum uptake at four hours, suggests Tc $^{99m}$ (v) DMSA is behaving, in part, like a bone scanning agent, and that uptake may be due to the similarity of the TcO$_4^{2-}$ pentavalent core to a phosphate molecule (Endo et al, 1985). The uptake in kidneys (with maximum uptake at six hours) probably reflects, not only the route of excretion of Tc $^{99m}$ (v) DMSA, but also binding in the proximal tubules of the kidney. Such a phenomenon indicates similar behaviour to Tc $^{99m}$ (III) DMSA and this could have been due, in part, to in-vivo conversion from Tc $^{99m}$ (v) DMSA to Tc $^{99m}$ (III) DMSA but this is unlikely on the basis of Tc $^{99m}$ (v) DMSA stability results (see Discussion, page 576). The biodistribution in the bladder wall with maximum uptake at six hours is likely to be due to mucosal adsorption, and/or urinary contamination rather than actual uptake.
The blood pool biodistribution results confirm the bi-exponential pharmacokinetic results previously discussed in this section. The radioactivity in the liver, cervical lymph nodes, nasal mucosa, lung and pituitary probably reflects the large blood flow in each of these tissues since each organ at necroscopy was washed but not perfused with saline prior to counting. Radioactivity in the bile indicates biliary excretion and radioactivity in the gall bladder at six hours and at 24 hours is probably a function of high blood flow, organ uptake and/or adsorption, biliary contamination or a combination of any of these factors.

The lacrimal glands are visualised on human scintigraphic images using Tc $^{99m}$ (v) DMSA. Uptake might have been expected in the rabbit lacrimal glands, and the lack of radioactivity within these glands may be due to the fact that rabbits do not produce tears. If there is a similarity between the PO$_4^{-}$ molecule and the pentavalent Tc $^{99m}$ (v) DMSA core, marrow uptake might have been expected to be greater than it was since phosphorus-32 phosphate is used to treat polycythaemia rubra vera in man due to its substantial marrow accumulation.
The thymus is a lymphoid organ with a high blood flow which might be expected to exhibit uptake of Tc $^{99m}$ (v) DMSA in a similar manner to cervical lymph glands. However, the thymus contains more adipose tissue than cervical lymph glands and although the majority of fat was removed from the thymus, some was inevitably left behind. This could have affected the biodistribution results since adipose tissue is not as vascular as the thymus gland.

In rabbits with tumours, significant radioactivity from Tc $^{99m}$ (v) DMSA was found in the bone, kidney and bladder wall to six hours, and in the bone and kidney to 24 hours. The finding that there was no significant difference ($p > 0.05$) in the organ biodistribution between the rabbits with no tumour and rabbits with tumour groups was a reflection of low tumour uptake (0.026%/g at two hours).

The radioactivity detected in the lung, nasal mucosa, pituitary, spleen, liver, cervical lymph glands and gall bladder is due to similar reasons to those discussed for the rabbits with no tumour group. The radioactivity in bile again reflects biliary excretion and reasons for the absence of uptake in the marrow and thymus have already been discussed.
The lack of evidence for tumour uptake of Tc $^{99m}$ (v) DMSA at either two, four, six or 24 hours probably reflects the poor blood supply of the tumours and the non-specific nature of Tc $^{99m}$ (v) DMSA for squamous cell carcinoma. The radioactivity detected in the tumours could be due to either blood pool and/or an increase in blood capillary permeability, both of which are thought to be important contributory factors in the uptake of Ga $^{67}$-Citrate into tumour cells (Tsan and Scheffel, 1986). Other factors which may be important include uptake by inflammatory cells and bacteria (when present), tumour pH, the similarity of the Tc $^{99m}$ (v) DMSA pentavalent core to the phosphate molecule which is avidly taken up by some tumour cells (Endo et al, 1985) and the presence of binding proteins such as lactoferrin and ferritin.

The uptake of Tc $^{99m}$ (v) DMSA at two hours in inflammatory tissue, which was similar to tumour uptake at that time, is probably due to the high vascularity of inflammatory tissue although other factors (which are thought to operate for Ga $^{67}$-Citrate (Tsan, 1985)) such as an increase in capillary permeability, leucocyte and bacterial uptake may also be important. These findings demonstrate the problem with human or animal scintigraphy in that uptake could be in either tumour, inflammatory tissue or both. The radioactivity in
inflammatory tissue at four, six and 24 hours reflects the pharmacokinetic results and confirms a blood pool effect. The reason that radioactivity appears in inflammatory tissue at two hours, and then diminishes radically at four, six and 24 hours respectively is probably due to the fact that the specimens were washed free of activity and therefore very little intrinsic blood content was left (in contrast to organs such as the liver and lung).

The finding that there was no significant difference in the uptake between whole, outside viable living and inside non-viable necrotic tumour at two, four and six hours is interesting. $^{67}$Ga-Citrate is taken up by experimental animal carcinoma tumour models and human squamous carcinoma (Hayes et al, 1970; Manfredi and Weiss, 1978) and uptake shows a high concentration in outside viable living tumour, which has a better blood supply compared with the less vascular inside non-viable necrotic tumour (Hayes et al, 1970; Tsan and Scheffel, 1986). However, in this study, whole (viable plus non-viable) tumour did show a prolonged washout phase of $^{99m}$Tc (v) DMSA when compared to the blood pool, and these results confirm earlier work (Watkinson, 1987). It was also suggested in that study that using $^{99m}$Tc (v) DMSA:$^{125}$HSA ratios, inside non-viable necrotic tumour appeared to wash out more slowly than outside viable
living tumour. These results were, however, only in 10 tumours and the data was not analysed statistically. Statistical analysis of the results in this thesis are therefore more accurate, and there was no significant difference between the inside non-viable and outside viable tumour radioactivity results. This phenomenon would explain not only the tumour:blood and tumour:muscle ratios, but also help to explain the approximate optimal imaging time of four hours when the tumour:blood ratio was approximately 1:1 and the tumour:muscle ratio was 6:1. By six and 24 hours the tumour:blood and tumour:muscle ratios had increased (six hours, 2:1 and 7:1; 24 hours, 5:1 and 15:1). There are, however, other factors apart from these ratios which contribute to the optimal imaging time such as the half-life of technetium-99m.

The disappointing lack of uptake of Tc\textsuperscript{99m} (v) DMSA in human tumours (similar to findings observed in rabbits) probably reflects the non-specific nature of Tc\textsuperscript{99m} (v) DMSA for squamous cell carcinoma and the poor vascularity of the tumours and limits the use of this radiopharmaceutical for detecting squamous carcinoma. The wide ranges in the tumour:blood and tumour:muscle ratios reflect the different natures of the tumours, since each tumour has its own intrinsic biological behaviour. Narrower ranges with similar
ratios for each of the tumours would have been surprising. Although the tumour:blood ratios for the laryngeal, tongue and maxilla tumours indicated very little uptake, the two patients with hypopharyngeal tumours had tumour:blood ratios of 2.5:1 and 4.1:1 respectively. Both hypopharyngeal tumours were biopsied approximately four hours post-injection. The former tumour was moderately differentiated, while the latter was well differentiated squamous carcinoma. The reason for the difference in the ratios between the hypopharyngeal tumours and those in the laryngeal, tongue and maxilla tumours is unclear.

All the biodistribution results for Tc $^{99m}$ DMSA in the rabbits with no tumour and rabbits with tumour groups support previously reported preliminary results (Westera et al, 1985; Ramamoorthy et al, 1987; Watkinson, 1987).

The animal pharmacokinetic and biodistribution (and the human pharmacokinetic) data was all analysed using parametric statistics. However, due to the small numbers studied in each group, it was impossible to define whether or not any normal distribution was present. Parametric statistics (student t-test) were therefore used and these did not show any significant difference between the results in the non-tumour
and tumour groups. If any significant difference had been shown, then the results would also have been analysed by non-parametric statistics. Since the percentage of the injected dose in tumours was always less than 1%, one would be surprised to observe any significant change in either the pharmacokinetics or biodistribution of Tc $^{99m}$ (v) DMSA when comparing non-tumour and tumour groups (particularly for the human results). With such low uptake of Tc $^{99m}$ (v) DMSA, if one wanted to show any significant change in either pharmacokinetic or biodistribution data by the methods used in this study, then larger numbers of patients and rabbits would be required in each group. Alternatively, whole body retention data obtained using a whole body counter would have been a more accurate way of assessing differences in radiopharmaceutical pharmacokinetic and biodistribution data (e.g. I $^{131}$ and differentiated thyroid cancer; Susan Clarke, Personal Communication, 1989).

**Animal Body and Thymus Weights**

There was no significant difference in mean body weight between the rabbit with no tumour group in this study and the value quoted in the literature (Kozma et al, 1974, p 56). However, there was a significant difference ($p < 0.025$) in
mean thymus wt/kg body weight between these two groups. Approximately equal numbers of rabbits were studied in both groups (20 with no tumour in this study; 23 in Kozma's series). In this study the thymus was not meticulously trimmed of fat and this may have contributed to the difference. In addition, all rabbits on arrival were young adults (2.5 kg, 3-4 months old) and therefore of similar age and weight to those used by Kozma et al. However, some rabbits with no tumours were not sacrificed for up to one month following arrival and were therefore older. This may have contributed to the higher mean thymus weight in rabbits with no tumour observed in this series, compared with that in the literature.

The significant difference in mean body weight in the rabbits with tumour group compared with the mean body weights in rabbits with no tumours in this, and Kozma's series, is probably due to the fact that some of the rabbits with tumours were older. On arrival, tumours were transplanted and the rabbits sacrificed at varying intervals (range 6-72 days, mean 29 ± 34). In view of this, one might expect the mean tumour thymus weight to be greater than in the rabbits with no tumour. In fact, there was no significant difference between the mean thymus weight in rabbits with tumours and
the mean thymus weight in rabbits with no tumours quoted in the literature (Kozma et al, 1974, p 56). The former thymus weight in rabbits with tumours was significantly less than the mean thymus weight in the rabbits with no tumours in this study.

These results, taken in conjunction with the mean body weight, suggest the thymus weights were significantly less in the rabbits with tumour group than in the rabbits with no tumour group. Multiple regression analysis showed that only 26% of this reduction was due to the tumour weight and time with tumour. Other possible factors which may have been responsible include physiological rabbit thymus atrophy, an increase in metabolic requirements and consequent reduction in thymic and total body fat.

**Distant Metastases**

The incidence of lymph node metastases (8% of rabbits) is lower than one would expect from human studies. However, nodes were only examined macroscopically. The incidence of distant metastases (13%) is similar to the figure that used to be quoted for humans (10-12%), but less than that in recent studies (46%, Section 1.3.6.). However, patterns of distribution were different. In humans, the commonest site
for distant metastases is the lung (80%) followed by mediastinal nodes (31%), liver (31%) and bone (31%). In this study the commonest site was the liver (80%) followed by the lung (20%). Due to small numbers it is difficult to comment on the influence of tumour weight, and time with tumour, on the incidence of lymph node and distant metastases. However, the rabbit with the greatest tumour mass was the only one to develop macroscopic metanchronous metastases. These results should be interpreted taking into account that only the lymph nodes, lungs and liver were examined macroscopically, and bone was not examined at all. False negatives are inevitable and, therefore the incidence of lymph node and distant metastases was almost certainly higher than that observed. Care should be taken when comparing the behaviour of superficially transplanted animal tumours in the thorax and trunk of a rabbit with human head and neck squamous carcinoma. In addition, other factors such as the host-tumour response are probably important, and would be different in both groups.

**Absorbed Finger Radiation Dose**

The absorbed finger radiation dose to the assistant surgeon during the biodistribution studies was low, and well within accepted limits.
Subcellular Biodistribution

The quantification results for subcellular biodistribution are similar to those quoted in the literature (Steck, 1972, p 81). The higher relative enzymic activity of tumour lactate dehydrogenase compared with that found in the liver reflects the characteristic anaerobic metabolism of squamous cell carcinoma. The ultracentrifuge has fostered powerful technological advances by which subcellular organelles and their membranes can be prepared and analysed. However, difficulties in purifying organelles and membranes arise from the overlap in their physical properties, and membrane isolation is further complicated by fragmentation (and the concomitant heterogeneity of the product), and by the tendency of membranes to adsorb or enclose extrinsic macromolecules. Differential centrifugation is the most widely used fractionation technique but its resolving power is limited. Rather than using any one organelle designation, it is better to use the terms "nuclear" fraction (which includes cell membranes, nuclear membranes etc) and "mitochondrial", "microsomal" and "cytosol" fractions.

There is considerable physical disruption of organelles which takes place during homogenisation, and the endoplasmic
reticulum is the most sensitive organelle (Steck, 1972, p 86). This structure is invariably reduced to small vesicles which generally populate the microsomal fraction. The disruption of nuclei and lysosomes by hypotonic lysis or too vigorous homogenisation frequently occurs (Steck, 1972, p 87), and the lysosomal and nuclear enzymes can be recovered from the soluble protein fraction within the cytosol. The "mitochondrial" fraction is rich in lysosomes, peroxisomes and may contain considerable plasma membrane, golgi vesicles and endoplasmic reticulum (Steck, 1972, p 79). The outer mitochondrial membrane may also be disrupted by homogenisation and appear in the "microsomal" fraction. The Golgi apparatus, itself a composite of associated vesicles and tubules, is often dispersed by homogenisation and can be recovered, not only in the "mitochondrial" fraction but also in the "microsomal" and "nuclear" fractions (Steck, 1972, p 87).

The plasma membrane is obviously disrupted by homogenisation. It can be recovered as either an intact, sealed envelope (or ghost), as large sheet-like fragments or as small sealed vesicles, and all these may appear in the "nuclear", "mitochondrial" or "microsomal" fractions. The "nuclear" fraction itself contains not only plasma membrane but also outer nuclear membrane and both rough and smooth endoplasmic reticulum.
A possible solution to avoid these misleading designations would be to refer to the various fractions as $10^4$, $10^5$ or $10^6$-min pellets etc. Techniques of separation can be enhanced by using rate zonal or equilibrium density gradient centrifugation, zonal density gradient electrophoresis, filtration, affinity chromatography and the use of adsorption and trapping to remove extraneous proteins (Steck, 1972).

Therefore the cell membrane (nuclear), mitochondrial, microsomal and cytosol fractions presented in this work are undoubtedly contaminated with the various fractions described above. However, the quantification results show that each of the tumour and liver fractions are enriched with the correct marker enzyme. Since the techniques were identical for rabbit and human tumour subcellular biodistribution, certain comparisons can be made, particularly since similar methodology has been used to study the subcellular localisation of radiopharmaceuticals such as Tc$^{99m}$ hexakisalkylisonitrile and Thallium-201 Thallous Chloride (Mousa et al, 1987).

The finding that radioactivity on the liver cell membrane fraction decreased sequentially with time is difficult to explain, particularly since none of the other liver or tumour
fractions showed similar relationships. One possible reason would be altered biodistribution due to tumour uptake but why the cell membrane should be selectively affected is unclear. Tc $^{99m}$ (v) DMSA is non-specific for the normal liver cell which explains the predominant accumulation of activity within the cytosol and the localisation of some radioactivity on the cell membrane. Hepatocytes are highly metabolic cells involved in drug and glucose metabolism. Dimercaptosuccinic acid is a low molecular weight organic acid and its incorporation in the glycolytic pathway would explain mitochondrial activity and its subsequent metabolism by conjugation of the sulfhydryl groups with glucoronic acid may explain microsomal activity.

Variations of the biodistribution of the individual liver fractions with time (particularly the mitochondrial and microsomal fractions) can be explained by the fact that many cellular drug metabolic reactions vary with the age, environment, diet and temperature of the animal, as well as the time of day the experiment is performed (Williams, 1971). The subcellular biodistribution studies were carried out on rabbits of the same sex, fed on similar diets, housed in a similar environment and which had not previously received Tc $^{99m}$ (v) DMSA or any other drug. All experiments were performed at approximately the same time of day but animals did vary in size.
It might be expected that the subcellular localisation of Tc $^{99m}$ (v) DMSA in the rabbit tumours would vary as a function of tumour age. Such variations may have been observed if different viable tumour depths had been sampled since, as the centre of a squamous cell carcinoma is approached, the cells become less vascular and more anoxic, and consequently vary in their rate and type of metabolism. The range of activities in each of the individual tumour fractions can be explained by the finding that, although all the tumours were squamous cell carcinomas, any one tumour had its own inherent biological behaviour at any one particular time. The presence of radioactivity within the tumour cytosol, and on the cell membrane, is probably due to similar reasons which were postulated to explain the liver cytosolic and cell membrane biodistribution, and again reflect the non-specific nature of Tc $^{99m}$ (v) DMSA. Squamous carcinoma cells are less vascular and less metabolically active than hepatocytes, and this may partly explain the lower radioactivity observed in the tumour mitochondrial and microsomal fractions.

It is difficult to make meaningful comments when comparing the human or rabbit subcellular biodistribution results particularly since subcellular drug metabolic activity can vary considerably from species to species (Williams, 1971).
In addition, although all the human tumours were squamous cell carcinomas, the degree of differentiation varied and they were all of different ages and from different head and neck sites.

However, since there were similarities between the rabbit and human pharmacokinetic and biodistribution results, certain comparisons may be valid. There was no evidence of selective specific human tumour cellular or intracellular accumulation and although the results show there is uptake of radioactivity into squamous carcinoma, the lack of any evidence of any specific intracellular localisation mechanism in the presence of good clinical images may mean the majority of Tc $^{99m}$ (v) DMSA is located in tumour extracellular fluid or inflammatory tissue or even in areas of microcalcification within the tumour. The majority of the radioactivity was located either non-specifically within the cytosol or on the cell membrane. These results in humans are similar to those observed in rabbits, although there was some variation in actual proportions. There was less cytosolic radioactivity, but more cell membrane radioactivity, observed in the human tumours. The similar amount of radioactivity within the human and rabbit tumour microsomal fractions may reflect a similar drug metabolic pathway. However, the microsomal fractions in both rabbits and humans could have contained
lysosomal contaminants which might represent the site of intracellular Tc $^{99m}$ (v) DMSA tumour localisation (in contrast to Ga $^{67}$-Citrate; Tsan and Scheffel, 1986). Since the microsomal fraction contained the least radioactivity of all the fractions in both rabbit liver, and human and rabbit tumour groups, such a mechanism of localisation is unlikely and may mean that Tc $^{99m}$ (v) DMSA is probably not specifically localised in lysosomes. Further work to include autoradiographic studies would be necessary to evaluate the exact site of intracellular localisation. The overall evidence in the literature is that Ga $^{67}$-Citrate is predominantly localised within the cytoplasm, and this study has shown a similar subcellular biodistribution for Tc $^{99m}$ (v) DMSA. Obviously an increase in capillary permeability is important for tumour uptake of both agents, but these findings may mean that the uptake of Tc $^{99m}$ (v) DMSA may be, in part, transferrin dependent (c.f. Endo et al, 1985; Yokoyama et al, 1985).

Dosimetry

Like all dosimetric calculations, a number of distinct assumptions had to be made in this study because of the incomplete and sparse biological results which were obtained by combining animal and human data. Human clearance from
the kidney and bladder was derived from results which made
the assumption that whole body retention (%) is
"100-urinary excretion (%)", a statement which may not be
entirely true since some biliary excretion of radioactivity
did occur in rabbits. The cumulative activity in the human
kidney was estimated from the rabbit biodistribution data,
corrections being made for both kidney mass and minimal bony
uptake observed in humans. Errors in estimating the bone
mass of a rabbit are inevitable, and the bone mass of a rabbit
with no tumour (137 g/kg body weight, 13.7% body weight)
estimated in this study is less than that previously reported
(17.1% body weight, Watkinson, 1987). However, although
this earlier value was obtained using African flesh eating
beetles, the carcass was removed prior to complete removal
of rabbit flesh. In this work, the flesh eating beetles were
left for three weeks and there was no visible remaining
flesh on the skeleton. Because of this, the value obtained
can be regarded as reasonably accurate, and one which is not
only similar to the expected value in the rabbit (10-11%
body weight; Ranch Rabbits Ltd., Personal Communication, 1987),
but also to that observed in humans (10% body weight,
Snyder et al, 1974, p 65).
The human pharmacokinetic, rabbit biodistribution and bone mass results used for the dosimetric calculations in this thesis are, in theory, more accurate than those used previously (Clarke et al, 1987; Watkinson, 1987). However, the pharmacokinetic data used by Clarke et al (1987) was derived from a mixture of patients with and without tumour (squamous cell carcinoma and medullary thyroid carcinoma) and no urine was collected after six hours post-injection. Watkinson (1987) used a compartmental model similar to that used in this work to calculate the effective dose equivalent in man. However, he used the same pharmacokinetic data used by Clarke et al (1987) to estimate the cumulative blood pool activity and whole body retention. In addition, the rabbit kidney biodistribution results represented mean data from two rabbits, and the rabbit bone mass was less accurate since all the flesh on the carcass was not completely removed.

Therefore the value of 5.1 uSv/MBq may be a more valid estimation of the effective dose equivalent of Tc $^{99m}$ (v) DMSA in man, and as such it is less than those figures quoted for other technetium labelled compounds (Tc $^{99m}$ DTPA, 10 uSv/MBq; Tc $^{99m}$ (III) DMSA 12.5 uSv/MBq. DHSS, 1988). This may reflect either a true lower radiation dose, or an underestimation resulting from the assumptions
previously described. Despite this, an adult dose of 
Tc $^{99m}$ (v) DMSA (370 MBq) would result in an effective dose 
equivalent of 1.9 mSv per scan which is still considerably 
less than the effective dose equivalent for Ga $^{67}$-Citrate 
(18 mSv/150 MBq. DHSS, 1988).

Radiopharmaceutical Stability

The radiopharmaceutical stability studies show 
Tc $^{99m}$ (v) DMSA is stable in-vitro to two hours post-preparation. 
An important question is its in-vivo stability and, in 
particular, whether or any any in-vivo conversion to 
Tc $^{99m}$ (III) DMSA occurs which would explain kidney uptake in 
both rabbits and humans. Current collaborative work using 
TLC and electrophoresis (Avtar Bharij, Personal Communication, 
1989) has shown that both Tc $^{99m}$ (v) DMSA and Tc $^{99m}$ (III) DMSA 
are stable to four hours in in-vitro blood from non-tumour 
and tumour patients and in in-vivo blood from non-tumour and 
tumour NZW rabbits. Tc $^{99m}$ (v) DMSA was stable in-vivo to 
four hours post-injection in non-tumour and tumour patients 
and non-tumour and tumour rabbits. Only Tc $^{99m}$ (v) DMSA 
was identified in the urine in all these four groups up to 
four hours post-injection. For Tc $^{99m}$ (III) DMSA in-vivo, 
there appeared to be some conversion to Tc $^{99m}$ (v) DMSA in 
tumour patients and tumour rabbits but not in non-tumour patients.
There was no evidence of any in-vivo blood conversion of Tc $^{99m}$ (v) DMSA to Tc $^{99m}$ (III) DMSA, but in the in-vivo blood non-tumour rabbit and human samples there was an extra cationic species identified which was not present in the corresponding tumour groups. Therefore, any tumour accumulation observed using Tc $^{99m}$ (v) DMSA may be due, in part, to this extra cationic species which is being taken up by tumour and which is then absent from the blood. Further work is underway to investigate this phenomenon.

**Optimal Imaging Time;** Tc $^{99m}$ (v) DMSA

The scintigraphic appearances in rabbits with and without tumours confirm the animal biodistribution results with a distribution to include bone, kidneys and bladder. The qualitative imaging time of four hours is a function of the biodistribution and pharmacokinetic results, together with the half-life of Technetium-99m. At 30 minutes, the eight false negatives were probably due to minimal tumour uptake together with high blood levels, both of which would have contributed to the observed low tumour:blood and tumour:background ratios. By four hours, optimum conditions with significant blood clearance allowed visualisation of all but the smallest tumour (0.5 x 0.5 cm), and the tumour:background (soft tissue) ratios
confirmed maximum quantitative uptake at between one and a half and five hours. This substantiates the prolonged tumour washout phase observed with the biodistribution results. The cause of the two false positive results is unclear. Uptake may have occurred in non-malignant nodes or nodes with microscopic disease but this is unlikely. The most likely reason is uptake in the tip of the scapula although the rabbits forearms were always extended to try and exclude this structure from the field of view.

For the human qualitative optimal imaging time, six patients with primary tumours all measuring greater than 4 cms were studied and there were six true positives at two, four and six hours. Two patients had cervical metastases which measured 3 x 3, 1.5 x 1.5 and 2 x 1.5 cm on CAT and none of these were detected by scintigraphy.

For the human quantitative optimal imaging time, maximum uptake occurred in all tumours at between two and four hours, and in only two tumours did the tumour:background (soft tissue) ratios exceed 1:1. The range of tumour:background (soft tissue) ratios were similar in both rabbits and humans at two hours (0.71:1-1.30:1 and 0.55:1-1.47:1 respectively).
At four and six hours, there was a wider range of tumour:background (soft tissue) ratios observed in humans when compared to rabbits (0.40:1-1.86:1 and 0.63:1-1.10:1 respectively at four hours; 0.48:1-1.73:1 and 0.42:1-0.65:1 respectively at six hours). However, the rabbit and human tumours were of different sizes and the human results were affected by the two patients whose ratios exceeded 1:1, and one of these had received radiotherapy. Previous irradiation may affect Tc $^{99m}$ (v) DMSA uptake since one patient who had received radiotherapy to a T2 laryngeal carcinoma, and who was diagnosed subsequently as having post-irradiation oedema and inflammation had the highest observed ratio (inflammation: soft tissue ratio. 1.89:1 at six hours).

Taking into account the difference in sizes between the rabbit and human tumours and the fact two patients with positive disease had received previous irradiation, the optimal imaging time in both groups are comparable and occur approximately between two and four hours. Such congruous findings may indicate that similar mechanisms exist for Tc $^{99m}$ (v) DMSA tumour uptake in both rabbits and humans with squamous cell carcinoma.
Optimal Imaging Characteristics: Tc $^{99m}\text{Tc}$ (v) DMSA

There are a number of factors that influence the detectability of a lesion on a scintigraphic image. These can be divided up into patient, gamma camera, image analysis and interpretation factors. With regards to the patient, detectability depends on lesion size, location and number, together with the imaging characteristics, background concentration and lesion uptake of the radiopharmaceutical. Patient immobility is important as is whether the lesion is "hot" or "cold". Intrinsic properties of the gamma camera system are crucial. Sensitivity, spatial resolution (intrinsic and extrinsic), spatial linearity, uniformity, choice of collimator and the presence of a SPECT facility are all important, as are analysis factors. Adequate counts for statistics are vital with subsequent correct analysis and interpretation. These are facilitated by suitable display, the knowledge and experience of the interpreter and the patient's history and examination. Radiopharmaceutical preparation, dose, route and speed of administration together with patient renal function and medication are also important.

In this study, the tumour rabbits were metabolically unstable with possible impaired renal function. Tumours were transplanted in the flank and shoulder regions well away
from bone, kidney and vascular structures to optimise tumour: blood and tumour:background ratios. Tumour uptake was "hot" and there was only one tumour in one specified site (i.e. loin or shoulder) when the optimal imaging time was being estimated. For the optimal imaging characteristics there was occasionally more than one tumour in one site. The Tc $^{99m}$ (v) DMSA was prepared by an experienced radiopharmacist and all rabbits were anaesthetised, positioned, examined and the tumours ensured to be in the field of view by the author using a Tc $^{99m}$ marker. A high resolution medium energy collimator was used and the hard copy planar images were reported by Dr. Susan Clarke who has considerable experience in tumour imaging using Tc $^{99m}$ (v) DMSA. These optimum factors are relevant to the finding that when evaluating the optimal imaging time, all tumours greater than 1.0 x 1.0 cm in size were detected. The smallest tumour (0.5 x 0.5 cm) was palpable, and a possible reason why it was not detected scintigraphically was that its size lay outside the overall spatial resolution of the gamma camera system.

For the optimal imaging characteristics, the overall sensitivity of 50% is predominantly a function of size since, of the 29 false negatives, 26 measured less than 2 cms. For these tumours (less than 2 cms), the overall sensitivity was 58% which increased to 89% for Class III and then dropped to
75% for Class IV and Class V tumours although only eight tumours in total were imaged in these two latter groups. The drop in sensitivity for these Class IV and Class V tumours was probably due to the fact that the majority of them overlay the kidney and as lateral views were not taken, some tumours may have been obscured by the predominant kidney uptake of Tc $^{99m}$ (v) DMSA. In addition to size, false negatives could have been due to tumour superimposition, since some sites contained more than one tumour. The low specificity (63%) reflects the false positive results. Possible sources of these in the shoulder include bone uptake in the scapula tip and/or uptake in normal lymph nodes or nodes with microscopic squamous carcinoma. Loin false positives are more difficult to explain. These may have been due to blood pool effects in the subcutaneous tissues or skin contamination from radioactive urine.

The rabbit palpation results show that palpation is better than scintigraphy in detecting the presence of superficially transplanted tumours in rabbits.

Although few comparisons can be made between palpating superficially transplanted rabbit tumours and lymph nodes in the neck, there are a number of important observations arising from this study. Tumours greater than 2 cms were
palpated by all observers and this is a similar finding to that observed in humans for neck palpation (Section 1.4.2. and 4.2.5.). For tumours less than 2 cms, there was no apparent interobserver variation in ability to detect tumour although the two highest sensitivities occurred with the two most experienced observers. What was interesting was that a preclinical medical student had a sensitivity of 83% which was greater than the two registrars and the houseman, and similar to that seen with the two more experienced observers. The incidence of false negative (32%) and false positive (12%) results is similar to mean values obtained from the literature for neck nodes (29% and 19% respectively), and this is surprising since the tumours were superficial and the rabbits were anaesthetised and therefore easier to examine.

Possible sources for false positive results in the neck which simulate a lymph node include the transverse processes of the first and second cervical vertebrae, the carotid bifurcation, the superior horn of the thyroid cartilage, the tail of the parotid gland and irradiated submandibular salivary glands. In the rabbit, false positive results could have arisen due to the kidneys, ribs, the tip of the scapula and faeces in the colon.
It appears that the ability to estimate tumour size is a function of experience and this ability is more accurate the smaller the tumour. There was no significant difference between the four otolaryngologists in their ability to estimate tumour size and all four were significantly more accurate than either the houseman or the medical student. The two more experienced observers (1 and 2) were the most accurate, not only in detecting the presence of tumour, but also in predicting the size of tumours which measured less than 2 cms. However, for the larger tumours (> 2 cms) these more experienced observers constantly under-estimated tumour size (as did observer 4, but to a lesser extent). It would appear that as an individual learns to assess tumour size, accuracy increases for smaller tumours but is accompanied by a tendency to under-estimate the larger ones.

There is a recognised error in measuring tumour size at necropsy depending on whether measurements are based on the largest dimension (R = 0.72), area (R = 0.97) volume (prolate sphere, R = 0.98) or water displacement (R = 1.00, Euhus et al, 1986). Errors occur when the largest dimension is used because some tumours contain loosely associated nodules, there is often difficulty deciding what represents the greatest longitudinal diameter and there is inter-observer variation.
in measurement techniques. In this study, all tumour measurements were performed by the author. Size was based on the largest dimension since this allowed a simple clinico-pathological classification and a correlation coefficient of 0.72 is acceptable for the purpose of this work.

One of the most important prognostic factors in head and neck cancer is the presence or absence, level and size of metastatic cervical lymphadenopathy. The surgeon must first detect the presence of any nodes and then assess their size. Individuals vary in their ability to palpate tumour. False positive and false negative results are inevitable and the evaluation and treatment of the clinically N\textsubscript{0} neck remains controversial (Sections 1.4 and 1.5). The introduction of new joint UICC-AJC staging criteria based on nodal size means that experienced observers may understage some necks in head and neck cancer patients and this may result in inappropriate treatment. Otolaryngologists should be aware of this dilemma and, in selected cases, nodal measurements using either computerised axial tomography or ultrasound may be used to supplement the clinical examination (Section 1.4).

The scintigraphic appearances in those patients with no tumours imaged with Tc\textsuperscript{99m} (v) DMSA confirm previously published data (Clarke et al, 1987), with a normal biodistribution
which includes the lacrimal glands, nasal mucosa, blood pool, kidney and bladder. Uptake of radioactivity in the lacrimal glands is also seen in humans with Ga\textsuperscript{67} and probably reflects a route of excretion via the tears. The biodistribution of Tc\textsuperscript{99m} (v) DMSA in the nasal mucosa reflects the high blood flow this organ receives and similar phenomena are also seen not only with Ga\textsuperscript{67}, but also Tc\textsuperscript{99m}-MDP. Uptake in the kidneys and the bladder predominantly reflects the route of excretion of Tc\textsuperscript{99m} (v) DMSA, rather than \textit{in-vivo} conversion to Tc\textsuperscript{99m} (III) DMSA which seems unlikely on the basis of current work in progress and on the \textit{in-vivo} stability of Tc\textsuperscript{99m} (v) DMSA (see previous Discussion, page 576).

Overall for detecting patients with cancer Tc\textsuperscript{99m} (v) DMSA planar scintigraphy was less efficient than clinical examination (80% sensitivity; 42% specificity, and 92% sensitivity; 96% specificity respectively), but these figures reflect both initial and follow-up evaluation. The absence of any difference in the scintigraphic results at two and four hours supports the theory that there is no active accumulation of Tc\textsuperscript{99m} (v) DMSA by squamous cell carcinoma and any improvement in image quality at four hours when compared to the two hour images is mainly due to previously discussed pharmacokinetic data.
For the initial evaluation of head and neck primary tumours using Tc $^{99m}$ (v) DMSA planar scintigraphy, both for patient imaging and lesion detection, the sensitivity and specificity (70% and 66%; and 71% and 43% respectively) was less than those values obtained from clinical evaluation alone (90% and 83%; 90% and 86% respectively) and overall, for the detection of primary tumours, clinical evaluation was a more efficient method of detecting primary head and neck tumours than Tc $^{99m}$ (v) DMSA planar scintigraphy although the scintigraphic results based on "T" staging alone are slightly biased since size is not the only determining factor of the "T" stage, and two embryonal rhabdomyosarcomas are included.

Of the five false negatives obtained by clinical examination, two had presumed occult primary head and neck squamous carcinomas (also undetected by CAT) and three had nasopharyngeal carcinomas (subsequently detected by CAT). The nasopharynx is well recognised as being a difficult area to evaluate clinically and some tumours may be submucosal. Occult primary tumours are even more difficult to detect. Many are small, submucosal and occur in so called "hidden areas". Some manifest at a later date, and some never appear at all although such results are biased since the majority of these patients subsequently receive head and neck irradiation.
The two false positive results occurred in patients who had previously received external beam radiotherapy and such findings highlight the difficulties which exist when trying to detect residual and recurrent disease following surgery and irradiation.

For Tc $^{99m}$ (v) DMSA planar scintigraphy of primary tumours, the decrease in sensitivity and specificity when compared to patient imaging reflects the increase in both false positive and false negative results. The false positive results occurred in patients who had carcinoma of the larynx which had been irradiated and uptake of Tc $^{99m}$ (v) DMSA could have occurred in either normal or inflamed thyroid cartilage. The explanation of this is unclear although uptake into the thyroid cartilage is recognised with Co $^{57}$-Bleomycin, and many metal chelates are taken up into immature bone (Yokayama and Saji, 1980) which is often present in varying amounts in the adult larynx during the normal process of ossification that occurs with ageing. The other false positives occurred in the mandible where positive uptake of Tc $^{99m}$ (v) DMSA was seen in the region of proven areas of periodontal disease. These findings confirm Tc $^{99m}$ (v) DMSA may be behaving, in part, like a bone scanning agent and it therefore suffers from similar limitations to Tc $^{99m}$-MDP when imaging the oral cavity, i.e. that it is impossible to reliably distinguish malignant tumour invasion of the mandible from benign dental disease.
For the evaluation of primary tumours, Tc$^{99m}$ (v) DMSA planar scintigraphy was affected by tumour size and location. Detection was best for the larger laterally placed tumours situated away from the floor of the mouth, nasopharynx and the blood pool and for those growths with any cartilage or bony involvement (for reasons previously discussed). False negative results occurred in the nasopharynx, presumably due to the superimposition of the nasal mucosa normal biodistribution blood pool effect and poor localisation in this area has been reported by other workers (Aw et al, 1986). False negative results also occurred with exophytic lesions of the oral cavity and supraglottis which may be due, in part, to the high surface area to low bulk ratio which is sometimes observed clinically with these tumours. The sensitivity and specificity for detecting head and neck primary tumours in this study with Tc$^{99m}$ (v) DMSA is less than that recently reported by others (Ohta et al, 1988; approximately 80% sensitivity and specificity). However, in Ohta's series no details are given of size or histology of the head and neck tumours (thyroid tumours are excluded) and Ohta et al make the point that the detection rate was influenced by tumour size, location and histology. Sensitivity was highest for larger squamous cell carcinomas situated in the region of the maxilla and mandible, i.e. those with direct or sympathetic bony involvement and that false positive results
were observed in the region of the floor of the mouth, gingiva and larynx (sub-site not specified). All the false negative primary tumours on Tc$^{99m}$ (v) DMSA scintigraphy lay within the theoretical spatial resolution of the gamma camera system used in this study. Compared with clinical evaluation, one occult primary tumour was detected using Tc$^{99m}$ (v) DMSA planar scintigraphy and no tumours were upstaged. Although one occult second primary was also discovered on planar imaging, the scintigraphic results clearly show there is no place for Tc$^{99m}$ (v) DMSA scintigraphy in the routine evaluation and staging of a head and neck primary squamous carcinoma.

One of the most important prognostic factors in head and neck squamous carcinoma is the presence or absence, level and size of metastatic cervical lymphadenopathy and its early detection is crucial to subsequent management. Overall, for palpating malignant cervical lymphadenopathy in this study, there were six false positives (16%) and 57 false negatives (36%). Both these figures lie within the ranges obtained from the literature for false positive (4-45%, mean 19) and false negative (4-60%, mean 29) nodes, and are not dissimilar from the resultant mean values from the literature or from the rabbit palpation results. Although few comparisons can be made between the literature values
for human palpation and values from the small number of patients studied in this work, (particularly as the mean follow-up time of 14 months is relatively short), the palpation results highlight the dilemmas facing the head and neck surgeon when evaluating the neck.

Clinically false positive neck nodes are important for two reasons. Firstly, a false positive node may result in unnecessarily aggressive treatment and secondly, a patient with a unilateral primary but bilateral palpable nodes may be condemned to inadequate treatment on the basis he or she has incurable disease. In this study, of the five patients (15%) with clinically false positive neck nodes, four had unnecessary neck dissections on the basis of the palpation results. However of these four, two were positive for neck nodes by CAT criteria and three had pectoralis flap reconstructions which necessitated neck dissection for access. Therefore only one patient had an unnecessary operation based on neck palpation, and he was also positive for neck disease on CAT criteria. The last patient had an inoperable T4 squamous carcinoma of the tongue with bilateral palpable nodes who subsequently turned out to have N1 disease. Although he would have been condemned to inadequate surgery on the basis of his neck disease, his primary was not unilateral and was deemed inoperable anyway.
Clinically false negative nodes are also of importance since failure to detect positive neck disease will have prognostic implications. The question must be asked how important are clinically false negative nodes in the clinically N-positive neck. In this study, for detecting lateral neck compartments with metastatic squamous carcinoma there were nine false negatives (9%, 5 N₁; 4 N₂b). Although the nodal palpation results highlight the well recognised errors in neck examination, the increase in the false negative rate (36%) when compared to the results for detecting lateral neck compartments with positive disease (9%) would not have affected any management decisions. However, these results may be important when calculating prognostic statistical data from patients who have clinical (and histologically proven) N₁ disease, but who do not have surgery, and who may indeed be N₂b.

In contrast to palpation, for the detection of malignant cervical lymphadenopathy using Tc⁹⁹ᵐ (v) DMSA planar scintigraphy, the sensitivity was 19% (91% specificity) and there were nine false positives (36%) and 67 false negatives (43%) (in contrast to lateral neck compartment scintigraphy. 21 false negatives, 19%). Although these results show that Tc⁹⁹ᵐ (v) DMSA planar imaging can detect metastatic cervical lymphadenopathy, the technique was inferior to palpation and size was a factor for detection
although the measurements used to distinguish nodal size i.e. $N_1 = 0-3$ cms etc, were rather crude. No nodes less than 2 cms were detected on planar scintigraphy, presumably since such lesions would have been outside the spatial resolution of the scintronix camera and also the intimate relationship that exists between many neck nodes and vascular structures such as the internal jugular vein. These findings support those of others (Cummings et al, 1981; Ohta et al, 1988) although there has been no previously controlled study relating the size and stage of malignant cervical lymphadenopathy to the scintigraphic uptake of any radiopharmaceutical. In all those lateral neck compartments which were positive scintigraphically, it was only possible to identify one area of increased radioactivity. This could have been because the other positive nodes were smaller, because one scintigraphically positive nodal mass represented a number of confluent nodal masses or because in any one nodal mass there was a metabolically dominant node. In addition, scintigraphic uptake could have occurred in inflammatory neck nodes which are associated with malignant nodes and uptake of Tc $^{99m}$ (v) DMSA into the inflammatory tissue which surrounds rabbit squamous carcinoma has been demonstrated in this study. Out of the four false positive and four true negative clinically palpable (and presumed inflammatory) nodes, half showed positive scintigraphic uptake,
and therefore the other five false positive results which occurred on planar Tc$^{99m}$ (v) DMSA scintigraphy could have resulted from uptake in non-palpable inflammatory nodes.

It follows from the above discussion that, using scintigraphy, no necks were upstaged from $N_1$ to $N_{2b}$. However, the argument applied to lesion palpation applies to lesion scintigraphy, i.e. would the increase in false negative rate have affected management decisions. This is of secondary importance for scintigraphy since both for lateral neck compartment and nodal detection, the sensitivity and specificity was inferior to palpation and as a result some patients would have received inadequate or inappropriate treatment. Only four clinically $N_0$ necks were upstaged on scintigraphy, one correctly from $N_0$ to $N_1$ and three incorrectly ($N_0$ to $N_1$; correct N status, $N_{2b}$) and this, together with the unacceptable low sensitivity means that Tc$^{99m}$ (v) DMSA planar scintigraphy has no role to play in the detection of malignant cervical lymphadenopathy in patients with head and neck carcinoma and, in particular, is of no value in the evaluation of the clinically $N_0$ neck. In contrast to the low sensitivity (19%), the apparent high specificity (91%) when using Tc$^{99m}$ (v) DMSA planar scintigraphy to detect malignant neck nodes reflects not only the small number of patients with palpable (presumed inflammatory) nodes but also the inability of planar scintigraphy to detect more than one
nodal mass. This is because there may have been many more
inpalpable inflammatory nodes in N₀ and N-positive necks
and some of these nodes may have exhibited uptake of
Tc ⁹⁹m (v) DMSA but were undetected as false positives since
more than one nodal mass could not be resolved.

The above discussion on palpation versus scintigraphy
is based on the overall findings in all the patients examined
in this study with cervical lymphadenopathy. The findings
in the smaller subgroup of 26 patients who had 31 neck
dissections substantiate the fact that palpation is more
accurate than scintigraphy as a method of identifying
lateral neck compartments with, and nodes within those necks
with, metastatic carcinoma. Although four lateral compartment
necks (13%) had their staging correctly changed by
Tc ⁹⁹m (v) DMSA planar scintigraphy and three were upstaged
from N₀ to N₁, in two of these latter lateral neck compartments
the actual nodal status was N₂b. In addition, one
lateral neck compartment was downstaged N₁ to N₀ on
scintigraphy. Is it not reasonable to suggest that a patient
with a clinically positive lateral neck compartment and a
negative Tc ⁹⁹m (v) DMSA scan needs an operation?
Although scintigraphy upstaged three (19%) clinically N₀ lateral neck compartments, a further three (19%) were incorrectly upstaged and would have had unnecessary surgery while four (25%) had positive disease undetected with Tc₁₉⁹ (v) DMSA. Therefore, Tc₁₉⁹ (v) DMSA planar scintigraphy has no role to play in routine lateral neck compartment staging of patients with head and neck squamous carcinoma (to include the clinically N₀ neck).

Planar scintigraphic imaging suffers from distinct disadvantages. Using a standard large field of view gamma camera to obtain planar antero-posterior and lateral views, a two dimensional image is obtained of a three dimensional object. This is often of great diagnostic value but has a number of disadvantages. It provides poor depth information, allows tissue superimposition and makes little allowance for gamma ray attenuation by the tissues. Using SPECT, since one dimensional views are taken of a two dimensional transverse axial slice, the algebraic sum of the activity within that slice can be mathematically reconstructed. This greatly improves depth interpretation and reduces tissue superimposition artefacts.
If Tc $^{99m}$ (v) DMSA is taken up by squamous cell carcinoma of the head and neck, then the use of SPECT might improve diagnostic sensitivity by increasing the detection rate for low volume disease which would be of particular value in the clinically N$_0$ neck, i.e. to detect clinically occult neck nodes since, by the time such pathology is detected by planar scintigraphy, the nodes are usually greater than 2 cms and therefore palpable in the large majority of cases.

In this study, for SPECT Tc $^{99m}$ (v) DMSA imaging of head and neck cancer the **overall**, **primary** tumour, **lateral** neck compartment and **nodal** sensitivity, together with image quality and spatial resolution, were all improved when compared to their planar scintigraphic counterparts. However, although SPECT increased both the **overall** and **primary** tumour scintigraphic sensitivity when compared to planar imaging, it decreased the specificity. As an imaging modality for detecting **patients** with head and neck carcinoma and patients with **primary** tumours, SPECT was inferior to clinical examination and, as such, it plays no role in the routine evaluation of these patients.

For detecting both **lateral** neck compartments with metastatic tumour and individual **nodes** within those
compartments, SPECT improved the image quality and spatial resolution of the investigation and was more effective than planar Tc $^{99m}$ Tc (v) DMSA imaging but less effective than palpation, and size was an important factor in the detection of nodal masses for both palpation, and planar and SPECT Tc $^{99m}$ Tc (v) DMSA evaluation. Of the 51 clinically N$_0$ lateral neck compartments which were evaluated with SPECT Tc $^{99m}$ Tc (v) DMSA, only three would have been correctly upstaged (two correctly to N$_1$; one incorrectly to N$_1$ (true status N$_{2b}$)) and only in the latter patient would this have affected subsequent management. However, three lateral neck compartments would have been incorrectly upstaged to N$_1$. Although SPECT Tc $^{99m}$ Tc (v) DMSA imaging can increase diagnostic sensitivity for low volume disease in head and neck carcinoma and is superior to planar imaging, it has no role to play in the routine evaluation of patients, when compared to clinical examination and palpation combined with a comprehensive clinical understanding of the philosophy of head and neck cancer management.

Of the four patients with distant metastases (three squamous carcinoma, one adenocarcinoma) examined and imaged with Tc $^{99m}$ Tc (v) DMSA planar scintigraphy, no occult primary or non-bony metastatic lesions were identified. Of the three patients with squamous carcinoma (five false negatives), two had occult primaries which may have been too small to
resolve with planar scintigraphy. However, the last patient with metastatic squamous carcinoma had three tumour deposits, all greater than 2 cms, in the liver, pancreas and sigmoid colon and although these should have been scintigraphically resolvable by size criteria, they were deep seated masses and detection would have been affected by gamma ray attenuation. Of the positive lesions (five true, two false), all but one occurred in bony lesions (metastatic squamous carcinoma (1), metastatic breast adenocarcinoma (4), fibrous dysplasia of the maxilla (1)). These findings support the theory that Tc\textsuperscript{99m} (v) DMSA is behaving, in part, like a bone scanning agent and, like Tc\textsuperscript{99m}-MDP, although highly sensitive, it is poorly specific and is therefore unable to distinguish benign from malignant disease. The one false positive result occurred in an inflamed biopsy site and shows Tc\textsuperscript{99m} (v) DMSA can accumulate at sites of inflammation in man and these findings support the animal findings in this thesis. It is apparent from these results that Tc\textsuperscript{99m} (v) DMSA whole body planar scintigraphy has no role to play in the routine evaluation and subsequent detection of distant metastases from primary and occult primary squamous carcinoma.
Of the patients scanned with benign inflammatory lesions, positive uptake was observed in those with periodontal disease (when uptake was seen in the region of the mandible), in three patients with four inflammatory neck nodes and in one patient who had had an excision biopsy of a squamous cell carcinoma from the right axilla. These findings support previously reported data (Ohta et al, 1988) that \( {^{99}Tc} \text{DMSA} \) is less sensitive but more specific than \( {^{67}Ga} \). However, results from this thesis suggest \( {^{99}Tc} \text{DMSA} \) may be found in inflammatory tissue which surrounds not only the rabbit Vx-2 squamous carcinoma and human squamous carcinoma, but also at the sites of inflammatory neck nodes in patients with head and neck squamous carcinoma. The animal data can be considered reasonably accurate since it is based on \textit{in-vitro} analysis, although one must then be cautious about comparing the behaviour of a superficially transplanted rabbit squamous carcinoma with human squamous cell carcinoma. The results from this thesis indirectly suggests that \( {^{99}Tc} \text{DMSA} \) is not actively accumulated by inflammatory tissue and that accumulation, if any, in inflammatory neck nodes is probably a function of increased vascularity. Further work would be necessary to calculate the percentage of the injected dose/g in human inflammatory tissue to confirm or refute these findings.
In humans, although uptake was reported in four palpable (presumed inflammatory) neck nodes in three patients, two nodes (two patients) were also positive by CAT criteria but negative on histology. They could have therefore been false negative pathological nodes and scintigraphic uptake explained as a true positive result. In addition, some false positive scintigraphic results occurred with SPECT in patients with both palpable and non-palpable (presumed inflammatory) nodes (see previous Discussion, page 593). Although such uptake could have occurred in such nodes, it is conceivable that there could have been some free pertechnetate in the Tc $^{99m}$ (v) DMSA injection although both the thyroid and salivary glands would have exhibited positive scintigraphic uptake.

Of those patients with benign tumours (glomus tumours (2); pleomorphic adenoma (1); squamous papilloma (1)), only one with a Stage IV glomus jugulare exhibited positive uptake of Tc $^{99m}$ (v) DMSA. Although it is very difficult to draw any conclusions from such a small number of patients, certain observations are possible. The uptake in the glomus jugulare reflects not only its size but significant vascularity. The other glomus tumour was a Stage I tympanicum (which was also negative on I $^{123}$-MIBG) and such a small
tumour was probably outside the resolution of the gamma camera. The squamous papilloma was a small 2 x 1 cm lesion in the oral cavity which was close to vascular structures and again would have been difficult to resolve with a gamma camera. The last patient had a large 5 x 4 cm pleomorphic adenoma of the parotid and one might have expected this to show positive uptake particularly since uptake has been reported not only in pleomorphic adenomas but also a large variety of benign tumours (Ohta et al, 1988). However, in the latter series no mention is made of tumour size! It seems reasonable to suppose that uptake in benign tumours (as for malignancy) is, in part, a function of size and whereas a 2 x 1 cm squamous papilloma of the oral cavity would remain undetected, a 7 x 7 cm tumour would not. Further studies are necessary on a large number of benign tumours relating uptake to size to confirm or dispute such findings. False positive results observed in those patients scanned with benign lesions occurred in the larynx of the patient with the squamous papilloma of the oral cavity (two scans; pre- and post-operation) and in the mandible of a patient with proven periodontal disease. Reasons for these findings have already been discussed.

Reasons for the accumulation of Tc $^{99m}$ (v) DMSA in the region of the human pituitary are unclear. Variations in
hormonal activity are unlikely since all patients (except
two) were over the age of 60 and there were approximately
equal numbers of male and female patients with positive
Tc\textsuperscript{99m} (v) DMSA pituitary accumulation. The pituitary gland
has a high blood flow and this phenomenon is reflected in the
rabbit biodistribution studies. The positive uptake observed
in humans could reflect blood pool and although no pituitary
uptake was visualised on any of the rabbit scintigrams, this
can be explained by the small size of the rabbit pituitary
(approximately 1 mm\textsuperscript{2}) and the significant bony accumulation
of Tc\textsuperscript{99m} (v) DMSA in the surrounding immature calvarium.

The accumulation of Tc\textsuperscript{99m} (v) DMSA in the patient who
had had a hypophysectomy could be explained by uptake in the
surrounding resultant inflammatory tissue although the patient
was not imaged pre-operatively and in another patient no
uptake of Tc\textsuperscript{99m} (v) DMSA was observed in the region of a
pituitary eosinophil adenoma which had recently been
irradiated. What is interesting is that another patient
with a Stage IV glomus jugulare and the empty Sella Syndrome
exhibited positive uptake of Tc\textsuperscript{99m} (v) DMSA in the region
of the pituitary gland.
Based on the above findings, it seems reasonable to suggest that the pituitary gland region in man should be included in the normal biodistribution of $\text{Tc}^{99m} (v) \text{DMSA}$.

$\text{Ga}^{67}$-Citrate

$\text{Ga}^{67}$-Citrate has been extensively evaluated as a tumour imaging agent in head and neck squamous carcinoma (Section 1.3.2.). However, it suffers from distinct disadvantages inherent in its low sensitivity and specificity, considerable expense and prolonged blood clearance resulting in a delayed imaging time and poor resolution which usually means lesions measuring less than 2 cms are not detected by scintigraphy.

In this study, the overall patient sensitivity for $\text{Ga}^{67}$-Citrate in the detection of head and neck cancer was 94% (in contrast to clinical examination, 100%; $\text{Tc}^{99m} (v) \text{DMSA scintigraphy}$, 75%). For detecting patients with head and neck cancer, head and neck primary tumours and both lateral neck compartments with, and cervical lymph nodes containing, metastatic squamous carcinoma $\text{Ga}^{67}$ was less efficient than clinical examination and palpation, but more efficient than planar $\text{Tc}^{99m} (v) \text{DMSA scintigraphy}$. 
For Ga$^{67}$, the highest sensitivity was observed when imaging primary lesions but this is predominantly a function of size since of the 15 tumours which were detected scintigraphically and "T" staged, 11 were either T$_3$ or T$_4$. The lowest sensitivity was observed in detecting metastatic cervical lymphadenopathy and this was again a function of size since the majority of the false negative nodes measured less than 2 cms.

Although Ga$^{67}$-Citrate was more sensitive than Tc$^{99m}$ (v) DMSA in detecting patients with head and neck squamous carcinoma, it was less specific. Uptake of Ga$^{67}$ occurred not only in patients with squamous carcinoma, but also in lymphoma and inflammatory lesions. Compared with clinical examination, Ga$^{67}$-Citrate scintigraphy would have upstaged the lateral neck compartments of two patients (one correctly N$_0$ to N$_1$; one incorrectly N$_0$ to N$_1$ (correct status N$_2b$)).

The above findings support those in the literature that Ga$^{67}$ has no role to play in the routine evaluation of patients with head and neck squamous carcinoma. However, it does have a role within the head and neck in the management of lymphoma (particularly in restaging following initial treatment with radiotherapy and chemotherapy) and in the detection and evaluation of occult infection.
Miscellaneous Scans

Of the six patients who had Tc\textsuperscript{99m}-MDP scans at presentation, five had proven bony lesions and all five were positive with both Tc\textsuperscript{99m}-MDP and Tc\textsuperscript{99m} (v) DMSA and these findings show the latter agent is behaving, in part, like a bone scanning agent. Although both agents are highly sensitive, they are poorly specific since they cannot reliably distinguish benign from malignant disease and this explains why Tc\textsuperscript{99m}-MDP (and therefore Tc\textsuperscript{99m} (v) DMSA) has no role to play in the evaluation of local malignant spread of head and neck carcinoma into areas such as the mandible. However, Tc\textsuperscript{99m}-MDP does have a role in the evaluation of distant bony metastases in diseases such as carcinoma of the breast or squamous carcinoma of the bronchus. Although Tc\textsuperscript{99m}-MDP is taken up by metastatic squamous carcinoma, distant bony metastases in patients with head and neck carcinoma at presentation are uncommon (particularly in the presence of a normal serum calcium) so staging to include Tc\textsuperscript{99m}-MDP scintigraphy is not necessary. Similar arguments apply to routine whole body Tc\textsuperscript{99m} (v) DMSA scintigraphy.

The one patient who had a negative in-vitro labelled red blood cell head and neck scan was positive on
$^{99m}$Tc (v) DMSA scintigraphy. Such a finding supports the human biodistribution data in so far as some patients may exhibit tumour accumulation of $^{99m}$Tc (v) DMSA over and above a blood pool effect.

Of the three patients who had MRI at presentation, one patient with a clinically $T_3N_1$ nasopharyngeal carcinoma had $^{99m}$Tc (v) DMSA planar and SPECT scintigraphy, CAT, MRI and ultrasound of the neck. Although SPECT and CAT correctly identified a nodal mass in level 1, MRI and ultrasound upstaged the neck to $N_{2b}$.

**Computerised Axial Tomography**

The computerised axial tomographic results shown that CAT was as good as clinical examination, but superior to $^{99m}$Tc (v) DMSA scintigraphy in the overall identification and primary tumour evaluation of patients with head and neck cancer. For the detection of lateral neck compartments with metastatic carcinoma, as well as individual nodes with cancer, CAT was superior to both clinical examination and $^{99m}$Tc (v) DMSA scintigraphy (planar and SPECT).
One of the crucial questions relevant to CAT evaluation is its exact role in patients with head and neck squamous carcinoma. In the USA, the majority of patients would have CAT evaluation at presentation but in this country this is neither practically nor financially feasible. Stell (1987) has stated that CAT investigation of patients with head and neck cancer can only be justified in the United Kingdom if it radically alters treatment, i.e. from radiotherapy to surgery or from radical surgery to no treatment at all.

Although the numbers of patients studied are relatively small in this thesis, the results support the overall concensus in this country's literature that CAT only has a role to play in the evaluation of a head and neck primary carcinoma in those areas where clinical examination can be unreliable. These include the examination of the nasopharynx, maxillary sinus and the petrous temporal bone, in the assessment of bony and cartilaginous involvement in tumours of the floor of the mouth and tongue, larynx and hypopharynx, and in the evaluation of the occult primary. Of those patients upstaged in this study, three had clinically occult nasopharyngeal carcinomas while two had laryngeal and two had hypopharyngeal carcinomas. However, CAT is
not 100% sensitive. Two occult primary tumours (presumed head and neck) were undetected and tumours of the floor of the mouth, tonsil and hypopharynx (2) were understaged since CAT failed to detect evidence of bony and cartilaginous involvement.

It has been stated many times in this study that one of the greatest prognostic factors in head and neck squamous carcinoma is the presence or absence, level and size of metastatic cervical lymphadenopathy. The errors of palpation have been highlighted, not only in the historical review but also in the palpation results. There is no doubt that CAT can detect nodes which are in palpable and therefore could play a role in the evaluation of the N\textsubscript{0} neck. The question is which patients in the UK should have CAT neck evaluation? Some would argue that there is no extra cost involved to include the neck in total CAT evaluation (Stevens et al, 1985) so that the question now becomes whether or not to instigate total CAT evaluation.

Overall in this study, CAT was superior to clinical examination in detecting not only which lateral neck compartments contained metastatic cancer but also the number of involved nodes within any one lateral neck compartment. Is this
important? - not really since management decisions rest on the question whether or not metastatic neck cancer is present although such phenomena could affect statistical analysis since, in the absence of histological neck dissection data survival data would be falsely based on N₁ and not true N₂₀ status.

In this thesis, CAT was superior to palpation (81% sensitivity (90% accuracy) vs 71% sensitivity (87% accuracy)) in predicting the presence of metastatic squamous carcinoma in the lateral neck compartments of 51 patients with head and neck cancer. In recent studies, Friedman et al (1984) studied 50 patients (50 neck dissections) and obtained a 96% sensitivity (90% accuracy) for CAT (82% accuracy for palpation) and similar results have been obtained by others (Stevens et al, 1985; 70% accuracy for palpation, 93% accuracy for CAT) although in the former series 1.0 cm nodal size criteria were used while in the latter, 1.5 cm was the cut-off point for malignancy. In contrast, Feinmesser et al (1987) studied 79 patients (100 neck dissections) and obtained a 60% sensitivity (72% accuracy) for CAT (in contrast to 62% (77% accuracy) for palpation). The reasons for the discrepancies between Friedman's and Feinmesser's results have been reviewed by Friedman (1988) who states that although both studies used
third generation scanners, he used bolus contrast in addition to continuous infusion, 5 mm as opposed to 10 mm sections, 10 mm rather than 15 mm nodal size criteria and all scans were reviewed by one experienced radiologist (in contrast to Feinmesser et al, 1987). In this thesis, 6 mm sections were used, a continuous infusion of contrast was utilised in all but one patient, 10 mm nodal size criteria were used and all scans were reviewed by two experienced CAT radiologists with an interest in the head and neck.

However, the results in this thesis on the 51 patients are based not only on histological examination of neck dissection specimens but also on FNAB and clinical follow-up. Certain errors are inevitable. In the subgroup of 26 patients who had 31 neck dissections the sensitivity for neck palpation was 63% (68% accuracy) and this increased to 68% for CAT (71% accuracy). Both the accuracy and the false-negative (44%) and false positive rates (20%) reflect the inevitable errors with palpation and as the latter values are within the ranges quoted in the literature they will not be discussed further. Although these CAT results are approximately similar to those of Feinmesser et al (1987), they are less accurate than those observed by Friedman et al (1984) and Stevens et al (1985). In addition, the false positive rate was not greatly increased by CAT
in this study or in those conducted by Friedman et al (1984) and Stevens et al (1985). However, in the study conducted by Feinmesser et al (1987) the false positive rate increased from 9% for clinical evaluation to 18% for CAT. On the basis of these results, it is apparent that with current state of the art CAT imaging to include 4-5 mm sections, bolus and continuous contrast infusion together with super-specialist interpretation (Friedman et al, 1984; Stevens et al, 1985), CAT can be superior to clinical evaluation. However, the use of less sophisticated scanning methods and less than super-specialist interpretation will result in an inevitable drop in CAT sensitivity and specificity.

Although a lot of argument exists in the literature as to whether the 10 mm or 15 mm nodal size criteria should be used for CAT evidence of malignancy, overall in this study only two nodal masses were CAT positive by size alone and both of these were greater than 1.5 cm.

In the studies conducted by Friedman et al (1984), Stevens et al (1985) and Feinmesser et al (1987) approximately 20-31% of clinically N\textsubscript{0} necks were correctly upstaged by CAT and in one series (Stevens et al, 1985) overall neck staging was correctly changed by 25%. In this thesis (for the subgroup who all had neck dissections), overall neck
staging was correctly changed in 10% (three patients, three lateral neck compartments; one clinically N₀; two clinically N₁). Of these clinically N₁ necks, one was correctly upstaged to N₂b, but this did not affect any management decisions while the other was downstaged from N₁ to N₀. Would not most head and neck surgeons operate on a patient with a clinically positive neck and a normal CAT scan? Although CAT correctly upstaged one (6%) of the clinically N₀ lateral neck compartments, one patient (6%) was incorrectly upstaged to N₁ and would have had unnecessary surgery while CAT missed positive disease in six patients (38%).

On the basis of the results in this thesis it would seem appropriate that some arguments that have been applied against elective neck dissection could be levelled against elective neck CAT i.e. if only approximately 5% of clinically N₀ necks were upstaged by CAT (and if false-positive results are inevitable in the presence of large inflammatory neck nodes), then CAT would play no role in the evaluation of the N₀ neck. Treatment should be based on a sound understanding of the natural history of the disease process in question. Surely it is cheaper and as effective to adopt a policy of "wait and see" and perform five neck dissections rather than 100 CAT scans,
particularly since CAT false-positives may increase the number of patients having unnecessary surgery and the natural history of the tomographically positive node is not known.

The question of false positive CAT nodes is interesting. Of the five false positive neck nodes that were histologically examined in this study, all were positive by size, peripheral enhancement and central necrosis criteria. Although fatty replacement can cause false positive results on central necrosis criteria, such nodes are usually of normal size. Other causes of false-positive results include inflammatory nodes (or masses) or pathologically false-negative nodes. All the neck dissection specimens in this study were examined by experienced pathologists and nodes measuring more than 1 cm were sectioned into 5 mm sections. CAT scans are rarely done to evaluate neck disease alone. Stevens et al (1985) state that the important issue is not whether or not to scan the neck, but whether to get a scan at all. In his series, 25% of patients had their neck staging correctly changed by CAT and Stevens et al (1985) concluded, as have others (Friedman et al, 1984), that CAT has an important role to play in the nodal staging of head and neck cancer. However, in this country it is neither feasible or financially practical to CAT all the necks of patients with head and neck cancer.
Which necks then should be scanned? It seems appropriate to suggest (based on the results in this thesis, on those of Feinmesser et al (1987) and on the recommendations of Stell (1987)) that CAT of the neck may be of value, firstly if the neck is being scanned as part of the evaluation of the primary tumour, i.e. larynx or hypopharynx. Secondly, if there is a significant incidence (15-25%; Lindberg, 1972) of occult ipsilateral or bilateral nodal disease when its detection will alter subsequent management, i.e. large primary tumours, tumours crossing the midline, tumours of the pyriform sinus etc. Thirdly, in the presence of an ipsilateral N₂ or N₃ neck when there is the possibility of either deep fixation to vital structures and/or contralateral nodal disease, both of which will significantly alter management. Fourthly, in those patients with short stocky necks or who, for other reasons, are difficult to examine and lastly for restaging of the neck following treatment.

**Residual and Recurrent Disease**

One of the major problems in head and neck cancer, apart from the detection and evaluation of metastatic cervical lymphadenopathy, is the detection of residual and recurrent disease following surgery and irradiation.
Although some head and neck surgeons in the USA advocate baseline and subsequent routine CAT follow-up to detect early recurrences, such a protocol is neither practically nor financially feasible in this country. At the present moment in time, most head and neck surgeons in the United Kingdom follow their patients up with regular clinical examination, followed by chest radiography, endoscopy and CAT as appropriate.

The concept of Ehrlich's magic bullet is an attractive one, particularly to image microscopic residual and recurrent disease since the clinical examination of such pathology is fraught with difficulty. Despite such a theoretical alluring idea, the use of many radiopharmaceuticals (to include monoclonal antibodies) has been singularly unsuccessful.

In those patients in this study who were imaged with Tc$^{99m}$ (v) DMSA scintigraphy following surgery (17 scans) there were 12 positive and five negative scans. Out of the 12 positive scans, although eight had positive resection margins, seven had had bony manipulation or resection and the scintigraphic accumulation of Tc$^{99m}$ (v) DMSA far exceeded the extent of any residual disease, and in all positive scans any scintigraphic accumulation of Tc$^{99m}$ (v) DMSA was almost
certainly a function of surgery together with possible uptake in surrounding inflammatory tissue. Out of those patients who had post-surgery Tc\(^{99m}\) (v) DMSA scintigrams and no other subsequent treatment there was one true positive, one false positive and two true negative scans. From these results it is apparent that due to the effect of surgery ± subsequent inflammation, Tc\(^{99m}\) (v) DMSA scintigraphy almost certainly has no role to play in the detection of residual and recurrent disease immediately following operation.

Of those patients followed up after treatment (surgery, surgery with radiotherapy (pre-operative and post-operative), radiotherapy, radiotherapy and chemotherapy) although Tc\(^{99m}\) (v) DMSA was taken up at sites of residual or recurrent disease, it was inferior to clinical examination and it was impossible to ascertain whether uptake was in tumour, inflammatory tissue or both. One patient with macroscopic residual disease had post-operative SPECT and although this improved the image quality and spatial resolution of the investigation, it was impossible to ascertain whether Tc\(^{99m}\) (v) DMSA accumulation was in tumour, inflammatory tissue or both. Further in-vitro studies are necessary to confirm or refute this point.
Therefore, Tc\textsuperscript{99m} (v) DMSA scintigraphy has no role to play in the routine follow-up of patients who have had treatment for head and neck cancer. Uptake observed in the salivary glands following radiotherapy is almost certainly due to similar factors which operate for the permanent accumulation of Ga\textsuperscript{67} in irradiated salivary tissue. Such factors include interstitial oedema, perivascular inflammation and subsequent interstitial fibrosis (Bekerman and Hoffer, 1976). Some of the patients having radiotherapy also had neck dissection to include the tail of the parotid. Although this could result in inflammation and/or hypertrophy of the tail of the parotid, and therefore accumulation of Tc\textsuperscript{99m} (v) DMSA, such uptake should be unilateral and in all cases it was bilateral. Uptake of Tc\textsuperscript{99m} (v) DMSA into salivary tissue following radiotherapy has not been reported by other workers and the reason for such discrepancies is unclear. Although Bekerman and Hoffer (1976) reported that the uptake of Ga\textsuperscript{67} into irradiated tissue was permanent, in all the patients studied in this thesis who were imaged more than once following treatment, uptake in the salivary glands decreased as a function of time. Reasons for this are unclear although it may reflect, in part, recovery of salivary tissue function following radiotherapy which is more likely to happen in the 1980's as opposed to the early 1970's due to improvement in radiotherapy techniques.
Of the four patients who showed uptake of Tc\textsuperscript{99m} (v) DMSA in the region of the sternoclavicular joint, all had radical neck dissection. In one patient, accumulation in the joint was confirmed with Tc\textsuperscript{99m}-MDP scintigraphy. One patient was imaged six months following surgery while another was imaged at one year. Two patients were imaged sequentially to one year and in these the Tc\textsuperscript{99m} (v) DMSA accumulation was a constant finding which did not appear to decrease as a function of time. Although the obvious cause of such uptake is recent surgery, one would expect the accumulation of Tc\textsuperscript{99m} (v) DMSA to decrease as a function of time. Another explanation would be the stiff shoulder which many patients (including the four above) experience following radical neck dissection. The resultant abnormal movements of the shoulder girdle puts unnecessary strain on the sternoclavicular joint which could result in Tc\textsuperscript{99m} (v) DMSA (and Tc\textsuperscript{99m}-MDP) accumulation since any minor change in bone or joint physiology is known to cause increased uptake of Tc\textsuperscript{99m}-MDP.

\textsuperscript{67}Ga-Citrate has been evaluated in patients being followed up after treatment for head and neck cancer in an attempt to identify residual and recurrent disease. It is, at present, of no proven value for squamous cell carcinoma due to a low sensitivity and specificity.
In this study, of the two patients followed up with Ga$^{67}$, one had a composite resection with flap repair and was subsequently shown to have positive resection margins. He was a diabetic and developed an inflammatory abscess under the flap which was negative on Tc$^{99m}$ (v) DMSA scintigraphy but positive with Ga$^{67}$ and the uptake of this latter agent far exceeded any accumulation which could have occurred in residual and recurrent disease. The other patient (who had positive margins following a composite resection) developed a haematoma under his flap. This was negative on both Tc$^{99m}$ (v) DMSA and Ga$^{67}$-Citrate scintigraphy but MRI clearly demonstrated a haematoma.

Due to the small number of patients followed up in this series with CAT and/or MRI, it is difficult to make any meaningful comments as to the role these modalities may have in the evaluation of residual and recurrent head and neck cancer following surgery and irradiation. Using these investigations, no patients were identified who were not suspected of having either hidden or overt residual or recurrent disease, and there was one false positive CAT scan. Although these imaging techniques are of obvious value in the assessment of residual and recurrent disease in certain instances such as in those areas which are difficult to examine (i.e. the nasopharynx, maxillary sinus, temporal bone
and cervical oesophagus) the fact that they cannot reliably
distinguish benign from malignant disease, together with
cost and availability may mean that clinical examination
(together with chest radiography and panendoscopy) will remain
the main method of follow-up in this country for the majority
of surgeons.

In this study, a successful animal tumour model system
has been established which has been used to evaluate the
pharmacokinetics, biodistribution and optimal imaging
characteristics of the new tumour imaging agent Tc $^{99m}$ (v) DMSA.
These parameters were then compared with those obtained
from a parallel study in humans and the animal and human
pharmacokinetic and biodistribution data combined to calculate
the effective dose equivalent in man.

Although there was no evidence of active tumour uptake
in the rabbit model, and palpation was more effective than
Tc $^{99m}$ (v) DMSA planar scintigraphy in detecting the presence
of superficially transplanted tumours, the successfully
established animal model system can now be used to evaluate
new tumour imaging radiopharmaceuticals.
The human work evaluated the pharmacokinetics and whole body retention of Tc$^{99m}$ (v) DMSA and the biodistribution results failed to demonstrate any evidence of any active tumour uptake. The normal scintigraphic biodistribution of Tc$^{99m}$ (v) DMSA was confirmed and, overall, Tc$^{99m}$ (v) DMSA scintigraphy was less efficient than clinical examination in the detection of head and neck squamous carcinoma. Although Tc$^{99m}$ (v) DMSA has no role to play in the management of patients with this disease, this study has calculated its effective dose equivalent in man which may be of value to those using this agent as a tumour imaging radiopharmaceutical in the diagnosis and management of patients with medullary carcinoma of the thyroid.

In the future there may be refinement in anatomical TNM staging (tumour thickness (Spiro et al, 1986) and nodal level (to include the size of the largest node detected)) as well as the addition of physiological (functional) staging (Lentle et al, 1985). There will be further developments in both anatomical and physiological diagnostic imaging (to include fine resolution and three dimensional CAT, MRI and spectroscopy (Figure 63), SPECT (Figure 64) and PET, all of which could be used either singly or in combination as image superimposition), as well as continued research into both
A coronal abdominal spin echo MR image (TR 700 msec; TE 100 msec) taken with a body coil (Philips Gyroscan 515) of a NZW rabbit with a 7 x 5 cm transplanted squamous carcinoma in the left flank. The tumour is clearly visualised (A) with an area of central necrosis (B).

$^31$P MR spectrum obtained in-vivo (Philips Gyroscan 515) on the same rabbit as above using a 5 cm surface coil. The phosphocreatine (PCR), inorganic phosphate (Pi), phosphomonoester (PME) and phosphodiester (PDE) together with $\alpha$, $\beta$ and $\gamma$-ATP peaks are clearly seen.
$^{67}$Ga SPECT in a patient with a $T_4$ squamous carcinoma of the left pyriform sinus. Strongly positive accumulation of radioactivity (arrowed) is seen at the site of known primary disease.

These advances together with the further evaluation of PET quantification for both diagnosis and treatment (Di Chiro and Brooks, 1988; Minn et al, 1988) and the development of animal models to evaluate residual and recurrent disease (Porter et al, 1988) can only lead to an increase in otolaryngological diagnostic sensitivity and specificity and ultimately to an overall improvement in the way we diagnose, stage and treat head and neck cancer.
SUMMARY OF RESULTS

ANIMAL STUDIES

1) A successful animal tumour model system was established which may be used to evaluate new tumour imaging radiopharmaceuticals.

2) Tc $^{99m}$ (v) DMSA had a bi-exponential blood clearance with no significant difference between non-tumour and tumour groups. The cumulative urine clearance of Tc $^{99m}$ (v) DMSA was bi-exponential with no apparent difference between non-tumour and tumour groups.

3) The major organ biodistribution of Tc $^{99m}$ (v) DMSA was in the bone, kidneys and blood pool. There was no significant difference in biodistribution between non-tumour and tumour groups. The main route of excretion of Tc $^{99m}$ (v) DMSA is in the urine via the kidneys and bladder.

4) There was no evidence of active tumour accumulation of Tc $^{99m}$ (v) DMSA. However, compared to the blood pool the tumours did exhibit a prolonged washout phase. There was
uptake in inflammatory tissue but this never exceeded blood pool radioactivity.

5) There was no evidence of any specific tumour intracellular localisation mechanism for Tc $^{99m}$ (v) DMSA in rabbit squamous cell carcinoma.

6) The optimal imaging time for Tc $^{99m}$ (v) DMSA was approximately four hours.

7) Palpation is more efficient than Tc $^{99m}$ (v) DMSA planar scintigraphy in detecting superficially transplanted tumours.

8) A preclinical medical student was as good as a Consultant ENT Surgeon in predicting which sites had cancer but the ability to assess tumour size was related to the experience of the clinician.

9) There was a significant difference in mean thymus weights between the non-tumour and tumour groups but no significant difference between the mean tumour thymus weight and the value for mean thymus weight in non-tumour rabbits available from the literature. In this study, 26% of the reduction of the thymus weight in the tumour group was due to time with tumour and tumour mass.
10) Eighteen percent of rabbits developed macroscopic distant metastases.

11) The bone mass of a non-tumour rabbit was 13.7% body weight.

**HUMAN STUDIES**

1) Tc\(^{99m}\) had a bi-exponential blood clearance and cumulative urine excretion with no significant difference between non-tumour and tumour groups.

2) There was no significant difference in whole body retention of Tc\(^{99m}\) (v) DMSA between non-tumour and tumour groups.

3) There was no evidence of any active tumour accumulation or any specific tumour intracellular localisation mechanism for Tc\(^{99m}\) (v) DMSA in human squamous cell carcinoma.

4) The optimal imaging time for Tc\(^{99m}\) (v) DMSA was between two and four hours.
5) Tc\(^{99m}\) (v) DMSA had a normal biodistribution to include the lacrimal glands, nasal mucosa, blood pool, kidneys and the bladder.

6) Clinical examination was superior to Tc\(^{99m}\) (v) DMSA planar imaging in detecting not only which patients had squamous carcinoma but also primary tumours, lateral neck compartments with, and nodes within those compartments with metastatic carcinoma as well as patients with residual and recurrent disease following surgery and irradiation. No primary tumours were upstaged using Tc\(^{99m}\) (v) DMSA planar scintigraphy.

7) Tc\(^{99m}\) (v) DMSA SPECT improved the sensitivity, image quality and spatial resolution of the investigation compared to planar imaging but was still inferior to clinical examination in detecting patients with squamous carcinoma, primary tumours, lateral neck compartments with, and nodes within those compartments with metastatic carcinoma. No primary tumours were upstaged by SPECT.

8) Tc\(^{99m}\) (v) DMSA imaging has no role to play in the management of patients with head and neck squamous carcinoma.
9) Ga$^{67}$-Citrate planar scintigraphy was more efficient than Tc$^{99m}$ (v) DMSA planar scintigraphy but less efficient than clinical examination in the overall detection of patients with squamous carcinoma as well as those with primary tumours, lateral neck compartments with, and nodes within those compartments with metastatic squamous carcinoma.

10) Ga$^{67}$-Citrate planar scintigraphy has no role to play in the initial management of patients with head and neck squamous carcinoma.

11) CAT was as effective as clinical examination (and both investigations were superior to Tc$^{99m}$ (v) DMSA planar imaging) in detecting patients with squamous cell carcinoma and those with primary tumours.

12) CAT was superior to clinical examination in staging tumours of the maxillary antrum, nasopharynx, floor of the mouth, tonsil, larynx and hypopharynx.

13) Palpation was as accurate as CAT in predicting which lateral neck compartments contained metastatic carcinoma.

14) CAT (like Tc$^{99m}$ (v) DMSA planar scintigraphy) has no role to play in the routine evaluation of the clinically N$_0$ neck.
15) Tc$^{99m}$ (v) DMSA is a cheap radiopharmaceutical with a safe radiation dose (5.1 uSv/MBq) which is stable to two hours post-preparation in-vitro.
A successful animal tumour model system has been established which may be used to evaluate new tumour imaging radiopharmaceuticals. Tc $^{99m}$ (v) DMSA is a cheap radiopharmaceutical with a safe radiation dose (5.1 uSv/MBq) which is stable to two hours post-preparation *in-vitro*. Tc $^{99m}$ (v) DMSA scintigraphy is inferior to current techniques available to assess patients with squamous carcinoma of the head and neck.
SUGGESTIONS FOR FURTHER WORK

A. ANIMAL STUDIES

(i) To use the established tumour model system to evaluate the pharmacokinetics, biodistribution and optimal imaging characteristics of new specific and non-specific tumour imaging radiopharmaceuticals.

B. HUMAN STUDIES

(i) To evaluate new specific and non-specific tumour imaging radiopharmaceuticals (in parallel to A (i)) using planar imaging, SPECT and PET.

(ii) Ga$^{67}$-Citrate SPECT.

(iii) Fine resolution CAT, MRI (in-vitro and in-vivo) to include anatomical and functional image superimposition.

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PRESENTATIONS AND PUBLICATIONS

PRESENTATIONS


3. $^{99}$Tc\textsuperscript{m} (v) Dimercaptosuccinic Acid - a new imaging agent for squamous carcinoma of the head and neck? UMDS Bi-Annual Surgical Meeting. April 1987.


12.* Imaging head and neck cancer with radioisotopes. The ENT Department, Lewisham Hospital, May 1989.


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PUBLICATIONS


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APPENDIX

Glossary and Explanation of Technical Terms

Tables 1A-26A

Figures 1A-7A
GLOSSARY AND EXPLANATION OF TECHNICAL TERMS

ABSORBED DOSE (\(\bar{D}\)) - The energy deposited at a point per unit mass of a material by ionising radiation. The unit of absorbed dose is the gray (Gy) which is defined as one joule per kilogram. Until recently, and still widely used in some countries, it was the rad (defined as 0.01 J per kilogram). The absorbed dose = dose rate per unit activity x cumulative activity

\[
\bar{D} = \frac{\Delta \phi \bar{A}}{M_v}
\]

(simplified equation)

where \(\bar{D}\) = absorbed dose (in grays)
\(\Delta\) = mean energy of radiation emitted per nuclear transformation
\(\phi\) = the fraction of energy emitted which is absorbed by the target organ
\(\bar{A}\) = cumulative activity (Bq/s)
\(M_v\) = mass (in kilograms) of the organ whose absorbed dose is being calculated.

ACTIVITY - A quantity of radioactive material. It describes the rate at which nuclear transformations occur. Unit:- becquerel (Bq). 1 Bq = 1 transformation per second.
ADVERSE REACTION - Any unexpected patient reaction that might be connected with the administration of a radiopharmaceutical.

ALPHA PARTICLE - A particle consisting of two protons and two neutrons, produced by the disintegration of heavy nuclei (atomic number > 52).

ANIONIC - Negatively charged species.

ANNIHILATION RADIATION - The photons produced when an electron and a positron unite, and then cease to exist. The annihilation of a positron - electron pair results in the production of two gamma photons, each of which has an energy of 0.51 MeV.

ATOM - The smallest particle of an element which is capable of entering into a chemical reaction.

ATOMIC MASS NUMBER (Z) - The number of protons in the nucleus of an atom.

ATTENUATION COEFFICIENT - The attenuation of a beam of X- or gamma rays is made up of two components, absorption and scatter. Attenuation depends on the nature of the atom and is proportional to the density of the material as well as being related to the atomic number of the radionuclide.
BECQUEREL (Bq) - The SI unit of activity. One becquerel is equal to one nuclear transformation per second.

BETA PARTICLE - A charged particle emitted from the nucleus of an atom. Its mass and charge equal those of an electron. A beta particle with a positive charge is called a positron.

BIODISTRIBUTION - The distribution of a material in a biologic system, such as in a patient or in an experimental model.

BIOLOGICAL HALF-LIFE ($T_b$) - The time by which one-half of an administered dose of a substance is eliminated by biological processes such as urinary and faecal excretion.

CATIONIC - Positively charged species.

CHELATE - A metal ion attached to a complexing agent at more than one point.

CHROMATOGRAPHY - A method of separation which depends for its efficiency upon the equilibrium distribution of solute molecules between two phases, one of which is stationary and, over which, the second one flows.

COLLIMATOR - A device used to confine a radiation beam within a specific field of view.
**COMPLEX** - A compound of two or more components in which the constituents are more intimately associated than in a simple mixture.

**CUMULATIVE ACTIVITY (A)** - The value for the total number of transformations which occur in a source organ. It has units of Bq's.

**CUMULATIVE HALF-TIME** - The time taken for radioactivity to increase by 50%, i.e. cumulative urine excretion half-time \( t_{50\%} \) is the time taken to excrete 50% of the injected radioactivity.

**CURIE (Ci)** - A unit of radioactivity. A curie is defined as \( 3.7 \times 10^{10} \) disintegrations per second. It has now been replaced by the S.I. unit, Becquerel.

**DAUGHTER** - Any nuclide that originates from another nuclide by radioactive decay.

**DECAY** - The process of spontaneous transformation of a radionuclide resulting in a decrease in radioactive activity.

**DECAY CONSTANT \((\lambda)\)** - The fraction of atoms of a radioactive element decaying per unit time. It is expressed as \( \lambda = 0.693/t_p \), where \( t_p \) is the physical half-life of the radionuclide.
**DECAY PRODUCT** — A nuclide or radionuclide produced by decay. It may be formed directly from a radionuclide or as the result of a series of successive decays through several radionuclides.

**DOSE** — A general term for the amount of a radiopharmaceutical administered in KBq or MBq.

**DOSE EQUIVALENT** — The quantity obtained by multiplying the absorbed dose by a factor to allow for the different effectiveness of the various ionising radiations in causing harm to tissue. Unit: Sievert, Sv. 1 Sv = 1 Jkg\(^{-1}\).

The dose equivalent (H) at any point in a tissue is given by:

\[ H = D Q N \]

where \( D \) = absorbed dose (Gy)
\( Q \) = radiation quality factor
\( N \) = product of all other modifying factors

currently,
\[ N = 1 \]
\[ Q = 1 \text{ for } x-, \text{ gamma rays and } B \text{ particles, } 10 \text{ for neutrons and } 20 \text{ for alpha particles.} \]

**DOSIMETRY** — The calculation or measurement of radiation absorbed dose
"E" - A mathematical term used to describe a number to the power 10, i.e. $10^2 = E2$ and $10^{-4} = E-4$.

**EFFECTIVE DOSE EQUIVALENT** - The quantity obtained by multiplying the dose equivalents to various tissues and organs by the risk weighting factors appropriate to each and summing the products. Expressed in Sieverts, Sv.

**EFFECTIVE HALF-LIFE** ($T_e$) - Time required for an initial administered dose to be reduced by one-half due to both physical decay and biologic elimination of a radionuclide.

**ELECTRON** ($e$) - A negatively charged particle with low mass, $1/1836$ that of a proton, circulating around the atomic nucleus.

**ELECTROPHORESIS** - The migration of charged particles in solution under the influence of an applied electrical field. The rate of migration depends on the strength of the electrical field, the net charge, molecular size and shape, and the viscosity, ionic strength and temperature of the medium in which the molecule migrates.

**ELEMENT** - A substance composed entirely of atoms of the same atomic number.
ELUANT - A liquid or gas used to "wash-off" an adsorbed substance from a solid adsorbing matter (e.g. an ion-exchange resin). 0.9% sodium chloride solution is the eluant for the Tc $^{99m}$-generator.

ELUATE - The solution containing a substance "washed-off" or eluted from a solid adsorbing matter (e.g. ion-exchange resin). Tc $^{99m}$-TcO$_4^-$ in 0.9% sodium chloride solution is the eluate from the Tc $^{99m}$-generator.

FREE TECHNETIUM - An impurity in a Tc $^{99m}$-radiopharmaceutical referring to Tc $^{99m}$ pertechnetate which has not been chemically reduced and bound to the pharmaceutical.

GAMMA CAMERA - A piece of equipment which detects gamma rays and converts them into a visible image showing the distribution of a radiopharmaceutical within an object.

GAMMA RAY - A discrete quantity of electromagnetic energy, without mass or charge. Emitted by a radionuclide.

GENERATOR - A device in which a short-lived daughter is separated chemically and periodically from a long-lived parent adsorbed on adsorbent material (e.g. an ion-exchange resin).
ION - An atom or group of atoms with a positive charge (cation) or a negative charge (anion).

IONISATION - The process of removing electrons from atoms or molecules, thereby creating ions.

IONISING RADIATION - Radiation that produces ionisation in matter, e.g. alpha and beta particles, X- and gamma rays and neutrons.

ISOMERS - One of two or more nuclides with the same number of neutrons and protons in the nucleus (same Z and A), but existing in different energy states and spins.

ISOMERIC TRANSITION - The process by which a state of an isomer, or daughter radionuclide, decays by emission of gamma rays to a lower energy state.

ISOTOPE - Nuclides of the same atomic number but different mass numbers. They have the same chemical properties.

KITS - Pre-packaged sets of sterile reagents, of guaranteed pharmaceutical quality, prepared with the purpose of giving a specific radiopharmaceutical of a given quality, if handled according to instructions for use.
LABELLED COMPOUND - A compound in which one or more of the atoms of a proportion of the molecules is replaced by a detectable radioactive isotope.

LIGAND - A chemical group, ion or molecule co-ordinated to a central atom or group in a complex or chelate.

MASS NUMBER (A) - The number of protons plus neutrons (nucleons) in the nucleus of an atom.

MEAN LIFE - The mean period of time a radionuclide exists on average, before disintegration. It is related to half-life and decay constant by: mean life = 1/λ = 1.44 t<sub>½</sub>.

MEGABECQUEREL (MBq) - A quantity of radioactivity equal to 10<sup>6</sup> becquerels.

METASTABLE STATE (m) - An excited state of a nucleus that returns to the ground state by the emission of a gamma ray. Ground state is achieved over a measurable half-life.

MILLICURIE (mCi) - A quantity of activity equivalent to 10<sup>-3</sup> Curie.

MOLYBDENUM BREAKTHROUGH - The unwanted appearance of Mo<sup>99</sup> in the Tc<sup>99m</sup>-TcO<sub>4</sub> eluate of a Mo<sup>99</sup>-Tc<sup>99m</sup> radionuclide generator.
**NEUTRON** - An elementary particle with no electric charge, and a mass approximately the same as that of a proton.

**NUCLEAR MEDICINE** - The application of radioactive materials to the diagnosis and treatment of patients, and the study of human disease.

**NUCLEON** - A common term applied to protons and neutrons in the nucleus.

**NUCLEUS** - The core of an atom occupying little of the volume, containing most of the mass and bearing a positive charge.

**NUCLIDE** - This is a species of atom with a specific atomic number $Z$, a neutron number $N$, and which is in a defined nuclear state. The nucleus is either stable or radioactive and is usually in the ground state. If it is metastable, the nuclide is represented by the superscript $m$ (e.g. Tc$^{99m}$).

**ORGAN, CRITICAL** - The organ that receives the highest radiation dose after administration of radioactivity.

**ORGAN, TARGET** - The organ intended to be imaged and expected to receive the greatest concentration of administered activity.
OXIDATION - A chemical process by which an atom or groups of atoms loses electrons: an increase in the oxidation state number of an element.

PARENT - A radionuclide which yields another nuclide on disintegration. The "daughter" may be radioactive or stable.

pH - The unit of hydrogen ion concentration. It is given by the negative common logarithm of the hydrogen ion concentration in a solution, pH = -log₁₀ [H⁺].

PHARMACOKINETICS - Relates to the blood clearance and subsequent urine excretion of a specific radiopharmaceutical.

PHYSICAL HALF-LIFE (T₁/₂) - The time in which the activity of a radionuclide decays to half its original value.

POSITRON - A particle having a mass equal to the electron, and having an equal but opposite charge.

POSITRON EMISSION TOMOGRAPHY (PET) - Emission tomography using positron emitters (e.g. Carbon-11), and the subsequent coincidence detection of annihilation photons.

PROTON - An elementary nuclear particle with a positive electric charge equal numerically to the charge of the electron.
**RAD** - A unit of absorbed dose. One rad is equal to 100 ergs per gram. Now largely replaced by the Gray. 1 rad = 0.01 Gy.

**RADIOACTIVE CONCENTRATION** - The activity per unit quantity of any material in which a radionuclide occurs, e.g. MBq/ml.

**RADIOACTIVITY** - The property of radionuclides of spontaneous emission of energy as ionising radiation, resulting in the formation of new nuclides: emission of one or more types of radiation occurs (alpha or beta particles, gamma radiation).

**RADIOISOTOPE** - A specific form of an element of the periodic table whose nucleus disintegrates spontaneously.

**RADIONUCLIDE** - A species of atom (i.e. nuclide) which does not have a stable combination of neutrons and protons and therefore undergoes radioactive decay and disintegrates by spontaneous fission or the emission of alpha or beta particles or gamma radiation.

**RADIONUCLIDE GENERATOR** - See Generator.

**RADIOPHARMACEUTICAL** - A medicinal product used in the investigation or treatment of human disease that contains a radionuclide as an integral part.
REDUCING AGENT - A chemical agent which brings about the process of reduction.

REDUCTION - A chemical process in which an atom, or groups of atoms, gains electrons to become more negatively charged. The opposite of oxidation.

REM (ROENTGEN EQUIVALENT MAN) - A unit of human biological dose as a result of exposure to one or many types of ionising radiation. It is equal to the absorbed dose in rads, multiplied by the Quality Factor of the type of radiation. Now largely replaced by the Sievert. 1 rem = 0.01 Sv.

RETARDATION FACTOR (R_f) - A ratio calculated from chromatography results relating the distance travelled by a compound to the distance travelled by the solvent front.

SCINTIGRAM - A photographic recording of the distribution of radioactivity in an area of interest in the body by the use of a scintillation gamma camera.
SCINTILLATION SCANNING OR IMAGING - A recording of the distribution of radioactivity in the body, or a section of the body, with the use of a sodium iodide (NaI) detector. The images may be planar (antero-posterior, lateral or oblique) or in tomographic form (transaxial, coronal or sagittal) using SPECT.

S.I. UNIT - The Système Internationale Unit. The Council of European Communities, C.E.C., made the use of the becquerel and the gray mandatory for member states by 21st April, 1978. The C.E.C. expected Bq, Gy and Sv to be the legal units of measurement from 1st October, 1981, and the curie, rad and rem to cease to be used by 31st December, 1985.

SIEVERT - The name proposed by the International Commitee on Radiation Protection (ICRP) as the S.I. unit of dose equivalent. 1 Sv = 100 rem.

SINGLE PHOTON EMISSION COMPUTERISED TOMOGRAPHY (SPECT) - Using a special gamma camera, a large number of one-dimensional planar scintigraphic views are taken of a two-dimensional transverse axial slice of a region of the body, and the activity distribution within that slice reconstructed mathematically using a computer. Views are obtained in the transaxial, coronal and sagittal planes. The technique is analagous to X-ray computerised tomography.
SPECIFIC ABSORBED FRACTION ("S") - Most of the biological data to calculate the absorbed dose are contained within the cumulative activity (\(\bar{A}\)). The remaining part of the equation (\(\Delta, \phi\), and \(M_v\)) involves physical and anatomical data. This has been brought together in one unit, "S", called the absorbed dose per unit accumulated activity (specific absorbed fraction). The absorbed dose (\(\bar{D}\)) then becomes \(\bar{A}S\). Tabulations for "S" for a wide range of radionuclides are available in the literature (MIRD pamphlet II, 1975).

SPECIFIC ACTIVITY - The activity per unit mass of an element or compound containing a radioactive nuclide, e.g. MBq/mg.

SULPHYDRYL (-SH) - Sulphur-hydrogen group found in some proteins.

T₁ and T₂ RELAXATION TIMES - In a perfectly uniform magnetic field, the envelope of decay of the magnetic resonance signal is an exponential with a time constant known as the T₂ relaxation time. The vertical component of magnetisation drops to zero immediately after the radiofrequency pulse and takes longer to return to equilibrium. This return to equilibrium is also an exponential with a decay constant which is referred to as the T₁ relaxation time.
THERMOLUMINESCENT DOSIMETER (TLD) - A means of measuring absorbed radiation which utilises materials which, having been irradiated, release light in proportion to the radiation absorbed, when subsequently heated. It is usually worn on the fingers or forehead.

THIN LAYER CHROMATOGRAPHY (TLC) - A technique which uses an adsorbent as the stationary phase (e.g. polyester backed silica gel) and the development of the chromatogram takes place as the mobile phase percolates through the adsorbent. It has several advantages over paper chromatography due to its convenience, rapidity, greater resolution and higher sensitivity.

TRACER - A radionuclide or a compound labelled with a radionuclide that may be used to follow its distribution or course through a chemical, physical or metabolic process.

X-RAY - A discrete quantity of electromagnetic energy, without mass or charge.
TABLE 1A
BLOOD LEVELS OF RADIOACTIVITY FOLLOWING AN INTRAVENOUS INJECTION OF
TC\textsuperscript{99m}(v) DMSA IN 5 NON-TUMOUR BEARING NZW RABBITS
(% INJECTED DOSE PER GRAM OF BLOOD)

<table>
<thead>
<tr>
<th>TIME</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mins</td>
<td>2.07 E-1</td>
<td>1.38 E-1</td>
<td>1.47 E-1</td>
<td>1.59 E-1</td>
<td>1.46 E-1</td>
</tr>
<tr>
<td>15 mins</td>
<td>1.42 E-1</td>
<td>9.37 E-2</td>
<td>9.46 E-2</td>
<td>1.12 E-1</td>
<td>8.60 E-2</td>
</tr>
<tr>
<td>45 mins</td>
<td>-</td>
<td>-</td>
<td>5.53 E-2</td>
<td>6.90 E-2</td>
<td>4.42 E-2</td>
</tr>
<tr>
<td>1.5 hrs</td>
<td>-</td>
<td>-</td>
<td>3.27 E-2</td>
<td>4.81 E-2</td>
<td>2.60 E-2</td>
</tr>
<tr>
<td>2 hrs</td>
<td>4.42 E-2</td>
<td>2.81 E-2</td>
<td>2.60 E-2</td>
<td>4.39 E-2</td>
<td>2.18 E-2</td>
</tr>
<tr>
<td>3 hrs</td>
<td>2.51 E-2</td>
<td>1.83 E-2</td>
<td>1.31 E-2</td>
<td>2.92 E-2</td>
<td>1.69 E-2</td>
</tr>
<tr>
<td>4 hrs</td>
<td>1.64 E-2</td>
<td>1.27 E-2</td>
<td>1.45 E-2</td>
<td>2.00 E-2</td>
<td>1.01 E-2</td>
</tr>
<tr>
<td>6 hrs</td>
<td>8.99 E-3</td>
<td>7.16 E-3</td>
<td>1.10 E-2</td>
<td>9.76 E-3</td>
<td>6.92 E-3</td>
</tr>
<tr>
<td>24 hrs</td>
<td>2.06 E-3</td>
<td>1.47 E-3</td>
<td>1.35 E-3</td>
<td>1.85 E-3</td>
<td>9.41 E-4</td>
</tr>
</tbody>
</table>
**TABLE 2A**

BLOOD LEVELS OF RADIOACTIVITY FOLLOWING AN INTRAVENOUS INJECTION OF TC\textsuperscript{99m}(v) DMSA IN 9 TUMOUR-BEARING NZW RABBITS

(\% Injected Dose Per Gram of Blood)

<table>
<thead>
<tr>
<th>TIME</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mins</td>
<td>2.20 E-1</td>
<td>2.18 E-1</td>
<td>1.29 E-1</td>
<td>3.01 E-1</td>
<td>1.24 E-1</td>
<td>1.57 E-1</td>
<td>3.04 E-1</td>
<td>2.19 E-1</td>
<td>3.14 E-1</td>
</tr>
<tr>
<td>15 mins</td>
<td>1.36 E-1</td>
<td>1.54 E-1</td>
<td>8.04 E-2</td>
<td>1.91 E-1</td>
<td>8.47 E-2</td>
<td>1.20 E-1</td>
<td>2.22 E-1</td>
<td>1.45 E-1</td>
<td>2.33 E-1</td>
</tr>
<tr>
<td>30 mins</td>
<td>9.88 E-2</td>
<td>1.18 E-1</td>
<td>6.04 E-2</td>
<td>1.40 E-1</td>
<td>6.39 E-2</td>
<td>9.45 E-2</td>
<td>1.74 E-1</td>
<td>1.13 E-1</td>
<td>1.95 E-1</td>
</tr>
<tr>
<td>45 mins</td>
<td>7.04 E-2</td>
<td>9.77 E-2</td>
<td>4.55 E-2</td>
<td>1.07 E-1</td>
<td>5.78 E-2</td>
<td>7.48 E-2</td>
<td>1.49 E-1</td>
<td>9.23 E-2</td>
<td>1.75 E-1</td>
</tr>
<tr>
<td>60 mins</td>
<td>4.97 E-2</td>
<td>8.50 E-2</td>
<td>3.74 E-2</td>
<td>8.71 E-2</td>
<td>4.86 E-2</td>
<td>6.70 E-2</td>
<td>1.39 E-1</td>
<td>7.98 E-2</td>
<td>1.63 E-1</td>
</tr>
<tr>
<td>1.5 hrs</td>
<td>3.61 E-2</td>
<td>7.13 E-2</td>
<td>2.58 E-2</td>
<td>6.26 E-2</td>
<td>4.00 E-2</td>
<td>5.54 E-2</td>
<td>1.16 E-1</td>
<td>6.60 E-2</td>
<td>1.49 E-1</td>
</tr>
<tr>
<td>2 hrs</td>
<td>2.57 E-2</td>
<td>5.83 E-2</td>
<td>1.94 E-2</td>
<td>4.83 E-2</td>
<td>3.00 E-2</td>
<td>4.51 E-2</td>
<td>9.96 E-2</td>
<td>5.27 E-2</td>
<td>1.41 E-1</td>
</tr>
<tr>
<td>3 hrs</td>
<td>1.52 E-2</td>
<td>3.86 E-2</td>
<td>1.21 E-2</td>
<td>3.18 E-2</td>
<td>2.44 E-2</td>
<td>3.48 E-2</td>
<td>7.23 E-2</td>
<td>4.24 E-2</td>
<td>1.31 E-1</td>
</tr>
<tr>
<td>4 hrs</td>
<td>1.12 E-2</td>
<td>2.72 E-2</td>
<td>7.78 E-2</td>
<td>2.30 E-2</td>
<td>1.79 E-2</td>
<td>2.88 E-2</td>
<td>5.93 E-2</td>
<td>3.25 E-2</td>
<td>1.15 E-1</td>
</tr>
<tr>
<td>6 hrs</td>
<td>6.46 E-3</td>
<td>1.25 E-2</td>
<td>5.01 E-3</td>
<td>1.10 E-2</td>
<td>1.26 E-2</td>
<td>1.74 E-2</td>
<td>3.42 E-2</td>
<td>2.58 E-2</td>
<td>9.39 E-2</td>
</tr>
<tr>
<td>24 hrs</td>
<td>8.89 E-4</td>
<td>2.15 E-3</td>
<td>1.42 E-3</td>
<td>1.34 E-3</td>
<td>1.29 E-3</td>
<td>9.43 E-3</td>
<td>4.46 E-3</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

TUMOUR WEIGHT (g)

1.68 4.60 5.20 6.34 9.75 0.24 0.40 0.77 0.99
TABLE 3A

URINE LEVELS OF RADIOACTIVITY FOLLOWING AN INTRAVENOUS INJECTION
OF Tc$^{99m}$ (v) DMSA IN 5 NON-TUMOUR NZW RABBITS

(% INJECTED DOSE PER GRAM OF URINE)

<table>
<thead>
<tr>
<th>TIME (HRS)</th>
<th>RABBIT NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>0.05</td>
<td>2.19E-1</td>
</tr>
<tr>
<td>1</td>
<td>1.17E-2</td>
</tr>
<tr>
<td>2</td>
<td>9.11E-2</td>
</tr>
<tr>
<td>3</td>
<td>2.72E-1</td>
</tr>
<tr>
<td>4</td>
<td>1.67E0</td>
</tr>
<tr>
<td>6</td>
<td>1.94E-1</td>
</tr>
<tr>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td>TIME (HRS)</td>
<td>RABBIT NUMBER</td>
</tr>
<tr>
<td>------------</td>
<td>---------------</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>3.07 E0</td>
</tr>
<tr>
<td>2</td>
<td>2.96 E0</td>
</tr>
<tr>
<td>3</td>
<td>1.68 E0</td>
</tr>
<tr>
<td>4</td>
<td>1.95 E0</td>
</tr>
<tr>
<td>6</td>
<td>5.32 E-1</td>
</tr>
<tr>
<td>24</td>
<td>1.80 E-1</td>
</tr>
<tr>
<td>TUMOUR WEIGHT (g)</td>
<td>1.68</td>
</tr>
<tr>
<td>ORGAN</td>
<td>MEAN</td>
</tr>
<tr>
<td>--------------</td>
<td>--------</td>
</tr>
<tr>
<td>BRAIN</td>
<td>1.01 E-3</td>
</tr>
<tr>
<td>PITUITARY</td>
<td>1.07 E-2</td>
</tr>
<tr>
<td>NASAL MUCOSA</td>
<td>1.22 E-2</td>
</tr>
<tr>
<td>THYROID</td>
<td>4.84 E-3</td>
</tr>
<tr>
<td>MUSCLE</td>
<td>4.26 E-3</td>
</tr>
<tr>
<td>HEART</td>
<td>7.92 E-3</td>
</tr>
<tr>
<td>LUNG</td>
<td>1.45 E-2</td>
</tr>
<tr>
<td>THYMUS</td>
<td>2.85 E-3</td>
</tr>
<tr>
<td>LIVER</td>
<td>9.83 E-3</td>
</tr>
<tr>
<td>ORGAN</td>
<td>MEAN</td>
</tr>
<tr>
<td>--------------------</td>
<td>---------</td>
</tr>
<tr>
<td>STOMACH</td>
<td>6.43 E-3</td>
</tr>
<tr>
<td>SPLEEN</td>
<td>6.77 E-3</td>
</tr>
<tr>
<td>SMALL BOWEL</td>
<td>6.74 E-3</td>
</tr>
<tr>
<td>GALL BLADDER</td>
<td>1.08 E-2</td>
</tr>
<tr>
<td>BILE</td>
<td>7.87 E-3</td>
</tr>
<tr>
<td>TESTIS</td>
<td>6.90 E-3</td>
</tr>
<tr>
<td>SUBMANDIBULAR GLAND</td>
<td>5.89 E-3</td>
</tr>
<tr>
<td>BONE</td>
<td>7.60 E-2</td>
</tr>
<tr>
<td>LACRIMAL GLANDS</td>
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<tr>
<td>ORGAN</td>
<td>MEAN</td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------</td>
</tr>
<tr>
<td>BLOOD</td>
<td>2.03 E-2</td>
</tr>
<tr>
<td>URINE</td>
<td>9.82 E-1</td>
</tr>
<tr>
<td>TONGUE</td>
<td>9.28 E-3</td>
</tr>
<tr>
<td>LARGE BOWEL</td>
<td>9.73 E-3</td>
</tr>
<tr>
<td>CERVICAL LYMPH NODE</td>
<td>1.27 E-2</td>
</tr>
<tr>
<td>MARROW</td>
<td>6.88 E-3</td>
</tr>
</tbody>
</table>
TABLE 6A

THE FOUR HOUR BIODISTRIBUTION OF Tc $^{99m}$ (v) DMSA IN FIVE NON-TUMOUR NZW RABBITS

(§ INJECTED DOSE PER GRAM OF TISSUE)

<table>
<thead>
<tr>
<th>ORGAN</th>
<th>MEAN</th>
<th>RANGE</th>
<th>STANDARD DEVIATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAIN</td>
<td>7.70 E-4</td>
<td>2.85 E-4 - 1.48 E-3</td>
<td>4.91 E-4</td>
</tr>
<tr>
<td>PITUITARY</td>
<td>5.82 E-3</td>
<td>3.57 E-3 - 9.09 E-3</td>
<td>2.01 E-3</td>
</tr>
<tr>
<td>NASAL MUCOSA</td>
<td>1.02 E-2</td>
<td>4.70 E-3 - 1.98 E-2</td>
<td>5.46 E-3</td>
</tr>
<tr>
<td>THYROID</td>
<td>3.49 E-3</td>
<td>2.50 E-3 - 5.23 E-3</td>
<td>1.07 E-3</td>
</tr>
<tr>
<td>MUSCLE</td>
<td>2.40 E-3</td>
<td>1.83 E-3 - 3.15 E-3</td>
<td>3.00 E-4</td>
</tr>
<tr>
<td>HEART</td>
<td>4.75 E-3</td>
<td>3.10 E-3 - 6.82 E-3</td>
<td>1.36 E-3</td>
</tr>
<tr>
<td>LUNG</td>
<td>7.32 E-3</td>
<td>5.16 E-3 - 1.04 E-2</td>
<td>1.90 E-3</td>
</tr>
<tr>
<td>THYMUS</td>
<td>1.92 E-3</td>
<td>1.58 E-3 - 2.46 E-3</td>
<td>3.45 E-4</td>
</tr>
<tr>
<td>ORGAN</td>
<td>MEAN</td>
<td>RANGE</td>
<td>STANDARD DEVIATION</td>
</tr>
<tr>
<td>------------------------</td>
<td>-------</td>
<td>---------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>LIVER</td>
<td>1.16E-2</td>
<td>9.80E-3 - 1.35E-2</td>
<td>1.23E-3</td>
</tr>
<tr>
<td>STOMACH</td>
<td>4.38E-3</td>
<td>2.50E-3 - 6.28E-3</td>
<td>1.45E-3</td>
</tr>
<tr>
<td>KIDNEY</td>
<td>6.61E-2</td>
<td>5.57E-2 - 8.20E-2</td>
<td>9.88E-3</td>
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<td>SPLEEN</td>
<td>1.35E-3</td>
<td>3.30E-3 - 6.88E-3</td>
<td>1.71E-2</td>
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<td>SMALL BOWEL</td>
<td>4.34E-3</td>
<td>3.08E-3 - 6.79E-3</td>
<td>1.41E-3</td>
</tr>
<tr>
<td>GALL BLADDER</td>
<td>6.44E-3</td>
<td>3.80E-3 - 1.10E-2</td>
<td>2.62E-3</td>
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<tr>
<td>BILE</td>
<td>4.56E-3</td>
<td>4.56E-3 - 6.86E-3</td>
<td>1.59E-3</td>
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### TABLE 7A
THE SIX HOUR BIODISTRIBUTION OF Tc $^{99m}$ (v) DMSA IN FIVE NON-TUMOUR NZW RABBITS

(%) INJECTED DOSE PER GRAM OF TISSUE)

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### TABLE 8A
THE 24 HOUR BIODISTRIBUTION OF Tc$^{99m}$ (v) DMSA IN FIVE NON-TUMOUR NZW RABBITS

(* INJECTED DOSE PER GRAM OF TISSUE*)

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TABLE 9A
THE TWO HOUR BIODISTRIBUTION OF TC$^{99m}$ (v) DMSA IN FIVE TUMOUR-BEARING NZW RABBITS
(% INJECTED DOSE PER GRAM OF TISSUE)

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## TABLE 12A

THE 24 HOUR BIODISTRIBUTION OF Tc\(^{99m}\) (V) DMSA IN FIVE TUMOUR-BEARING NZW RABBITS

(% INJECTED DOSE PER GRAM OF TISSUE)

<table>
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<th>MEAN</th>
<th>RANGE</th>
<th>STANDARD DEVIATION</th>
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TABLE 13A

THE TUMOUR: SOFT TISSUE QUANTITATIVE BACKGROUND RATIOS

IN 4 TRANSPLANTED TUMOURS*

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<th>STANDARD DEVIATION</th>
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* DATA FROM 2 RABBITS
- NO TUMOURS DETECTED
### Table 14A

PALPATION RESULTS FOR SIX INDEPENDENT OBSERVERS IN 14 TUMOUR-BEARING NZW RABBITS

(DATA FROM 58 TUMOURS)

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* Largest Dimension
Table 14A (Cont’d)

PALPATION RESULTS FOR SIX INDEPENDENT OBServers IN 14 TUMOUR-BEARING NZW RABBITS
(DATA FROM 58 TUMOURS)

<table>
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<tr>
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* Largest Dimension
Table 14A (Cont’d)

PALPATION RESULTS FOR SIX INDEPENDENT OBSERVERS IN 14 TUMOUR-BEARING NZW RABBITS

(DATA FROM 58 TUMOURS)

<table>
<thead>
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<th>MEASURED TUMOUR SIZE* (mm)</th>
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* Largest Dimension
### Table 14A (Cont'd)

PALPATION RESULTS FOR SIX INDEPENDENT OBSERVERS IN 14 TUMOUR-BEARING NZW RABBITS

(Data from 58 Tumours)

<table>
<thead>
<tr>
<th>MEASURED TUMOUR SIZE* (mm)</th>
<th>PALPATED TUMOUR SIZE</th>
<th>OBSERVER NUMBER</th>
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</thead>
<tbody>
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<td>SIZE (mm)</td>
<td>BIAS (mm)</td>
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<td>-7</td>
</tr>
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<td>10</td>
<td>-7</td>
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* Largest Dimension
Table 14A (Cont’d)

PALPATION RESULTS FOR SIX INDEPENDENT OBSERVERS IN 14 TUMOUR-BEARING NZW RABBITS

(DATA FROM 58 TUMOURS)

<table>
<thead>
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<th>PALPATED TUMOUR SIZE</th>
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<tr>
<td></td>
<td>SIZE (mm)</td>
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<td>18</td>
<td>15</td>
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<tr>
<td>22</td>
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<td>23</td>
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* Largest Dimension
Table 14A (Cont’d)

PALPATION RESULTS FOR SIX INDEPENDENT OBSERVERS IN 14 TUMOUR-BEARING NZW RABBITS

(DATA FROM 58 TUMOURS)

<table>
<thead>
<tr>
<th>MEASURED TUMOUR SIZE* (mm)</th>
<th>PALPATED TUMOUR SIZE</th>
<th>OBSERVER NUMBER</th>
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</thead>
<tbody>
<tr>
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<td>SIZE (mm)</td>
<td>BIAS (mm)</td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>30</td>
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<td>24</td>
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<td>25</td>
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<td>26</td>
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<td>28</td>
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<td>+2</td>
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<td>33</td>
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<td>-24</td>
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* Largest Dimension
Table 14A (Cont’d)

PALPATION RESULTS FOR SIX INDEPENDENT OBSERVERS IN 14 TUMOUR-BEARING NZW RABBITS

(DATA FROM 58 TUMOURS)

<table>
<thead>
<tr>
<th>MEASURED TUMOUR SIZE* (mm)</th>
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</tr>
<tr>
<td>SIZE (mm)</td>
<td>BIAS (mm)</td>
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<td>52</td>
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</tr>
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<tr>
<td>55</td>
<td>50</td>
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<td>71</td>
<td>50</td>
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* Largest Dimension
<table>
<thead>
<tr>
<th>BODY WEIGHT (kg)</th>
<th>THYMUS WEIGHT (g)</th>
<th>THYMUS WT/BODY WT (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.35</td>
<td>3.10</td>
<td>1.32</td>
</tr>
<tr>
<td>2.35</td>
<td>7.20</td>
<td>3.06</td>
</tr>
<tr>
<td>2.42</td>
<td>3.90</td>
<td>1.61</td>
</tr>
<tr>
<td>2.43</td>
<td>3.40</td>
<td>1.40</td>
</tr>
<tr>
<td>2.46</td>
<td>4.50</td>
<td>1.83</td>
</tr>
<tr>
<td>2.53</td>
<td>6.80</td>
<td>2.69</td>
</tr>
<tr>
<td>2.66</td>
<td>6.00</td>
<td>2.26</td>
</tr>
<tr>
<td>2.68</td>
<td>5.20</td>
<td>1.94</td>
</tr>
<tr>
<td>2.70</td>
<td>5.00</td>
<td>1.85</td>
</tr>
<tr>
<td>2.81</td>
<td>4.30</td>
<td>1.53</td>
</tr>
<tr>
<td>2.81</td>
<td>4.60</td>
<td>1.64</td>
</tr>
<tr>
<td>2.83</td>
<td>5.20</td>
<td>1.84</td>
</tr>
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<td>2.83</td>
<td>5.60</td>
<td>1.98</td>
</tr>
<tr>
<td>2.86</td>
<td>4.80</td>
<td>1.68</td>
</tr>
<tr>
<td>2.86</td>
<td>9.60</td>
<td>3.36</td>
</tr>
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<td>2.91</td>
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<tr>
<td>3.12</td>
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<td>1.51</td>
</tr>
<tr>
<td>3.14</td>
<td>7.80</td>
<td>2.48</td>
</tr>
<tr>
<td>3.18</td>
<td>4.80</td>
<td>1.51</td>
</tr>
<tr>
<td>* Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.75 ± 0.52</td>
<td>5.32 ± 3.00</td>
<td>1.94 ± 1.08</td>
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* ± 2 standard deviations
TABLE 16A
THYMUS WEIGHTS IN 29 TUMOUR-BEARING NZW RABBITS

<table>
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<th>RABBIT</th>
<th>THYMUS WT (kg)</th>
<th>TUMOUR WT (g)</th>
<th>THYMUS WT/BODY WT (g/kg)</th>
<th>TIME WITH TUMOUR (DAYS)</th>
<th>METASTASES</th>
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<tr>
<td>3.72</td>
<td>3.80</td>
<td>98.7</td>
<td>1.02</td>
<td>63</td>
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</tr>
<tr>
<td>3.43</td>
<td>3.90</td>
<td>73.1</td>
<td>1.14</td>
<td>56</td>
<td>-</td>
</tr>
<tr>
<td>4.20</td>
<td>4.90</td>
<td>24.0</td>
<td>1.17</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td>2.72</td>
<td>4.70</td>
<td>0.50</td>
<td>1.73</td>
<td>22</td>
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</tr>
<tr>
<td>3.49</td>
<td>6.50</td>
<td>0.40</td>
<td>1.86</td>
<td>21</td>
<td>-</td>
</tr>
<tr>
<td>3.80</td>
<td>4.10</td>
<td>5.20</td>
<td>1.08</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>3.08</td>
<td>4.60</td>
<td>1.40</td>
<td>1.49</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>2.80</td>
<td>2.90</td>
<td>98.8</td>
<td>1.04</td>
<td>31</td>
<td>+</td>
</tr>
<tr>
<td>3.14</td>
<td>4.20</td>
<td>57.3</td>
<td>1.34</td>
<td>72</td>
<td>-</td>
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<tr>
<td>3.55</td>
<td>7.20</td>
<td>1.20</td>
<td>2.03</td>
<td>19</td>
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<tr>
<td>4.09</td>
<td>4.50</td>
<td>40.0</td>
<td>1.10</td>
<td>47</td>
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</tr>
<tr>
<td>3.73</td>
<td>3.60</td>
<td>6.30</td>
<td>0.97</td>
<td>26</td>
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</tr>
<tr>
<td>3.48</td>
<td>2.70</td>
<td>77.3</td>
<td>0.78</td>
<td>50</td>
<td>-</td>
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<tr>
<td>3.45</td>
<td>4.10</td>
<td>5.20</td>
<td>1.19</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>2.95</td>
<td>5.10</td>
<td>1.00</td>
<td>1.73</td>
<td>13</td>
<td>+</td>
</tr>
<tr>
<td>3.58</td>
<td>4.90</td>
<td>54.5</td>
<td>1.37</td>
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<td>18.8</td>
<td>1.70</td>
<td>29</td>
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<tr>
<td>3.56</td>
<td>3.70</td>
<td>36.2</td>
<td>1.04</td>
<td>29</td>
<td>+</td>
</tr>
<tr>
<td>2.78</td>
<td>4.80</td>
<td>1.20</td>
<td>1.73</td>
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<tr>
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<td>4.00</td>
<td>5.30</td>
<td>1.21</td>
<td>21</td>
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<td>3.13</td>
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<td>2.14</td>
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<td>1.66</td>
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<tr>
<td>2.86</td>
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<td>1.20</td>
<td>1.57</td>
<td>6</td>
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</tr>
<tr>
<td>2.87</td>
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<td>1.53</td>
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<td>3.28</td>
<td>4.90</td>
<td>3.80</td>
<td>1.49</td>
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<tr>
<td>3.31</td>
<td>6.50</td>
<td>11.0</td>
<td>1.96</td>
<td>20</td>
<td>-</td>
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</table>

* Mean

\[
\begin{align*}
3.34 & \pm 0.75 \\
4.90 & \pm 2.70 \\
24 & \pm 62 \\
1.48 & \pm 0.82 \\
29 & \pm 34
\end{align*}
\]

* + 2 standard deviations + = Metastases present - = No metastases detected
TABLE 17A

SEQUENTIAL BLOOD LEVELS FOLLOWING INTRAVENOUS INJECTION OF
$\text{Tc}^{99m} \text{(v)} \text{ DMSA}$ IN 5 NON-TUMOUR PATIENTS

($\%$ INJECTED DOSE PER GRAM)

<table>
<thead>
<tr>
<th>TIME</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mins</td>
<td>1.1 E-2</td>
<td>6.5 E-3</td>
<td>8.2 E-3</td>
<td>1.4 E-2</td>
<td>1.1 E-2</td>
</tr>
<tr>
<td>15 mins</td>
<td>8.9 E-3</td>
<td>4.3 E-3</td>
<td>6.4 E-3</td>
<td>1.1 E-2</td>
<td>8.4 E-3</td>
</tr>
<tr>
<td>30 mins</td>
<td>7.2 E-3</td>
<td>4.1 E-3</td>
<td>5.0 E-3</td>
<td>9.2 E-3</td>
<td>6.8 E-3</td>
</tr>
<tr>
<td>45 mins</td>
<td>6.0 E-3</td>
<td>3.2 E-3</td>
<td>4.3 E-3</td>
<td>8.5 E-3</td>
<td>6.6 E-3</td>
</tr>
<tr>
<td>1 hr</td>
<td>5.3 E-3</td>
<td>3.1 E-3</td>
<td>4.0 E-3</td>
<td>8.0 E-3</td>
<td>6.3 E-3</td>
</tr>
<tr>
<td>1.5 hrs</td>
<td>4.9 E-3</td>
<td>2.8 E-3</td>
<td>3.4 E-3</td>
<td>7.2 E-3</td>
<td>5.7 E-3</td>
</tr>
<tr>
<td>2 hrs</td>
<td>4.6 E-3</td>
<td>2.4 E-3</td>
<td>2.6 E-3</td>
<td>6.6 E-3</td>
<td>5.2 E-3</td>
</tr>
<tr>
<td>3 hrs</td>
<td>3.6 E-3</td>
<td>2.0 E-3</td>
<td>2.3 E-3</td>
<td>5.5 E-3</td>
<td>4.3 E-3</td>
</tr>
<tr>
<td>4 hrs</td>
<td>3.1 E-3</td>
<td>1.7 E-3</td>
<td>2.0 E-3</td>
<td>4.7 E-3</td>
<td>3.7 E-3</td>
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<tr>
<td>6 hrs</td>
<td>2.7 E-3</td>
<td>1.5 E-3</td>
<td>1.6 E-3</td>
<td>3.8 E-3</td>
<td>3.0 E-3</td>
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<td>24 hrs</td>
<td>4.7 E-4</td>
<td>2.6 E-4</td>
<td>2.1 E-4</td>
<td>5.4 E-4</td>
<td>5.1 E-4</td>
</tr>
</tbody>
</table>
**TABLE 18A**

SEQUENTIAL BLOOD LEVELS FOLLOWING INTRAVENOUS INJECTION OF 

Tc⁹⁹m (v) DMSA IN 5 TUMOUR PATIENTS 

(\% INJECTED DOSE PER GRAM)

<table>
<thead>
<tr>
<th>TIME</th>
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<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mins</td>
<td>1.4 E-2</td>
<td>1.2 E-2</td>
<td>1.2 E-2</td>
<td>1.1 E-2</td>
<td>4.7 E-3</td>
</tr>
<tr>
<td>15 mins</td>
<td>1.0 E-2</td>
<td>1.1 E-2</td>
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<tr>
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<td>5.6 E-3</td>
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**Primary Site**

- Maxilla
- Buccal Mucosa
- Nasopharynx
- Occult
- Tongue
- Metastatic
- Neck Disease

**Histology**

- G2
- G2
- G3
- G4
- G1

*UICC, 1987
TABLE 19A
SEQUENTIAL CUMULATIVE URINE AND WHOLE BODY RETENTION VALUES FOLLOWING INTRAVENOUS INJECTION OF Tc$^{99m}$\(\text{v}\) DMSA IN 5 NON-TUMOUR PATIENTS.

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>% Injected Dose/g</th>
<th>% Injected Dose</th>
<th>Sum% Injected Dose</th>
<th>*Whole body retention %</th>
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* Whole Body Retention = 100 - Σ% Injected Dose Excreted
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<th>% Injected Dose</th>
<th>Whole body retention %</th>
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* Whole body Retention = 100 - Σ% Injected Dose Excreted
TABLE 19A (CONT'D)

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<th>% Injected Dose</th>
<th>Σ% Injected Dose</th>
<th>*Whole body retention %</th>
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* Whole Body Retention = 100 - Σ% Injected Dose Excreted
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<th>% Injected Dose</th>
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* Whole Body Retention = 100 - $\sum$% Injected Dose Excreted
**TABLE 20A**

SEQUENTIAL CUMULATIVE URINE AND WHOLE BODY RETENTION VALUES FOLLOWING INTRAVENOUS INJECTION OF Tc$^{99m}$ DMSA IN 5 TUMOUR PATIENTS.

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>% Injected Dose/g</th>
<th>% Injected Dose</th>
<th>Σ% Injected Dose</th>
<th>*Whole body retention %</th>
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* Whole Body Retention = 100 - Σ% Injected Dose Excreted
TABLE 20A (CONT'D)

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<th>Time (mins)</th>
<th>% Injected Dose/g</th>
<th>% Injected Dose</th>
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* Whole Body Retention = 100 - Σ% Injected Dose Excreted
TABLE 20A (CONT'D)

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<th>% Injected Dose</th>
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* Whole Body Retention = 100 - Σ% Injected Dose Excreted
TABLE 20A (CONT'D)

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<th>Time (mins)</th>
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<th>% Injected Dose</th>
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* Whole Body Retention = 100 - Σ% Injected Dose Excreted.
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<th>PATIENT NO</th>
<th>AGE (YEARS)</th>
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<th>PRIMARY SITE AND HISTOLOGY</th>
<th>*TNM CLASSIFICATION</th>
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<tr>
<td>1</td>
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<td>Occult G2</td>
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<td>A, B</td>
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<td>$rT_2 N_0 M_0$</td>
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<tr>
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<td>Nasopharynx G3</td>
<td>$T_3 N_1 M_0$</td>
<td>A, B ( + MRI and neck ultrasound)</td>
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*UICC, 1987  + Previous Surgery  # Previous irradiation
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*UICC, 1987  + Previous surgery
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*UICC, 1987

+ Previous surgery

# Previous irradiation

99mTc (V) DMSA (A), CAT (B), Ga^{67} (C).
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<tr>
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* Previous surgery
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<tr>
<td>43</td>
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*UICC, 1987  + Previous surgery  # Previous irradiation

Tc 99M(V)DMSA (A), CAT (B), Ga 67 (C).
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*UICC, 1987 + Previous surgery # Previous irradiation
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<td>T_{X'N'M}</td>
<td>T_{X'N'M}</td>
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<td>T_{1N'M}0</td>
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<td>#60</td>
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<td>rT_{2N'M}0</td>
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<td>T_{2N'M}0</td>
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<td>66</td>
<td>56</td>
<td>M</td>
<td>Larynx G2 (Supraglottis)</td>
<td>T_{2N'M}0</td>
<td>T_{2N'M}0</td>
</tr>
</tbody>
</table>

*UICC, 1987  + Previous surgery  # Previous irradiation
<table>
<thead>
<tr>
<th>PATIENT NO</th>
<th>AGE (YEARS)</th>
<th>SEX MALE (M) FEMALE (F)</th>
<th>PRIMARY SITE AND *HISTOLOGY</th>
<th>*TNM CLASSIFICATION</th>
<th>INVESTIGATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>67</td>
<td>42</td>
<td>M</td>
<td>Oral cavity G2 (Floor of mouth) Adenocarcinoma</td>
<td>T4N2aM0 T4N2aM0 C0</td>
<td>A, B, C.</td>
</tr>
<tr>
<td>68</td>
<td>75</td>
<td>F</td>
<td>Lymphoma of cervical oesophagus</td>
<td>- -</td>
<td>A, B, C.</td>
</tr>
<tr>
<td>69</td>
<td>63</td>
<td>F</td>
<td>Mandible GX (embryonal Rhabdomyosarcoma)</td>
<td>T1N0M0 T1N0M0</td>
<td>A, B + Tc99M - MDP.</td>
</tr>
<tr>
<td>70</td>
<td>19</td>
<td>M</td>
<td>Maxilla G4 (embryonal Rhabdomyosarcoma)</td>
<td>T2N0M0 T2N0M0</td>
<td>A, B.</td>
</tr>
<tr>
<td>+71</td>
<td>62</td>
<td>F</td>
<td>Metastatic bony breast adenocarcinoma GX</td>
<td>rTxNX0 rTxNX1</td>
<td>A + (Tc99M - MDP).</td>
</tr>
</tbody>
</table>

*UICC, 1987 + Previous surgery # Previous irradiation
TABLE 22A

PATIENTS IMAGED WITH HEAD AND NECK CANCER AT PRESENTATION USING TC\textsuperscript{99m}(V) DMSA (PLANAR AND SPECT)

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>pTNM</th>
<th>PLANAR TC\textsuperscript{99m}(V) DMSA PRIMAR SITE</th>
<th>NECK R</th>
<th>L</th>
<th>SPECT TC\textsuperscript{99m}(V) DMSA PRIMARY SITE</th>
<th>NECK R</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1</td>
<td>T\textsubscript{x}N\textsubscript{3} (R1)</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>Not Done</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>T\textsubscript{2}N\textsubscript{0} (RO)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Not Done</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>T\textsubscript{x}N\textsubscript{2b} (R1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Not Done</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*4</td>
<td>T\textsubscript{3}N\textsubscript{0} (R0)</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>Not Done</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>T\textsubscript{x}N\textsubscript{2}</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>T\textsubscript{3}N\textsubscript{x} (R2)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>*7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>T\textsubscript{x}N\textsubscript{2a} (R0)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>T\textsubscript{3}N\textsubscript{1} (R0)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>12</td>
<td>rT\textsubscript{x}N\textsubscript{2b} (R1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>T\textsubscript{4}N\textsubscript{2b} (R1)</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tc\textsuperscript{99m}(V) DMSA; ++ = Strongly Positive  + = Positive  - = Negative  *Pituitary Sign Present  R = Residual disease
TABLE 22A (Contd.)

PATIENTS IMAGED WITH HEAD ANDNECK CANCER AT PRESENTATION USING TC$^{99m}$m(V) DMSA (PLANAR AND SPECT)

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>pTNM</th>
<th>PLANAR TC$^{99m}$m(V) DMSA PRIMARY SITE</th>
<th>SPECT TC$^{99m}$m(V) DMSA PRIMARY SITE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R</td>
<td>L</td>
</tr>
<tr>
<td>14</td>
<td>T$_4$N$_0$ (RO)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>15*</td>
<td>T$_4$N$_3$ (R1)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>rT$_x$N$_2b$ (RO)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20*</td>
<td>T$_4$N$_0$ (Rx)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>21</td>
<td>T$_2$N$_1$ (RO)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22</td>
<td>T$_x$N$_1$ (RO)</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>23</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>25*</td>
<td>T$_4$N$_0$ (R1)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>26*</td>
<td>T$_3$N$_0$ (RO)</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Tc$^{99m}$m(V) DMSA; ++ = Strongly Positive  + = Positive  - = Negative  *Pituitary Sign  R = Residual Disease
<table>
<thead>
<tr>
<th>PATIENT</th>
<th>pTNM</th>
<th>PLANAR TC\textsuperscript{99m} (V) DMSA</th>
<th>SPECT TC\textsuperscript{99m} (V) DMSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRIMARY SITE NECK R L</td>
<td>PRIMARY SITE NECK R L</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>rT\textsubscript{2}N\textsubscript{0} (R0)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>28</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>29</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>T\textsubscript{4}N\textsubscript{2c} (R1)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>31</td>
<td>T\textsubscript{4}N\textsubscript{2c} (R1)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>32</td>
<td>T\textsubscript{2}N\textsubscript{0} (R0)</td>
<td>+</td>
<td>+</td>
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<tr>
<td>33</td>
<td>T\textsubscript{x}N\textsubscript{0} (R1)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>34</td>
<td>T\textsubscript{4}N\textsubscript{0} (R0)</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>35</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>36</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>37</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>38</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

\textsuperscript{99m} (V) DMSA; ++ = Strongly Positive  + = Positive  - = Negative  *Pituitary Sign Present  R = Residual Disease
TABLE 22A (Contd.)

PATIENTS IMAGED WITH HEAD AND NECK CANCER AT PRESENTATION USING Tc^{99}m(V) DMSA (PLANAR AND SPECT)

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>pTNM</th>
<th>PLANAR Tc^{99}m(V) DMSA PRIMARY SITE</th>
<th>NECK R</th>
<th>L</th>
<th>SPECT Tc^{99}m(V) DMSA PRIMARY SITE</th>
<th>NECK R</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>T_{42}N_{2c} (R1)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Not Done</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>T_{2}N_{3}M_{0}</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Not Done</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Occult Second Primary</strong></td>
<td><strong>(T_{2}) Right Lung</strong></td>
<td><strong>(C5)</strong></td>
<td>+</td>
<td>Not Done</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Not Done</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>*43</td>
<td>T_{4}N_{0} (R1)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Not Done</td>
<td></td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>T_{4}N_{0} (R0)</td>
<td>++</td>
<td>-</td>
<td>+</td>
<td>Not Done</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>T_{4}N_{2b} (R0)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>46</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Not Done</td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Not Done</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Not Done</td>
<td></td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Not Done</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tc^{99}m(V) DMSA; ++ = Strongly Positive  + = Positive  - = Negative  *Pituitary Sign Present  R = Residual Disease
TABLE 22A (Contd.)

PATIENTS IMAGED WITH HEAD AND NECK CANCER AT PRESENTATION USING Tc$^{99m}$ (V) DMSA (PLANAR AND SPECT)

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>pTNM</th>
<th>PLANAR Tc$^{99m}$ (V) DMSA PRIMARY SITE</th>
<th>NECK R L</th>
<th>SPECT Tc$^{99m}$ (V) DMSA PRIMARY SITE</th>
<th>NECK R L</th>
</tr>
</thead>
<tbody>
<tr>
<td>*50</td>
<td>rT$_3$N$_0$ (RO)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Not Done</td>
</tr>
<tr>
<td>*51</td>
<td>rT$_3$N$_0$ (RO)</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>52</td>
<td>- (Second Lung Primary)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Not Done</td>
</tr>
<tr>
<td>*53</td>
<td>- Primary Larynx Second Primary (Lung)</td>
<td>+</td>
<td>-</td>
<td>Not Done</td>
<td></td>
</tr>
<tr>
<td>54</td>
<td>- (Second Lung Primary)</td>
<td>+</td>
<td>-</td>
<td>Not Done</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>- (See Table 43)</td>
<td>-</td>
<td>-</td>
<td>Not Done</td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>T$_0$N$_0$M$_1$ (R1, C5)</td>
<td>-</td>
<td>-</td>
<td>Not Done</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>- (See Table 43)</td>
<td>-</td>
<td>-</td>
<td>Not Done</td>
<td></td>
</tr>
<tr>
<td>*58</td>
<td>- (See Table 43)</td>
<td>-</td>
<td>-</td>
<td>Not Done</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>- (See Table 43)</td>
<td>-</td>
<td>-</td>
<td>Not Done</td>
<td></td>
</tr>
<tr>
<td>60</td>
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<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>*61</td>
<td>T$_0$N$_0$ (RO)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Tc$^{99m}$ (V) DMSA; ++ = Strongly Positive + = Positive - = Negative *Pituitary Sign R = Residual Disease
TABLE 22A (Contd.)

PATIENTS IMAGED WITH HEAD AND NECK CANCER AT PRESENTATION USING TC\textsuperscript{99m}(V) DMSA (PLANAR AND SPECT)

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>pTNM</th>
<th>PLANAR TC\textsuperscript{99m}(V)</th>
<th>DMSA NECK R</th>
<th>DMSA NECK L</th>
<th>SPECT TC\textsuperscript{99m}(V)</th>
<th>DMSA PRIMARY SITE</th>
<th>NECK R</th>
<th>NECK L</th>
</tr>
</thead>
<tbody>
<tr>
<td>*62</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Not Done</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>67</td>
<td>T\textsubscript{4}N\textsubscript{2c} (R1)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>*68</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>Not Done</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>T\textsubscript{1}N\textsubscript{x}</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*71</td>
<td>-</td>
<td>Not Applicable (See Table 43)</td>
<td>-</td>
<td>-</td>
<td>Not Done</td>
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<td></td>
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<tr>
<td>*72</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td></td>
<td></td>
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<td>76</td>
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<td>-</td>
<td>-</td>
<td>Not Done</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tc\textsuperscript{99m}(V) DMSA; ++ = Strongly Positive  + = Positive  - = Negative  *Pituitary Sign Present  R= Residual Disease
**TABLE 23A**

**THE CLINICAL STATUS OF THE PATIENTS STUDIED IN THIS THESIS UPTO MARCH 1ST 1989**

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>TREATMENT</th>
<th>CLINICAL STATUS - MARCH 1ST 1989</th>
<th># (scans)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>*+</td>
<td>Alive and well (26 months FU)</td>
<td>(5 scans)</td>
</tr>
<tr>
<td>2</td>
<td>*</td>
<td>Alive and well (34 months FU)</td>
<td>(4 scans)</td>
</tr>
<tr>
<td>3</td>
<td>*+</td>
<td>Alive and well (6 months FU)</td>
<td>(3 scans)</td>
</tr>
<tr>
<td>4</td>
<td>*</td>
<td>Alive and well (25 months FU)</td>
<td>(2 scans)</td>
</tr>
<tr>
<td>5</td>
<td>*+</td>
<td>Alive and well (21 months FU)</td>
<td>(3 scans)</td>
</tr>
<tr>
<td>6</td>
<td>*+</td>
<td>Died at 9 months</td>
<td>(5 scans)</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>Alive and well (11 months FU)</td>
<td>(3 scans)</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>Alive and well (16 months FU)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>*+</td>
<td>Alive and well (16 months FU)</td>
<td>(7 scans)</td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td>Died at 8 months</td>
<td>(4 scans)</td>
</tr>
<tr>
<td>11</td>
<td>*</td>
<td>Alive and well (30 months FU)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>*</td>
<td>Alive and well (15 months FU)</td>
<td>(4 scans)</td>
</tr>
</tbody>
</table>

* Surgery + Radiotherapy # Follow up Tc$^{99m}$ (v) DMSA scintigraphy FU = Follow Up
### TABLE 23A (Contd.)

**THE CLINICAL STATUS OF THE PATIENTS STUDIED IN THIS THESIS UPTO MARCH 1ST 1989**

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>TREATMENT</th>
<th>CLINICAL STATUS - MARCH 1ST 1989</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>++</td>
<td>Recurrent disease at 7 months. Inoperable. Alive at 10 months. # (3 scans)</td>
</tr>
<tr>
<td>14</td>
<td>*</td>
<td>Alive and well (13 months FU) # (6 scans)</td>
</tr>
<tr>
<td>15</td>
<td>*</td>
<td>Recurrence right neck after 9 months. Treated by surgery. Now has distant bony metastases (21 months FU) # (1 scan)</td>
</tr>
<tr>
<td>16</td>
<td>+</td>
<td>Alive and well (9 months FU)</td>
</tr>
<tr>
<td>17</td>
<td>+</td>
<td>Residual/recurrent disease at 6 months. Died at 12 months. # (3 scans)</td>
</tr>
<tr>
<td>18</td>
<td>*</td>
<td>Alive and well (11 months FU) # (9 scans)</td>
</tr>
<tr>
<td>19</td>
<td>+</td>
<td>Alive and well (20 months)</td>
</tr>
<tr>
<td>20</td>
<td>++</td>
<td>Died at 6 months # (1 scan)</td>
</tr>
<tr>
<td>21</td>
<td>++</td>
<td>Local recurrence at 9 months. Surgical treatment planned. # (2 scans)</td>
</tr>
<tr>
<td>22</td>
<td>++</td>
<td>Residual and recurrent disease. No further treatment. Died at 6 months. # (2 scans)</td>
</tr>
</tbody>
</table>

* Surgery + Radiotherapy  # Follow up Tc$^{99m}$ DMSA scintigraphy  FU = Follow Up
### TABLE 23A (Contd.)

THE CLINICAL STATUS OF THE PATIENTS STUDIED IN THIS THESIS UPTO MARCH 1ST 1989

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>TREATMENT</th>
<th>CLINICAL STATUS - MARCH 1ST 1989</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>-</td>
<td>Died at 6 months</td>
</tr>
<tr>
<td>24</td>
<td>-</td>
<td>Died at 6 months</td>
</tr>
<tr>
<td>25</td>
<td>++</td>
<td>Alive and well (5 months FU)</td>
</tr>
<tr>
<td></td>
<td></td>
<td># (1 scan)</td>
</tr>
<tr>
<td>26</td>
<td>++</td>
<td>Alive and well. Developed subglottic stenosis following tracheostomy. Further tracheostomy resulted in intractable overspill. Treated by laryngectomy at 9 months. Alive and well (28 months FU)</td>
</tr>
<tr>
<td>27</td>
<td>*</td>
<td>Stomal recurrence at 6 months.</td>
</tr>
<tr>
<td></td>
<td></td>
<td># (2 scans)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treated by surgery. Then developed nodal metastasis left neck. No further treatment. Died at 30 months.</td>
</tr>
<tr>
<td>28</td>
<td>-</td>
<td>Died at 6 months</td>
</tr>
<tr>
<td>29</td>
<td>+</td>
<td>Residual and recurrent disease at 3 months. # (1 scan) Died at 7 months.</td>
</tr>
<tr>
<td>30</td>
<td>*</td>
<td>Residual/recurrent disease at 9 months left neck. No further treatment. Died at 11 months.</td>
</tr>
</tbody>
</table>

* Surgery + Radiotherapy - No treatment

# Follow up Tc^{99m}(v) DMSA scintigraphy

FU = Follow Up
**TABLE 23A (Contd.)**

**THE CLINICAL STATUS OF THE PATIENTS STUDIED IN THIS THESIS UPTO MARCH 1ST 1989**

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>TREATMENT</th>
<th>CLINICAL STATUS - MARCH 1ST 1989</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>++</td>
<td>Alive and well (7 months FU)</td>
</tr>
<tr>
<td>32</td>
<td>++</td>
<td>Residual/recurrent disease at 8 months. Died at 12 months.</td>
</tr>
<tr>
<td>33</td>
<td>++</td>
<td>Died at 7 months. Post-mortem examination showed no residual head and neck disease but a T4 bladder tumour.</td>
</tr>
<tr>
<td>34</td>
<td>+</td>
<td>Alive and well (14 months FU)</td>
</tr>
<tr>
<td>35</td>
<td>+</td>
<td>Recurrence at 7 months. Treated by surgery. Now alive and well. (24 months FU)</td>
</tr>
<tr>
<td>36</td>
<td>-</td>
<td>Died at 3 months</td>
</tr>
<tr>
<td>37</td>
<td>+</td>
<td>Alive and well (11 months FU)</td>
</tr>
<tr>
<td>38</td>
<td>-</td>
<td>Refused treatment. Died at 9 months.</td>
</tr>
<tr>
<td>39</td>
<td>++</td>
<td>Residual disease at 6 months. Died at 9 months.</td>
</tr>
</tbody>
</table>

* Surgery + Radiotherapy - No treatment # Follow up Tc$^{99m}$ (v) DMSA scintigraphy FU = Follow Up
**TABLE 23A** (Contd.)

THE CLINICAL STATUS OF THE PATIENTS STUDIED IN THIS THESIS UPTO MARCH 1ST 1989

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>TREATMENT</th>
<th>CLINICAL STATUS - MARCH 1ST 1989</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>+</td>
<td>Residual disease following treatment with radiotherapy and Hyperbaric oxygen. Post-mortem examination showed occult second primary in apex of right lung. Died at 2 months.</td>
</tr>
<tr>
<td>41</td>
<td>-</td>
<td>Died at 4 months</td>
</tr>
<tr>
<td>42</td>
<td>+</td>
<td>Residual disease. Died at 7 months.</td>
</tr>
<tr>
<td>43</td>
<td>*+</td>
<td>Alive and well (29 months FU)</td>
</tr>
<tr>
<td>44</td>
<td>*+</td>
<td>Alive and well (14 months FU)</td>
</tr>
<tr>
<td>46</td>
<td>-</td>
<td>Died at 5 months</td>
</tr>
<tr>
<td>47</td>
<td>-</td>
<td>Died at 2 months. Carotid blow out.</td>
</tr>
</tbody>
</table>

* Surgery + Radiotherapy — No treatment  # Follow up Tc⁹⁹m(v) DMSA scintigraphy  FU = Follow Up
### TABLE 23A (Contd.)

**THE CLINICAL STATUS OF THE PATIENTS STUDIED IN THIS THESIS UPTO MARCH 1ST 1989**

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>TREATMENT</th>
<th>CLINICAL STATUS - MARCH 1ST 1989</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>-</td>
<td>Residual and recurrent disease in larynx and right neck. Alive at 2 months.</td>
</tr>
<tr>
<td>49</td>
<td>-</td>
<td>Recurrent disease. Alive (2 months FU)</td>
</tr>
<tr>
<td>50</td>
<td>*</td>
<td>Alive and well (17 months FU) # (4 scans)</td>
</tr>
<tr>
<td>51</td>
<td>*</td>
<td>Recurrent disease at 14 months. Treated by local surgery. Now has further recurrence at 26 months. # (1 scan)</td>
</tr>
<tr>
<td>52</td>
<td>-</td>
<td>Died at 3 months</td>
</tr>
<tr>
<td>53</td>
<td>-</td>
<td>Died at 4 months</td>
</tr>
<tr>
<td>54</td>
<td>-</td>
<td>Died at 1 month</td>
</tr>
<tr>
<td>55</td>
<td>+</td>
<td>Died at 9 months</td>
</tr>
<tr>
<td>56</td>
<td>-</td>
<td>Died at 3 months. Post-mortem. Distant metastases in liver, pancreas and sigmoid colon (resected).</td>
</tr>
<tr>
<td>57</td>
<td>-</td>
<td>Died at 4 months. Developed bony metastases. No post-mortem.</td>
</tr>
<tr>
<td>58</td>
<td>-</td>
<td>Died at 12 months</td>
</tr>
<tr>
<td>* Surgery</td>
<td>+ Radiotherapy</td>
<td>- No treatment # Follow up Tc$^{99m}$ (v) DMSA scintigraphy FU = Follow Up</td>
</tr>
</tbody>
</table>
TABLE 23A (Contd.)

THE CLINICAL STATUS OF THE PATIENTS STUDIED IN THIS THESIS UPTO MARCH 1ST 1989

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>TREATMENT</th>
<th>CLINICAL STATUS – MARCH 1ST 1989</th>
</tr>
</thead>
<tbody>
<tr>
<td>59</td>
<td>+</td>
<td>Alive and well (30 months FU)</td>
</tr>
<tr>
<td></td>
<td></td>
<td># (3 scans)</td>
</tr>
<tr>
<td>60</td>
<td>-</td>
<td>Alive and well (40 months FU)</td>
</tr>
<tr>
<td></td>
<td></td>
<td># (4 scans)</td>
</tr>
<tr>
<td>61</td>
<td>*</td>
<td>Alive and well (23 months FU)</td>
</tr>
<tr>
<td>62</td>
<td>-</td>
<td>Alive and well (2 months FU)</td>
</tr>
<tr>
<td>63</td>
<td>*</td>
<td>Alive and well (11 months FU)</td>
</tr>
<tr>
<td>64</td>
<td>*</td>
<td>Died after 24 hours due to cerebro-vascular accident consequent upon carotid artery ligation</td>
</tr>
<tr>
<td>65</td>
<td>**</td>
<td>Alive and well (13 months FU)</td>
</tr>
<tr>
<td>66</td>
<td>**</td>
<td>Alive and well (11 months FU)</td>
</tr>
<tr>
<td>67</td>
<td>**</td>
<td>Developed an oro-cutaneous fistula. # (5 scans)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No treatment. Alive and well. (25 months FU)</td>
</tr>
<tr>
<td>68</td>
<td>-</td>
<td>Died at 3 months</td>
</tr>
<tr>
<td>69</td>
<td>*</td>
<td>Died of recurrent disease at 12 months # (3 scans)</td>
</tr>
</tbody>
</table>

* Surgery + Radiotherapy - No treatment # Follow up Tc^{99m}(v) DMSA scintigraphy  
FU = Follow Up
### TABLE 23A (Contd.)

**THE CLINICAL STATUS OF THE PATIENTS STUDIED IN THIS THESIS UPTO MARCH 1ST 1989**

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>TREATMENT</th>
<th>CLINICAL STATUS - MARCH 1ST 1989</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>+ and chemotherapy</td>
<td>Died of distant metastases at 30 months</td>
</tr>
<tr>
<td>71</td>
<td>*</td>
<td>Died from distant metastases at 11 months</td>
</tr>
<tr>
<td></td>
<td>Hormonal manipulation</td>
<td>to include hypophysectomy</td>
</tr>
<tr>
<td>72</td>
<td>+</td>
<td>Alive and well (8 months FU)</td>
</tr>
<tr>
<td>73</td>
<td>*</td>
<td>Alive and well (5 months FU)</td>
</tr>
<tr>
<td>74</td>
<td>Antibiotics</td>
<td>Alive and well (31 months FU)</td>
</tr>
<tr>
<td>75</td>
<td>*</td>
<td>Alive and well (26 months FU)</td>
</tr>
<tr>
<td>76</td>
<td>-</td>
<td>Alive and well (25 months FU)</td>
</tr>
<tr>
<td>77</td>
<td>-</td>
<td>Alive and well (27 months FU)</td>
</tr>
<tr>
<td>78</td>
<td>Antibiotics</td>
<td>Alive and well (27 months FU)</td>
</tr>
<tr>
<td>79</td>
<td>*</td>
<td>Alive and well (31 months FU)</td>
</tr>
<tr>
<td>80</td>
<td>-</td>
<td>Alive and well (8 months FU)</td>
</tr>
<tr>
<td>81</td>
<td>-</td>
<td>Alive and well (29 months FU)</td>
</tr>
<tr>
<td>* Surgery</td>
<td>+ Radiotherapy</td>
<td>No treatment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PATIENT NUMBER</td>
<td>PRIMARY</td>
<td>RIGHT</td>
</tr>
<tr>
<td>---------------</td>
<td>---------</td>
<td>-------</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GA67-CITRATE VS TC99m(V) DMSA PLANAR SCINTIGRAPHY

- 5, Positive
- +++, Strongly Positive
- , Negative
<table>
<thead>
<tr>
<th>PATIENT NUMBER</th>
<th>NUMBER OF POSITIVE NODES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PALPATION</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>26</td>
<td>1</td>
</tr>
<tr>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>31</td>
<td>1</td>
</tr>
<tr>
<td>31</td>
<td>-</td>
</tr>
<tr>
<td>32</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = Positive or abnormal    ++ = Strongly Positive    - = Negative or normal
<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Palpation</th>
<th>Planar TC\textsuperscript{99m}(V) DMSA</th>
<th>CAT</th>
<th>Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/2</td>
</tr>
<tr>
<td>34</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/58</td>
</tr>
<tr>
<td>39</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>3/23</td>
</tr>
<tr>
<td>43</td>
<td>-</td>
<td>1, +</td>
<td>-</td>
<td>0/4</td>
</tr>
<tr>
<td>43</td>
<td>-</td>
<td>1, +</td>
<td>-</td>
<td>0/51</td>
</tr>
<tr>
<td>44</td>
<td>1</td>
<td>1, +</td>
<td>2</td>
<td>0/51</td>
</tr>
<tr>
<td>51</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0 (Total number unknown)</td>
</tr>
<tr>
<td>61</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0 (Total number unknown)</td>
</tr>
<tr>
<td>67</td>
<td>1</td>
<td>1, +</td>
<td>1</td>
<td>2/34</td>
</tr>
<tr>
<td>67</td>
<td>-</td>
<td>1, +</td>
<td>-</td>
<td>1/30</td>
</tr>
</tbody>
</table>

+ = Positive  
- = Negative or normal
TABLE 26A

THE VALUES OF THE WEIGHTING FACTORS TO BE USED FOR THE
CALCULATION OF THE EFFECTIVE DOSE EQUIVALENT TO THE
WHOLE BODY IN MAN

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>WEIGHTING FACTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonads</td>
<td>0.25</td>
</tr>
<tr>
<td>Breast</td>
<td>0.15</td>
</tr>
<tr>
<td>Red bone marrow</td>
<td>0.12</td>
</tr>
<tr>
<td>Lung</td>
<td>0.12</td>
</tr>
<tr>
<td>Thyroid</td>
<td>0.03</td>
</tr>
<tr>
<td>Bone surfaces</td>
<td>0.03</td>
</tr>
<tr>
<td>Remainder</td>
<td>0.30 (ie 0.06 each)</td>
</tr>
<tr>
<td>(Bladder, kidney, uterus, small and large intestine).</td>
<td></td>
</tr>
</tbody>
</table>

Internal Commission on Radiation Units and Measurements
Publication 26, 1977
CLINICAL EXAMINATION AND TC\textsuperscript{99m}[\text{V}] DMSA PLANAR IMAGING: EVALUATION AT PRESENTATION

**FIGURE 1A**

MALIGNANCY 67 [OR PAST HISTORY OF MALIGNANCY] [TABLES 37 AND 43]

OTHERS 5 [ALL HEAD AND NECK]

EMBRYONAL Rhabdomyosarcoma [2] 2T\textsubscript{2}

ADENOCARCINOMA T\textsubscript{4}N\textsubscript{2}C [5 LESIONS]

LYMPHOMA [CERVICAL OESOPHAGUS [1]

METASTATIC BREAST ADENOCARCINOMA [1]

NUMBER OF LESIONS, 13 [12 MALIGNANT, 1 BENIGN]

---

NO OF PATIENTS 77

POSITIVE DISEASE

55

NEGATIVE DISEASE [TREATED PREVIOUSLY WITH DXT] 3

NEGATIVE DISEASE AT PRIMARY SITE [PREVIOUS DXT] BUT PRESENTED WITH RECURRENT NECK DISEASE [IE, SEE IPSILATERAL NECK NODES ALONE] 49

3 PATIENTS TREATED FOR PRIMARY HEAD AND NECK CANCER WITH NO SIGN OF RESIDUAL DISEASE

TWO HAD SECOND LUNG PRIMARIES AND ONE HAD DISTANT METASTASES IN THE LIVER, PANCREAS AND SIGMOID COLON LESIONS [8; 5 MALIGNANT, 3 BENIGN]

OVERALL: PREVIOUS RADIOTHERAPY 12

PREVIOUS SURGERY 8 [TO INCLUDE 3 TRACHEOSTOMIES]

PREVIOUS SURGERY AND RADIOTHERAPY 5

OVERALL: LESIONS = 189

MALIGNANT 151; BENIGN 38

"T" STAGING IN 53 TUMOURS

2T\textsubscript{1}, 13T\textsubscript{2}, 16T\textsubscript{3}, 22T\textsubscript{4}

77 STUDIES: 77 HEAD AND NECK IMAGES

5 HEAD, NECK AND THORAX IMAGES

SQUAMOUS CARCINOMA 62

OTHERS 4 CARCINOMA OF THE LUNG [1]

OCCULT PRIMARY'S [2]

MIDDLE THIRD OF OESOPHAGUS [1]

5 TUMOURS [1T\textsubscript{3}, 1T\textsubscript{4}, 2T\textsubscript{x}: 8 LESIONS; 5 ONE METASTASIS

MALIGNANT, 3 BENIGN]

INCLUDES

PERIODONTAL DISEASE, 5 [4 PATIENTS]

INFLAMMATORY NECK NODES 8 [7 PATIENTS]

OCCIPITAL ARTERIOVENOUS MALFORMATION 1

PRIMARY TUMOUR ALONE [24 PATIENTS]

PRIMARY TUMOUR WITH NODES

16 IPSILATERAL 7 BILATERAL

[8N\textsubscript{1} 1N\textsubscript{2} 2a 2b 2b' 4N\textsubscript{2} 1N\textsubscript{3} 6N\textsubscript{2} 2a 1N\textsubscript{3}]

24 NODES]

CONTRA-LATERAL NODES

NONE

IPSILATERAL NODES ALONE 5 [4N\textsubscript{2} 1N\textsubscript{3} 25 NODES]

TOTAL NUMBER OF NODES = 79
FIGURE 2A

Tc-99m [v] DMSA IMAGING: PLANAR VS SPECT

NUMBER OF PATIENTS 34

HEAD AND NECK MALIGNANCY 31

SQUAMOUS CARCINOMA 28

OTHERS

EMBRYONAL Rhabdomyosarcoma [2] 1T1, 1T2
ADENOCARCINOMA OF THE ORAL CAVITY
T4 N2c [4 LESIONS]
TOTAL NUMBER OF LESIONS = 6

NEGATIVE DISEASE 1

POSITIVE DISEASE 27

PRIMARY DISEASE ALONE 12 3T2, 5T3, 4T4

PRIMARY DISEASE WITH CERVICAL METASTASES 12
2T2, 3T3, 6T4, 1T0 and 5N1, 1N2a, 3N2b, 1N2c,
2N3 [31 NODES; 29 MALIGNANT, 2 BENIGN]

CERVICAL METASTASES ALONE 3 N2b [15 NODES]
[INCLUDES 1 PATIENT WITH AN OCCULT PRIMARY AND 2
WITH PREVIOUSLY SUCCESSFULLY TREATED PRIMARY SITES
WITH DXT, 1 TONGUE, 1 NASOPHARYNX]

TOTAL NUMBER OF LESIONS = 87 [75 MALIGNANT, 12 BENIGN]

NUMBER OF LESIONS = 69 MALIGNANT, 7 BENIGN
[INCLUDES 3 PROVEN AREAS OF PERIODONTAL DISEASE]
FIGURE 3A

GALLIUM - $^{67}$ CITRATE VS Tc$^{99m}$ [V] DIMERCAPTOSUCCINIC ACID : PLANAR SCINTIGRAPHY IN 17 PATIENTS AT PRESENTATION

PAROTID PLEOMORPHIC ADENOMA

LYMPHOMA OF CERVICAL OESOPHAGUS [1]

17 PATIENTS

BENIGN 1

MALIGNANT 16

HEAD AND NECK SQUAMOUS CARCINOMA [15]

1 PATIENT HAD AN OCCULT PRIMARY

1 PATIENT HAD A SECOND LUNG PRIMARY

15 PATIENTS WITH 16 PRIMARY LESIONS

[1 OCCULT]

$9T_4$, $2T_3$, $3T_2$, $1T_1$, $1T_x$

4 PATIENTS - PRIMARY TUMOURS ALONE

11 PATIENTS - PRIMARY TUMOUR WITH CERVICAL METASTASES

$3N_1$, $3N_2b$, $4N_2c$, $1N_3$

[ie, 16 LATERAL COMPARTMENT NECKS $5N_1$, $10N_2b$, $1N_3$]

1 PATIENT - IPSILATERAL METASTASES ALONE

$[N_2b]$

TOTAL NUMBER CERVICAL LYMPH NODE MASSES = 38 [2 BENIGN, 36 MALIGNANT]

2 PATIENTS WITH SQUAMOUS CELL CARCINOMA HAD BENIGN LESIONS [3 PROVEN AREAS OF PERIODONTAL DISEASE]

TOTAL BENIGN LESIONS = 4

TOTAL NUMBER OF LESIONS 58

[52 MALIGNANT; 6 BENIGN]

2 PATIENTS HAD FOLLOW UP SCANS AND, OF THESE, ONLY 1 WAS IMAGED AT PRESENTATION

[SEE RESIDUAL AND RECURRENT DISEASE]

BASED ON DATA FROM 12 NECK DISSECTIONS; THE INFORMATION FROM THE OTHER PATIENTS WAS OBTAINED FROM CAT SCANS [12], CLINICAL EXAMINATION AND SUBSEQUENT FOLLOW UP
Figure 4A

Clinical Evaluation, Tc⁹⁹ᵐ DMSA Scintigraphy and CAT Scanning of 54 Patients with a Possible Head and Neck Tumour

Number of Patients 54 [See Table 21A]

- 51 Malignant [Past or Present]
  - 2T₁, 10T₂, 13T₃, 18T₄
- 3 Benign
  - Glomus Jugulare [1]
  - Glomus Tympanicum [1]
  - Branchial Cyst [1]

Others [All Head and Neck]

Embryonal Rhabdomyosarcoma [2]
- 1T₁, 1T₂

Lymphoma of Cervical Oesophagus

Adenocarcinoma T₄N₂c [1]

- 2 Negative Disease
  - [Treated Previously with DXT]
  - [2 Negative at Primary Site but Presented with Recurrent Neck Disease]
  - [i.e., See Ipsilateral Nodes]

Positive 43
- Primary or Locally Recurrent Tumours 37

"T" Staging Possible in 37 Patients [38 Lesions]
- [1T₁, 8T₂, 13T₃, 16T₄]
  - Includes One Second Lung Primary

Primary Tumour Alone 21

Primary Tumour with Nodes

2 Occult Primaries

- 12 Ipsilateral Ear Tumours [2]
  - [7N₁, 1N₂a, 3N₂b, 1N₃]
  - Bilateral 6N₂c

Previous DXT [4]

Previous Surgery [5]

Previous Surgery & DXT [1]

Contralateral None

Ipsilateral Nodes Alone 4
- 1N₂a, 2N₂b, 1N₃

One Patient Had CAT of the Neck and Chest

And One Had CAT of the Chest and Lower Neck.

Two Patients Had Follow Up CAT Scans
Figure 5A
COMPARTMENTAL DOSIMETRY MODEL

% Injected dose in bone (Rabbit, 28%, Section 4.1.2, Table 19)
Bone mass rabbit = 137 g/kg
(Section 4.1.6)
% injected dose/g = 7.58 x 10^{-2}

TO CALCULATE HUMAN KIDNEY UPTAKE

% Injected dose in kidney (Rabbit, 0.92%, Section 4.1.2)
Correct for mass of human Kidney \( \frac{0.92 \times 4.4}{5.2} \)
= 0.78%,

And for rabbit bone uptake \( \frac{0.78}{100-28} \times 100 \)

Uptake in human kidney = 1.08%

* RABBIT KIDNEY = 5.2 g/kg body weight (Section 4.1.2, Table 19)
* HUMAN KIDNEY = 4.4 g/kg body weight (Snyder et al, 1974, p174)
Figure 6A

**CUMULATIVE ACTIVITIES (\( \tilde{A} \)) IN SOURCE ORGANS**

\[
A_{WB} = \left[ \left( -\lambda_A t \right) + \left( -\lambda_B t \right) \right] e^{-\lambda T_c t}
\]

\[
\tilde{A}_{WB} = \int_{0}^{\infty} A_{blood} dt = \left[ \frac{Ae^{-\left(\lambda_A + \lambda T_c\right)t}}{\lambda_A + \lambda T_c} + \frac{Be^{-\left(\lambda_B + \lambda T_c\right)t}}{\lambda_B + \lambda T_c} \right]_0^\infty
\]

\[
\tilde{A}_{WB} = \left[ \frac{A}{\lambda A + \lambda T_c} + \frac{B}{\lambda B + \lambda T_c} \right] \text{hrs}
\]

\( \lambda = \text{Decay Constant} \)

\[
\lambda = 0.693 \quad \text{hrs}^{-1}
\]

\( \lambda T_c = 0.115 \text{hr}^{-1} \)

The curves produced by Log Linear plots of whole body retention vs time were fitted to monoeponential (patients 4, 5 and 9) or biexponential (patients 7,8 and 10) terms. Similar plots for patients 1,2, and 3 appeared to indicate incomplete urine collection, and more than 100% excretion in patient 6, and were therefore excluded.

**WHOLE BODY RETENTION CURVES (Section 4.2.1.) i.e., WHOLE BODY (t) =Ae^{-\lambda A t} + Be^{-\lambda B t}**

1) Incomplete urine Collection
2) More than 100% excretion

<table>
<thead>
<tr>
<th>Patient</th>
<th>Patient Number</th>
<th>( \lambda_A )</th>
<th>( \lambda_B )</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>4.679</td>
<td>1.157E-3</td>
<td>5.479E-3</td>
</tr>
<tr>
<td>5</td>
<td>3.775</td>
<td>2.488E-3</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4.930</td>
<td>3.379E-3</td>
<td>5.479E-4</td>
</tr>
<tr>
<td>8</td>
<td>4.711</td>
<td>3.235E-3</td>
<td>3.679E-4</td>
</tr>
<tr>
<td>9</td>
<td>4.424</td>
<td>3.229E-3</td>
<td>1.097E-4</td>
</tr>
<tr>
<td>10</td>
<td>4.24</td>
<td>3.229E-3</td>
<td>1.097E-4</td>
</tr>
</tbody>
</table>
Figure 6A (Continued)

**KIDNEY**

\[ A_{\text{Kidney}} = \int_0^\infty \left( C e^{-\lambda Tc} \right) dt = \frac{C}{\lambda Tc} \]

\[ \therefore A_{\text{Kidney}} = \frac{0.0108}{0.115} = 0.094 \text{ hrs} \]

**BLADDER**

\[ \tilde{A}_{\text{bladder}} = \text{Area under urine excretion plot} \]

\[ \text{ie.} \]

% injected dose in the bladder

Assumes complete bladder emptying and linear rise in radioactivity from one bladder emptying to another

<table>
<thead>
<tr>
<th>patient</th>
<th>Bladder ( \tilde{A} ) (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0.260</td>
</tr>
<tr>
<td>5</td>
<td>0.848</td>
</tr>
<tr>
<td>7</td>
<td>0.905</td>
</tr>
<tr>
<td>8</td>
<td>0.345</td>
</tr>
<tr>
<td>9</td>
<td>0.365</td>
</tr>
<tr>
<td>10</td>
<td>0.521</td>
</tr>
</tbody>
</table>
DOSEMETER CALCULATIONS IN SIX PATIENTS using The MIRD* S* values, weighting factors \( W_T \) for the relevant Target organs and the Whole Body, Bladder and kidney as Source organs.

<table>
<thead>
<tr>
<th>MIRD * S* Factors rads / ( \mu )Ci hrs</th>
<th>( W_T )</th>
<th>Whole Body</th>
<th>Bladder</th>
<th>kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>GONAD</td>
<td>0.25</td>
<td>2.4 E - 6</td>
<td>7.3 E - 6</td>
<td>1.1 E - 6</td>
</tr>
<tr>
<td>BREAST</td>
<td>0.15</td>
<td>2.0 E - 6</td>
<td>2.4 E - 8</td>
<td>8.5 E - 7</td>
</tr>
<tr>
<td>LUNG</td>
<td>0.12</td>
<td>2.0 E - 6</td>
<td>2.4 E - 8</td>
<td>8.5 E - 7</td>
</tr>
<tr>
<td>MARROW</td>
<td>0.12</td>
<td>2.9 E - 6</td>
<td>2.2 E - 6</td>
<td>3.8 E - 6</td>
</tr>
<tr>
<td>THYROID</td>
<td>0.03</td>
<td>1.5 E - 6</td>
<td>2.1 E - 9</td>
<td>4.8 E - 8</td>
</tr>
<tr>
<td>BONE</td>
<td>0.03</td>
<td>2.5 E - 6</td>
<td>9.2 E - 7</td>
<td>1.4 E - 6</td>
</tr>
<tr>
<td>BLADDER</td>
<td>0.06</td>
<td>2.3 E - 6</td>
<td>1.6 E - 4</td>
<td>2.8 E - 7</td>
</tr>
<tr>
<td>KIDNEY</td>
<td>0.06</td>
<td>2.2 E - 6</td>
<td>2.6 E - 7</td>
<td>1.9 E - 4</td>
</tr>
<tr>
<td>UTERUS</td>
<td>0.06</td>
<td>2.6 E - 6</td>
<td>1.6 E - 5</td>
<td>9.4 E - 7</td>
</tr>
<tr>
<td>LARGE INTESTINE</td>
<td>0.06</td>
<td>2.3 E - 6</td>
<td>7.4 E - 6</td>
<td>7.2 E - 7</td>
</tr>
<tr>
<td>SMALL INTESTINE</td>
<td>0.06</td>
<td>2.5 E - 6</td>
<td>3.0 E - 6</td>
<td>2.9 E - 6</td>
</tr>
</tbody>
</table>

\[ \sum W_T S_T = \frac{2.322 \times 10^{-6}}{13.323 \times 10^{-6}} = 1.2694 \times 10^{-6} \]

\[ 1 \mu Sv / MBq hrs = 2.7 \times 10^{-5} \frac{1 \text{ rad}}{\mu \text{Ci hrs}} \]

\[ \text{Whole Body} \quad \sum W_T S_T = 6.276 \times 10^{-1} \mu Sv / MBq hrs \]

\[ \text{Bladder} \quad \sum W_T S_T = 3.6008 \mu Sv / MBq hrs \]

\[ \text{Kidney} \quad \sum W_T S_T = 3.4308 \mu Sv / MBq hrs \]

Effective Dose equivalent = \[ 6.276 \times 10^{-1} \left( \text{Whole Body Activity} + 3.6008 \left( \text{Bladder Activity} + 3.4308 \left( \text{Kidney Activity} \right) \right) \right) \]

\( (\mu \text{ Sv/MBq}) \) =

- Patient 4, 4.19
- Patient 5, 5.79
- Patient 7, 6.08
- Patient 8, 4.66
- Patient 9, 4.59
- Patient 10, 4.98

Mean effective dose equivalent = 5.1 \( \mu \text{ Sv/MBq} \)
LAUS DEO SEMPER
EDITORIAL

The evaluation of the solitary thyroid nodule

Thyroid nodules are common in the UK and the majority are benign. Up to 15.5% of the population have palpable nodules in their thyroid glands with 3.2% being solitary in women and 0.8% solitary in men. By contrast, thyroid cancer is comparatively uncommon with an approximate incidence in the UK and USA of 4 per 100,000 per annum.1 By far the commonest way for thyroid cancer to present is as a palpable solitary thyroid nodule in a euthyroid patient when the likelihood of malignancy is between 5 and 10%,2 although the exact figure varies considerably from series to series depending on selection criteria.

Because of this increased risk of malignancy, there has been much interest recently devoted to the evaluation of the palpable solitary thyroid nodule.1-3 Some authors prefer to evaluate a nodule by palpation and fine needle aspiration (FNA),1,4,5 others use palpation with ultrasound and FNA, while some favour the more conventional approach of palpation with thyroid scintigraphy followed by ultrasound and FNA.2

All patients with a palpable solitary thyroid nodule should have their routine thyroid function checked. Although the majority of patients with malignancy are euthyroid, occasionally a nodule may be malignant in the presence of thyroiditis or thyrotoxicosis, but it is extremely uncommon for a neoplastic lesion to cause thyrotoxicosis. Thyroid antibodies are often raised in both thyroiditis and malignancy and are therefore non-specific. Measurement of serum thyroglobulin is of little value in the preoperative detection of malignancy and calcitonin is not routinely measured unless there is a family history to suggest medullary carcinoma of the thyroid or bilateral cold nodules on scintigraphy.

The fundamental and crucial question which faces the surgeon is how to detect the 10% of solitary thyroid nodules with cancer without the need to perform unnecessary operations on the other 90%. A process of selection based on clinical evaluation has distinct limitations. There is a well recognized error in neck palpitation,6 and 25% of solitary nodules subsequently turn out to be more easily palpated dominant nodules in a multinodular goitre. The function of all preoperative assessment of the thyroid gland is to increase the likelihood of malignancy at operation by improving the positive predictive accuracy without any loss in sensitivity. There are many investigations currently available to assess thyroid nodules. Correct evaluation is crucial to subsequent management. How then should solitary thyroid nodules be assessed?

There is considerable disagreement about which test or tests to perform in order to select the patients to refer for surgery and there is absolutely no consensus of opinion in the literature. Some argue that all the tests currently available are so poor as predictors of malignancy that most patients with a nodule should have a hemithyroid-


ectomy,7 and total thyroidectomy if malignancy is subsequently discovered.

Thyroid scintigraphy has been the simplest and most widely used method for investigating the thyroid nodule. Technetium-99m (99mTc) pertechnetate (TcO₄⁻) is used for thyroid imaging because it is trapped by the thyroid gland in a similar manner to the iodide and the perchlorate ion. It is cheap, readily available, and the radiation dose is low. However, pertechnetate uptake does not always match the physiological distribution of iodide since its uptake is low (0.4–4%). This contributes to high background activity and, in addition, TcO₄⁻ is trapped, but not organified, by the thyroid. Iodine-123 (123I) is probably the optimal radionuclide for thyroid imaging because of its physiological properties but, since it is cyclotron produced, availability and cost remain important considerations. With either 99mTcO₄⁻ or 123I, the majority of nodules greater than 5 mm in diameter can be identified and palpation at the time of scintigraphy, together with the use of nodule markers and oblique views, can all contribute to an increase in sensitivity. False-negative results are often associated with smaller lesions in the isthmus, but these are usually easy to palpate and, therefore, do not cause a real problem.²

When a palpable solitary thyroid nodule is investigated, the scintigram may show a solitary non-functioning or hypofunctioning area (i.e., a 'cold' or 'warm' nodule); a functioning area (i.e., a 'hot' nodule) or a multinodular goitre. The probability of malignancy is increased to approximately 20% if the scan demonstrates a solitary or dominant 'cold' or 'warm' nodule in a euthyroid or hyperthyroid patient,⁸ but decreased to less than 1% if it shows a 'hot' nodule or a multinodular goitre.² A solitary 'cold' or 'warm' nodule should be investigated further by FNA and/or ultrasound. There are occasionally some nodules which function on the pertechnetate scan but are non-functioning on the iodine scan. These probably reflect an ability to trap but not organify iodine and problems from such rare discrepancies between pertechnetate and iodine studies can be avoided by performing 123I scans with perchlorate discharge on any nodule which concentrates TcO₄⁻ or, if 123I is not available, either an ultrasensitive TSH assay or a TRH test may be performed. If a nodule is functioning, then no further investigations are necessary. Such findings will avoid possible complications from operating on a mildly thyrotoxic patient or precipitating thyrotoxicosis by starting suppressive therapy with T₄. Not only can thyroid scintigraphy differentiate between functional and non-functional nodules, it can also identify solitary or dominant 'cold' and 'warm' nodules in patients who have Graves' disease or a multinodular goitre, which no other investigation is capable of doing.

The main criticism of using thyroid scintigraphy to differentiate between benign and malignant lesions has arisen because of occasional reports of malignancy in hot nodules.⁴ Some authors have reported a 9% malignancy rate in warm and hot nodules.⁹ Nagai et al. reported 3 cases of malignancy in hot nodules on 123I scans,¹⁰ while Evans reported that 44% of patients with thyroid cancer presented with warm nodules.¹¹ If only those nodules that suppress TSH or cause a flat TRH test response, or remain hot on an 123I scan after a perchlorate discharge test or delayed imaging (20 h) are called 'functioning', then the incidence of malignancy is very low (less than 0.1%). The confusion in the literature about the possibility of malignancy in 'hot' and 'warm' nodules arises because, as stated above, some trap but do not organify iodine. In addition, some adenomas and carcinomas have a high blood volume and therefore contain radio-tracer and appear warm. This fact has been used by some to differentiate cystic from solid lesions.⁸ Lastly, some so-called neoplastic 'hot' nodules are, in fact, incidental cancers adjacent to the 'hot' nodule.

There are other radiopharmaceuticals which have a role to play in the evaluation of the solitary thyroid nodule. Very occa-
ionally, the $^{99m}$TcO$_4^-$ or $^{123}$I scan may demonstrate bilateral symmetrical cold nodules. This raises the possibility of familial medullary carcinoma and subsequent scanning with either $^{99m}$Tc (v) dimercaptosuccinic acid (DMSA) or $^{131}$I/$^{123}$I metaiodobenzylguanidine (MIBG) may be diagnostic. The multiple endocrine neoplasia syndrome type II, which is associated with bilateral medullary carcinoma and phaeochromocytoma, should then be excluded pre-operatively to avoid an anaesthetic disaster. A gallium ($^{67}$Ga)-citrate scan may be of diagnostic value in patients with long-standing Hashimoto’s disease who develop a solitary thyroid nodule. This may be lymphoma and, as such, shows avid accumulation of $^{67}$Ga.

Thallium-201 ($^{201}$Tl)-chloride is being increasingly used for thyroid imaging because it is accumulated by functional thyroid tissue and is less dependent on TSH stimulation for this to occur. $^{201}$Tl has been used in solitary thyroid nodules to differentiate benign from malignant disease and although early results are encouraging, its exact role to date is unclear. However, it may be used to demonstrate supressed thyroid tissue which is not visualized on either a $^{99m}$TcO$_4^-$ or $^{123}$I scan and an example of this would be a toxic solitary nodule with suppression of the contralateral lobe.

Another method which has been used to reduce the surgical rate for thyroid nodules is X-ray fluorescence. This measures stable ($^{127}$I) within a nodule and, in one series, identified benign disease with a 63% sensitivity and 99% specificity. However, at present, this technique is not widely available.

Ultrasound has proved to be a valuable tool for demonstrating thyroid abnormalities and, in particular, to discriminate between solid and cystic lesions. It can measure thyroid volume and has been claimed to be able to detect cystic lesions 1 mm in diameter and some 3-mm solid lesions, and is more accurate for small lesions than either clinical examination or scintigraphy. Fifteen per cent of thyroid nodules are cystic and the majority of these are benign. Most malignancies are echogenic but, in one series, 19% of thyroid carcinomas were cystic. The probability of an occult neoplasm in the wall of the cyst is no greater than its probability in the remainder of the thyroid. Although ultrasound may be more accurate than either palpation or scintigraphy in diagnosing multinodular goitre, and can distinguish cystic from solid lesions, it cannot reliably distinguish benign from malignant disease nor differentiate between functioning and non-functioning nodules.

Probably the most important recent development as an adjunct to thyroid imaging is fine needle aspiration (FNA) of a nodule for cytological examination. This test is now becoming widely available and can increase the preoperative predictive accuracy of malignancy to more than 90%, although the technique is limited in its ability to detect follicular neoplasms. A review of the world literature on 3500 patients showed a false-negative rate of less than 10% and a false-positive rate of under 2%, though better results are now possible. Several groups have shown a reduction in surgery rate as a consequence of routine FNA of cold nodules, and it has been suggested that all patients with a solitary thyroid nodule should have FNA as their initial investigation and that a thyroid scan or ultrasound is not required. However, clinical examination frequently fails to detect that the ‘clinically solitary nodule’ is the more easily palpable nodule in a multinodular goitre. Although FNA can distinguish benign from malignant disease, it cannot differentiate between functional and non-functional nodules and results depend on the skill of the operator as well as the experience of the pathologist. Some authors state that FNA of all nodules could lead to an increase in the surgical rate, as functional nodules often have suspicious cytological features, and conclude that FNA is an efficient method for detecting cancer in patients who have a cold nodule on the thyroid
scan, but not in an unscreened population.17 There is currently no consensus of opinion as to how the solitary thyroid nodule should best be evaluated. Routine removal of all clinically apparent thyroid nodules is no longer justified and results in a great deal of unnecessary surgery. A reasonable strategy would be a $^{99m}$TcO$_4$ or $^{123}$I scan in the first instance, which is a cheap, accurate, and widely available test. Functional nodules will be identified and these patients should have a sensitive TSH or TRH test to confirm true physiological function with subsequent follow-up and treatment for thyrotoxicosis as appropriate. Patients with familial medullary carcinoma may be detected and all should be screened preoperatively to exclude a coexistent phaeochromocytoma as part of the MEN II syndrome.

All truly non-functioning solitary nodules should have FNA cytology because this results in a well documented reduction in unnecessary surgery. Clinical decisions based on FNA should not be made until 100 procedures have been undertaken and reviewed. Simple cysts can be aspirated and recurrences considered for treatment by injection with sclerosants, such as tetracycline.18 Some would argue that ultrasound is necessary only as an adjunct to the scan when FNA is not available. A recent review of 60 patients with non-functioning nodules on $^{123}$I scans showed that ultrasound missed surgically proved nodules in 32% of cases, and the authors concluded that ultrasound was not helpful in routine management.16 While it is excessive to evaluate all solitary nodules with scintigraphy, FNA, and ultrasound, the latter investigation may provide complementary information which would otherwise be unavailable. For example, the sensitivity for diagnosing multinodular goitre is increased and some nodules may have both cystic and solid components and this information can increase the accuracy of FNA. In addition, if a nodule is known to be purely cystic, one is more likely to persist to obtain a 'dry-tap'.

Although FNA and ultrasound may be as accurate as scintigraphy and FNA in predicting malignancy, the former combination cannot diagnose a functional nodule. This problem can be overcome by performing ultrasensitive TSH assays on all nodules but, as previously stated, FNA of functional nodules often gives rise to more suspicious features. If scintigraphy is not available (which is uncommon), then ultrasound and FNA with TSH testing is an acceptable alternative.

The treatment of the solitary thyroid nodule is less controversial. It is now generally agreed that the correct management of a truly non-functioning nodule is hemithyroidectomy,19 and not, as previously practised, suppressive therapy with thyroxine. Indeed, recent studies have shown no measurable decrease in nodule size in those patients treated with T$_4$.20

This editorial finishes where it began. Thyroid nodules are common. Thyroid cancer is uncommon. There is nothing to justify performing FNA alone on the solitary nodule and it is recommended that all patients with a solitary thyroid nodule should have a thyroid scan as their initial investigation. Patients with truly non-functioning nodules should be given the benefits of FNA and/or ultrasound as appropriate to improve diagnostic sensitivity so that a precise cytological diagnosis can be used to offer safe effective surgery to those who really require it.

J. C. Watkinson

References

99Tc\textsuperscript{m} (v) DMSA and \textsuperscript{67}Ga-citrate imaging in patients with head and neck squamous carcinoma: a clinical and scintigraphic study

J.C. WATKINSON\textsuperscript{1, \#} C.R. LAZARUS\textsuperscript{2}, M.N. MAISEY\textsuperscript{2} and S.E.M. CLARKE\textsuperscript{2}

The Departments of \textsuperscript{1}Otolaryngology and \textsuperscript{2}Nuclear Medicine, Guy's Hospital, St Thomas Street, London, SE1 9RT, UK

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Summary

Technetium-99m (\textsuperscript{99}Tc\textsuperscript{m}) (v) dimercaptosuccinic acid (DMSA) is a new tumour imaging agent which has been used to image medullary carcinoma of the thyroid and squamous cell carcinoma (SCC) of the head and neck. This study was undertaken to compare planar scintigraphy in patients with head and neck SCC using \textsuperscript{99}Tc\textsuperscript{m} (v) DMSA and the established tumour imaging agent gallium-\textsuperscript{67} citrate (\textsuperscript{67}Ga). Seventeen patients were studied of whom 16 had a head and neck malignancy.

Clinical examination was more sensitive and accurate than \textsuperscript{67}Ga scintigraphy, which in turn was more sensitive and accurate than \textsuperscript{99}Tc\textsuperscript{m} (v) DMSA in detecting patients with cancer, patients with primary tumours and patients with metastatic neck carcinoma.

Neither \textsuperscript{67}Ga or \textsuperscript{99}Tc\textsuperscript{m} (v) DMSA planar scintigraphy has any role to play in the routine evaluation at presentation of patients with head and neck SCC.

Introduction

\textsuperscript{99}Tc\textsuperscript{m} (v) DMSA is a new tumour imaging agent which is now used to evaluate medullary carcinoma of the thyroid [1]. Recent reports have described its uptake in patients with head and neck squamous carcinoma [2–3]. There are many radiopharmaceuticals which have been used to investigate patients with head and neck SCC and \textsuperscript{67}Ga is the one which has been evaluated the most [4–5]. The aim of this

\# Author to whom all correspondence should be addressed.

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study was to compare the uptake of $^{99}$Tc$^m$ (v) DMSA with $^{67}$Ga in patients with head and neck SCC.

**Materials and methods**

This study was carried out as part of a larger study evaluating the role of $^{99}$Tc$^m$ (v) DMSA in the management of patients with head and neck SCC [6]. Ethical committee approval was obtained to use $^{99}$Tc$^m$ (v) DMSA.

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**Fig. 1.** Details of the 17 patients studied in this series using $^{67}$Ga and $^{99}$Tc$^m$ (v) DMSA planar scintigraphy.

Seventeen patients (16 with malignancy, 10 male, 7 female; age range 42–81 years, mean 59: Fig. 1) were examined clinically and then imaged using $^{99}$Tc$^m$ (v) DMSA and then $^{67}$Ga planar head and neck scintigraphy. Patients with malignancy were staged using current UICC criteria [7]. Of the 16 patients with malignancy, 15 had SCC and one had a lymphoma of the cervical oesophagus. Of those patients with SCC, one had an occult primary, one had a second lung primary and two patients had proven periodontal disease (three lesions). All malignant tumours were confirmed histologically. One patient had a pleomorphic adenoma of the parotid gland.

$^{99}$Tc$^m$ (v) DMSA was prepared using an in-house method [8]. The purity of the complex was analysed by thin layer chromatography (Merck Silica Gel, developed with n-butanol/acetic acid/H$_2$O (3:2:3)) and no free pertechnetate or other $^{99}$Tc$^m$ derivative was detected. Patients were
injected intravenously with $^{99m}$Tc (v) DMSA (370 MBq) and anterior, right and left lateral head and neck planar images acquired at the optimal imaging time, that is between 2 and 4 h [9] using a Scintronix Digicamera interfaced to a Scintronix Data Processor. Similar images were then acquired within one week using $^{67}$Ga (1.8 MBq kg$^{-1}$ given intravenously and images acquired at 48 h). $^{67}$Ga was obtained from Amersham International. All images were reported by two of the authors (JCW and SEMC) without prior knowledge, where possible, of the patients condition, histology or previous treatment. Uptake of $^{99m}$Tc (v) DMSA and $^{67}$Ga was reported as being either positive or negative and each lateral compartment of the neck was reported separately.

Two patients had received previous surgery and one had had previous surgery and radiotherapy. Following imaging, three patients had surgery, three had radiotherapy and five had combined treatment. Six patients had no treatment.

Results

The normal head and neck biodistribution of $^{99m}$Tc (v) DMSA was observed with radioactivity observed in the nasal mucosa and of $^{67}$Ga with radioactivity observed in the lacrimal and salivary glands and nasal mucosa.

For detecting patients with malignancy at presentation using $^{99m}$Tc (v) DMSA, there were 12 true positives, 4 false negatives and 1 true negative (75% sensitivity, 100% specificity; 100% positive predictive accuracy, 20% negative predictive accuracy). For $^{67}$Ga, there were 15 true positives, 1 true negative and 1 false negative (94% sensitivity, 100% specificity; 100% positive predictive accuracy, 50% negative predictive accuracy) and for clinical examination there were 16 true positives and 1 true negative (100% sensitivity, specificity, positive and negative predictive accuracy).

For detecting primary tumours (includes one second primary and three areas of proven periodontal disease) using $^{99m}$Tc (v) DMSA there were 12 true positives (Fig.

![Image](https://via.placeholder.com/150)

**Fig. 2.** $^{99m}$Tc (v) DMSA (a) and $^{67}$Ga (b) anterior planar images in a patient with a T4 squamous carcinoma of the oral cavity (right retromolar trigone). Positive accumulation of radioactivity (A) is seen at the site of known primary disease on both images. Note the normal biodistribution of the two radiopharmaceuticals in the nasal mucosa.
2), 1 true negative, 3 false positives and 5 false negatives (71% sensitivity, 25% specificity; 80% positive predictive accuracy, 17% negative predictive accuracy). For $^{67}$Ga, there were 13 true positives (Fig. 2), 4 true negatives and 4 false negatives (76% sensitivity, 100% specificity; 100% positive predictive accuracy; 50% negative predictive accuracy) and for clinical examination there were 15 true positives, 4 true negatives and 2 false negatives (88% sensitivity, 100% specificity; 100% positive predictive accuracy, 67% negative predictive accuracy).

Each lateral compartment of the neck was reported as a separate site. For identifying lateral neck compartments with metastatic squamous carcinoma using $^{99}$Tc$^m$ (v) DMSA, there were 6 true positives (3–6 cm (5), > 6 cm (1)), 14 true negatives, 2 false positives and 10 false negatives (Fig. 3, 0–3 cm (5), 3–6 cm (5); 38% sensitivity, 88% specificity, 75% positive predictive accuracy, 58% negative predictive accuracy). For $^{67}$Ga, there were 8 true positives (Fig. 3, 0–3 cm (4), 3–6 cm (3) and > 6 cm (1)), 18 true negatives and 8 false negatives (0–3 cm (4); 3–6 cm (4); 50% sensitivity, 100% specificity; 100% positive predictive accuracy, 69% negative predictive accuracy) and for clinical examination there were 13 true positives (0–3 cm (5), 3–6 cm (6), > 6 cm (1)), 17 true negatives, 1 false positive and 3 false negatives (0–3 cm (3)); 81% sensitivity, 94% specificity; 93% positive predictive accuracy, 85% negative predictive accuracy).

Within these 34 lateral neck compartments (based on data from 12 neck dissections, 12 CT scans and subsequent follow-up) there were 38 cervical lymph node masses (2 benign, 36 malignant). For identifying individual lymph node masses using $^{99}$Tc$^m$ (v) DMSA, there were 6 true positives, 1 true negative, 1 false positive and 30 false negatives (17% sensitivity, 50% specificity; 81% positive predictive accuracy, 3% negative predictive accuracy). For $^{67}$Ga, there were 8 true positives, 2 true negatives and 28 false negatives (22% sensitivity, 100% specificity; 100% positive predictive accuracy, 7% negative predictive accuracy) and for clinical examination there were 17 true positives, 2 false positives and 19 false negatives (47% sensitivity, 0% specificity; 89% positive predictive accuracy, 0% negative predictive accuracy).

In none of the lateral compartment necks evaluated was $^{99}$Tc$^m$ (v) DMSA or $^{67}$Ga scintigraphy able to identify more than one nodal mass (Fig. 3).

**Discussion**

Over the last fifteen years head and neck surgeons have been attracted by the idea of using radiopharmaceuticals to image head and neck SCC in an attempt to identify not only primary and occult primary disease with cervical metastases but also residual and recurrent disease following surgery and irradiation. They have, however, been frustrated in their efforts using not only $^{67}$Ga [4] but many other radiopharmaceuticals [10] due to a low sensitivity and specificity, considerable expense and prolonged blood clearance which often delays the scanning time up to 48 h.

At present, the majority of head and neck surgeons in this country stage their patients at presentation by clinical examination supplemented by further investiga-
Fig. 3. $^{67}\text{Ga}$ (a) and $^{99}\text{Tcm}$ (v) DMSA (b) planar images (left lateral) in a patient with an occult head and neck primary and a N$_{2b}$ (two palpable nodes) left lateral compartment neck mass. Uptake is seen in the neck with $^{67}\text{Ga}$ (A) but the $^{99}\text{Tcm}$ (v) DMSA image is a false negative study. A subsequent left neck dissection showed four positive nodes. Note the normal biodistribution of $^{67}\text{Ga}$ in the lacrimal glands (B).

Recent studies have confirmed that $^{99}\text{Tcm}$ (v) DMSA is taken up at sites of head and neck SCC [3, 5, 6, 9, 12]. In one of these studies, Ohta et al. [5] evaluated the uptake of $^{99}\text{Tcm}$ (v) DMSA in 112 head and neck tumours (80 malignant, 32 benign (excluding the thyroid)) and, of these, 34 (27 malignant, 7 benign) also had $^{67}\text{Ga}$ scintigraphy. The patient sensitivity for $^{99}\text{Tcm}$ (v) DMSA was 80% (75% specificity) and for $^{67}\text{Ga}$ it was 89% (29% specificity). In Ohta's study (as in all previously published studies in imaging head and neck cancer with radioisotopes) no attempt was made to correlate tumour size and staging with scintigraphic uptake. However, the false negatives in Ohta's series were in the region of the floor of mouth, larynx and gingiva and were all less than 2 cm. False positive results occurred in patients with either inflammatory or bony pathology.

In this study, the overall patient sensitivities for $^{99}\text{Tcm}$ (v) DMSA (75%) and $^{67}\text{Ga}$...
(94%) were similar to those observed by Ohta et al. [5]. The 100% specificity for $^{99m}$Tc $\text{cm} (v)$ DMSA and $^{67}$Ga reflects the small number of patients with inflammatory lesions or benign tumours who were imaged.

Although $^{67}$Ga was more sensitive than $^{99m}$Tc $\text{cm} (v)$ DMSA in the overall detection of patients with cancer, patients with primary tumours and patients with metastatic neck carcinoma, it was always inferior to clinical examination. In Ohta’s study [5], there was no attempt made to separately assess scintigraphy of the primary tumour and that of the neck or to compare scintigraphy with clinical examination. Most primary head and neck tumours can be seen either directly or indirectly with the naked eye so that the emphasis is not necessarily on tumour detection but on upstaging. If one of the greatest prognostic factors in head and neck cancer is the presence or absence, level and size of metastatic neck disease [13] then one of the biggest problems facing the head and neck surgeon is the detection of clinically occult neck disease. Therefore, it is relevant to properly evaluate techniques which may be able to detect and accurately localize such pathology.

In this study, size was an important factor for scintigraphic detection. For $^{99m}$Tc $\text{cm} (v)$ DMSA, of the five false negatives, one had an occult primary, one had lymphoma and there were 2T2 and 1T3 SCC tumours. In the neck, of the 10 false negative lateral compartment neck masses, five were less than 3 cm and five measured between 3 and 6 cm. For $^{67}$Ga, there were 4 false negative primary tumours (one occult primary and 1T1, 1T2 and 1T3 SCC growths) while of the 8 false negative lateral compartment neck masses, 4 were less than 3 cm and 4 measured between 3 and 6 cm.

Using $^{99m}$Tc $\text{cm} (v)$ DMSA and $^{67}$Ga in this study, no occult primary tumour was detected and no tumours were upstaged using current UICC criteria. Of the 20 patients with clinically N0 (no palpable nodes) lateral compartment necks, none were upstaged using $^{67}$Ga compared with two for $^{99m}$Tc $\text{cm} (v)$ DMSA (one correctly, one incorrectly). Therefore, neither $^{99m}$Tc $\text{cm} (v)$ DMSA nor $^{67}$Ga scintigraphy has any role to play in the evaluation and detection of clinically occult neck disease.

The results in this study confirm those of Ohta et al.[5] that false positive uptake of $^{99m}$Tc $\text{cm} (v)$ DMSA can occur in benign bony pathology such as periodontal disease and may explain why Ohta suggested that visualization of head and neck tumours with $^{99m}$Tc $\text{cm} (v)$ DMSA was best in the region of the mandible and maxilla. This is because tumours of these areas often exhibit bony involvement or because the maxilla contains nasal mucosa which is part of the normal biodistribution of $^{99m}$Tc $\text{cm} (v)$ DMSA. Such findings may mean $^{99m}$Tc $\text{cm} (v)$ DMSA is behaving in part like a bone scanning agent and, as such (like $^{99m}$Tc $\text{m}$-MDP), it may be impossible to distinguish scintigraphically between benign periodontal disease and malignant tumour invasion of the mandible by a floor of mouth tumour. In this study, of the true positives with $^{99m}$Tc $\text{cm} (v)$ DMSA, nine had known bony or cartilaginous involvement while nine of the smaller false negatives exhibited such pathology. This may also explain why in Ohta’s series [5] small tumours away from or with no bone or cartilage involvement were not visualized scintigraphically while the larger tumours which often invade bone or cartilage were detected.
Conclusion

This study has confirmed that $^{99m}$Tc$^m$ (v) DMSA is taken up at sites of head and neck SCC. It has shown that $^{67}$Ga is both more sensitive and accurate than $^{99m}$Tc$^m$ (v) DMSA (but less sensitive and accurate than clinical examination) in detecting patients with cancer, patients with primary tumours and patients with metastatic neck carcinoma. This small series is the first to relate scintigraphic uptake to tumour staging for $^{67}$Ga. It confirms $^{67}$Ga planar scintigraphy has no role to play in the routine evaluation at presentation of patients with SCC although it may be of value in the assessment of lymphoma (particularly for restaging following treatment) and for the detection of occult infection [10].

Although $^{99m}$Tc$^m$ (v) DMSA is taken up at sites of head and neck SCC, it is less sensitive and accurate than both $^{67}$Ga and clinical examination and this means it, like $^{67}$Ga, has no role to play in the evaluation at presentation of patients with head and neck SCC.

In the future, further studies using both specific and nonspecific radiopharmaceuticals, SPECT, PET and fine resolution CT (to include image superimposition) are necessary to accurately relate scintigraphic and radiological detection to tumour size and stage. This can only lead to an increase in head and neck diagnostic sensitivity and specificity and ultimately improve the way head and neck surgeons diagnose, stage and treat head and neck cancer.

Acknowledgements

The authors wish to thank the staff and patients of the Head and Neck Oncology Unit and Nuclear Medicine Department at Guy's Hospital and to Sally Williams who typed the manuscript.

References


6. Watkinson JC, Lazarus CR, Maisey MN and Clarke SEM. \(^{99}\text{Tc}^m\) (v) DMSA planar scintigraphy: does it have a role in the management of patients with head and neck squamous carcinoma? *Nucl Med Commun* (in press).


Technetium-99m (99Tcm) dimercaptosuccinic acid (DMSA) is a new tumour imaging agent which has been used to image squamous cell carcinoma (SCC) of the head and neck. This study was undertaken to compare planar versus SPECT 99Tcm (v) DMSA scintigraphy in patients with head and neck SCC. Thirty-four patients were studied. Twenty-eight had SCC, and of these, four had received previous treatment with surgery or irradiation. SPECT was as sensitive and as accurate as clinical examination (but more sensitive and accurate than planar scintigraphy) in detecting which patients had cancer and which patients had primary tumours. SPECT was more sensitive and more accurate than planar scintigraphy (but less sensitive and accurate than clinical examination) in detecting lateral neck compartments with metastatic carcinoma. SPECT correctly upstaged 6% of clinically N0 necks. Although SPECT 99Tcm (v) DMSA scintigraphy improved the image quality, sensitivity and spatial resolution of the investigation, it has no role to play in the routine evaluation of patients with head and neck SCC (to include the clinically N0 neck).

Introduction

99Tcm (v) DMSA is a new tumour imaging agent which is now used to evaluate medullary carcinoma of the thyroid [1]. Recent reports have described its uptake in patients with head and neck squamous cell carcinoma (SCC) and some workers have...
claimed an improvement in image quality, sensitivity and spatial resolution using single photon emission computerized tomography (SPECT) [2-3].

The aim of this study was to compare planar versus SPECT $^{99m}$Tc (v) DMSA scintigraphy in patients with head and neck SCC.

**Materials and methods**

This study was carried out as part of a larger study evaluating the role of $^{99m}$Tc (v) DMSA in the management of patients with head and neck SCC [4]. Ethical committee approval was obtained to use $^{99m}$Tc (v) DMSA.

Thirty-four patients (31 with malignancy, 24 male, 10 female, age range 19–82 years, mean 58: Fig. 1) were examined clinically and then imaged using $^{99m}$Tc (v) DMSA (planar and SPECT). All patients with malignancy were staged using current UICC criteria [5]. Of the 31 patients with malignancy, 28 had head and neck SCC. Two patients had embryonal rhabdomyosarcomas and one patient had an adenocarcinoma of the oral cavity. All malignant tumours were confirmed histologically. Of the 27 patients with positive head and neck SCC, 12 had primary disease alone, 12 had primary disease with cervical metastases and three had cervical metastases alone (Fig. 1). Three patients had benign lesions. One had a Glomus Jugulare, one had a Glomus Tympanicum and one had an infected branchial cyst. In addition, three patients had four proven areas of periodontal disease.

$^{99m}$Tc (v) DMSA was prepared using an in-house method [6]. The purity of the complex was analysed by thin layer chromatography (Merck Silica Gel, developed with n-butanol/acetic acid/H$_2$O (3:2:3)) and no free pertechnetate or other $^{99m}$Tc derivative was detected. Patients were injected intravenously with $^{99m}$Tc (v) DMSA (370 MBq) and anterior, right and left lateral head and neck planar images acquired at the optimal imaging time, that is between 2 and 4 h [7] using a Scintronix Digicamera with a rotating SPECT facility interfaced to a Scintronix Data

![Diagram](image)

**Fig. 1.** Details of the 34 patients studied using planar and SPECT $^{99m}$Tc (v) DMSA.
Processor. SPECT imaging was performed using a parzen filter 1.5, elliptical orbits (where possible) and 64 projections were acquired over 360°, each view being for a 5.6° rotation of the camera.

All images were reported blind by two of the authors (J.C.W. and S.E.M.C.) without prior knowledge, where possible, of the patients condition, histology or previous treatment. Uptake of $^{99m}$Tc m (v) DMSA was reported as being either strongly positive, positive or negative, and each side of the neck (lateral neck compartment) was reported separately.

Three patients had received previous radiotherapy and one had had surgery. Following imaging, 12 patients underwent surgery, nine had radiotherapy and one had combined treatment. One patient had radiotherapy and chemotherapy and two patients had no treatment.

Results

Overall, for detecting patients with head and neck malignancy using SPECT there were 27 true positives, two true negatives; three false positives and two false negatives (Table 1) compared with planar imaging (22 true positives, two true negatives; two false positives and eight false negatives) and clinical examination (30 true positives, three true negatives and one false positive). Of the three false positives using SPECT, two patients had recently received irradiation to squamous carcinomas of the tongue (external beam (1) and iridium-192 (1)) and both showed positive uptake in the region of the previously treated tumour. One of these patients was clinically free of local recurrence while the other was thought to have local recurrence which subsequently turned out to be an area of radiation fibrosis. Both patients remain alive and well. The last false positive result occurred in a patient who had a highly vascular stage IV Glomus Jugulare tumour.

Of the two false negatives, one occurred in a patient with a T2 tumour of the buccal mucosa while the other occurred in a patient with a T4 tumour of the retromolar trigone which had been positive on planar imaging.

For detecting primary lesions using SPECT (33 patients, 37 lesions; 30 malignant,

Table 1. Clinical examination compared with planar v. SPECT $^{99m}$Tc m (v) DMSA scintigraphy in 34 patients.

<table>
<thead>
<tr>
<th>%</th>
<th>Clinical examination</th>
<th>Planar scintigraphy</th>
<th>SPECT</th>
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<tbody>
<tr>
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<td>93</td>
</tr>
<tr>
<td>Specificity</td>
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<td>50</td>
<td>40</td>
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<tr>
<td>Positive predictive accuracy</td>
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<td>92</td>
<td>90</td>
</tr>
<tr>
<td>Negative predictive accuracy</td>
<td>100</td>
<td>20</td>
<td>50</td>
</tr>
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</table>
Table 2. Clinical examination compared with planar v. SPECT 99Tcm (v) DMSA scintigraphy in 33 patients with 37 primary lesions (30 malignant, seven benign).

<table>
<thead>
<tr>
<th>%</th>
<th>Clinical examination</th>
<th>Planar scintigraphy</th>
<th>SPECT</th>
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</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>89</td>
<td>75</td>
<td>86</td>
</tr>
<tr>
<td>Specificity</td>
<td>89</td>
<td>33</td>
<td>22</td>
</tr>
<tr>
<td>Positive predictive accuracy</td>
<td>96</td>
<td>78</td>
<td>77</td>
</tr>
<tr>
<td>Negative predictive accuracy</td>
<td>73</td>
<td>30</td>
<td>33</td>
</tr>
</tbody>
</table>

seven benign) there were 24 true positives (22 strongly positive, Fig. 2), two true negatives; seven false positives and four false negatives (Table 2) compared with planar imaging (21 true positives (five strongly positive), three true negatives; six false positives and seven false negatives) and clinical examination (25 true positives, eight true negatives; one false positive and three false negatives). Of the seven false positives with SPECT, three have previously been mentioned. The other four occurred in three patients with four areas of proven periodontal disease. Of the four false negatives, two have been previously mentioned. The other two occurred in patients with a T3 nasopharyngeal primary and an occult primary SCC.

In those patients with primary malignant tumours evaluated by scintigraphy, 'T'
staging was possible in 26 (24 carcinomas, two embryonal rhabdomyosarcomas). For SPECT (Table 3), there were three false negatives (one T2 (buccal mucosa), one T3 (nasopharynx) and one T4 retromolar trigone tumour (all detected clinically)) compared with planar imaging (six false negatives; three T2 (buccal mucosa, supraglottis and tongue) and three T3; nasopharynx (2), tonsil (1)) and clinical evaluation (two false negatives; both nasopharynx (one T2, one T3)).

The results for imaging lateral compartment necks in 34 patients (54 cervical masses, 47 malignant, seven benign; all lymph nodes except one infected branchial cyst) are based on data from 23 neck dissections, 30 CT scans and subsequent follow-up.

For lateral compartment neck SPECT imaging, there were 11 true positives (nine strongly positive, Fig. 3), 45 true negatives; four false positives (Fig. 4) and eight false negatives (Table 4) compared with planar imaging (seven true positives, 44 true negatives; five false positives, 12 false negatives) and clinical examination (13 true positives, 45 true negatives; four false positives and six false negatives). Of the four false positives, two patients had inflammatory neck nodes thought to be positive.

Table 4. Clinical examination compared with planar and SPECT $^{99m}$Tc DMSA scintigraphy in 34 patients with 54 cervical masses (47 malignant, seven benign).

<table>
<thead>
<tr>
<th>Necks with metastatic carcinoma (each side of the neck reported separately)</th>
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<th>Planar scintigraphy</th>
<th>SPECT</th>
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<tbody>
<tr>
<td>%</td>
<td>Sensitivity</td>
<td>68</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>Specificity</td>
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<td>90</td>
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<td>79</td>
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<table>
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<tr>
<th>Positive nodes within the neck (each side of the neck reported separately)</th>
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<th>SPECT</th>
</tr>
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<tbody>
<tr>
<td>%</td>
<td>Sensitivity</td>
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<tr>
<td></td>
<td>Specificity</td>
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<td>Positive predictive accuracy</td>
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<td></td>
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<td>6</td>
<td>9</td>
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clinically. These exhibited positive $^{99m}$Tc (v) DMSA uptake on planar imaging and subsequently were shown to be benign. The two other false positives occurred in clinically N0 necks although one of these patients subsequently received radiotherapy to both sides of the neck and remains alive and well. Of the false negatives, there were four N1 and four N2b necks (compared with planar imaging (12 false negatives, five N1; seven N2b) and clinical examination (six false negatives, six N1. Table 5).

For nodal SPECT imaging of each lateral neck compartment (51 lesions), there were 11 true positives, five true negatives; two false positives and 36 false negatives (Table 4) compared with planar imaging (seven true positives, four true negatives; three false positives and 40 false negatives) and clinical examination (18 true positives, two true negatives; five false positives and 29 false negatives). No nodes less than 1.5 cm were detected on SPECT, more than one nodal mass within the neck was never identified and it was impossible to outline normal capsular outline or extracapsular spread.

Table 5. A comparison of clinical examination, planar and SPECT $^{99m}$Tc (v) DMSA scintigraphy in 16 patients (19 lateral neck compartments) with 'N' stageable head and neck carcinoma.

<table>
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<tr>
<td>FN</td>
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</tbody>
</table>

TP = True positive; FN = False negative.

Fig. 2. $^{99m}$Tc (v) DMSA SPECT study in a patient with a T4 squamous carcinoma of the hypopharynx. Strongly positive accumulation of radioactivity is seen at the site of known primary disease (A). Note the normal biodistribution of $^{99m}$Tc (v) DMSA in the nasal mucosa (B).

Fig. 3. $^{99m}$Tc (v) DMSA SPECT study in a patient with a T3N2b squamous carcinoma of the nasopharynx. Strongly positive accumulation of radioactivity is seen at the site of known primary disease (A) and in a nodal mass in the left lateral neck compartment (B).

Fig. 4. $^{99m}$Tc (v) DMSA SPECT study in patient with a R3N0 squamous carcinoma of the left maxillary antrum. The primary tumour is not visualized on these pictures but there is false positive accumulation of radioactivity in the right lateral neck compartment (A).
Discussion

Head and neck cancer remains a significant disease with considerable morbidity and mortality in the adult population. The majority of head and neck surgeons in this country stage their patients at presentation by clinical examination supplemented by further investigations to include computerized tomography, ultrasound and scintigraphy as appropriate. In the United Kingdom such investigations can only be justified if they will significantly alter treatment, that is from radiotherapy to surgery or from surgery to no treatment at all [8] or if the exact anatomical extent of a tumour is required for radiotherapy planning.

Recent studies have confirmed that \( ^{99}\text{Tc}^{m} \) (V) DMSA is taken up at sites of known head and neck SCC [4]. However, these have shown planar scintigraphy has no role to play in investigation and subsequent management since it was less accurate than clinical examination, only two clinically occult primary tumours were detected and size was an important factor in tumour detection as small primary tumours and low volume neck disease remained undetected. Other recent studies [9, 10] have shown that SPECT \( ^{99}\text{Tc}^{m} \) (V) DMSA can increase the image quality, sensitivity and spatial resolution of the investigation compared with planar scintigraphy.

In this study, SPECT undoubtedly improved image quality (Figs 2-4) with the number of strongly positive studies being much higher when compared with the planar group. Although SPECT improved the sensitivity of the investigation when compared with planar scintigraphy, it was always inferior to clinical examination in detecting patients with cancer, patients with primary tumours and patients with metastatic neck disease. In addition, specificity decreased for the detection of primary tumours. Using both planar and SPECT imaging, one clinically occult tumour was detected but no clinically apparent primary tumour would have been upstaged using current UICC criteria. Size was again an important factor in scintigraphic tumour detection but although SPECT was more accurate than planar scintigraphy it was less accurate than clinical examination. In addition, two patients with primary tumours treated previously with radiotherapy were incorrectly upstaged by SPECT (one on planar scintigraphy) while another patient with a primary tumour which was positive on planar imaging was negative on SPECT. Of the other false positive studies (planar and SPECT) one occurred in a patient with a Stage IV Glomus Jugulare tumour (which can be explained by the high vascularity of the tumour) while the others occurred in four areas of periodontal disease which may mean \( ^{99}\text{Tc}^{m} \) (V) DMSA is behaving, in part, like a bone scanning agent.

The above findings mean that SPECT \( ^{99}\text{Tc}^{m} \) (V) DMSA imaging (like planar evaluation) has no role to play in the evaluation of patients with a head and neck primary SCC.

One of the biggest prognostic factors in head and neck SCC is the presence or absence, level and size of metastatic cervical lymphadenopathy [11] and one of the greatest problems facing the surgeon is the detection of clinically occult neck disease. Recent reports have suggested SPECT \( ^{99}\text{Tc}^{m} \) (V) DMSA may be more accurate than
both clinical examination and planar scintigraphy and therefore have a role in the evaluation of neck disease to include the assessment of the clinically N₀ (no palpable lymphadenopathy) neck [3, 10].

In this study, although SPECT was more sensitive, specific and accurate in detecting which lateral neck compartments contained metastatic carcinoma as well as detecting positive nodes within each compartment when compared with planar scintigraphy, it was always inferior to clinical examination. For planar scintigraphy, although there were areas of increased radioactivity noted in the region of positive neck nodes, and some necks which contained positive nodes measuring less than 2 cm were identified, no distinct individual nodal features could be outlined and no nodes or nodal masses which measured less than 2 cm were accurately detected. Of those patients with 51 clinically N₀ lateral neck compartments, two (4%) were correctly upstaged using planar scintigraphy while three (6%) were incorrectly upstaged.

Using SPECT ⁹⁹Tcm (v) DMSA lateral neck compartment imaging undoubtedly improved the image quality and sensitivity of the investigation. In addition, although spatial resolution was improved (Table 5) and one clinically occult metastatic lymph node measuring approximately 1.5 cm in size was detected, only three (6%) of clinically N₀ lateral neck compartments were correctly upstaged while three (6%) were incorrectly upstaged. Of those lateral neck compartments that were correctly upstaged, two were correctly upstaged to N₁, one incorrectly to N₁ (correct status N₂b) and only in the latter case would upstaging have altered subsequent management. Although there were areas of increased radioactivity noted in the region of positive neck nodes, never more than one nodal mass was identified and nodal features to include capsular outline or extracapsular spread were never identified.

Although both planar scintigraphy and SPECT correctly downstaged one lateral compartment neck, would not most head and neck surgeons operate on a neck with a clinically positive node and a normal scan? Since correct upstaging occurred in only approximately 5% of lateral neck compartments, surely it is cheaper and as effective to adopt a policy of ‘wait and see’ and perform five neck dissections rather than 100 ⁹⁹Tcm (v) DMSA scans.

The above results show that SPECT ⁹⁹Tcm (v) DMSA imaging (like planar scintigraphy) has no role to play in the management of patients with metastatic neck carcinoma (to include the clinically N₀ neck).

**Conclusion**

This study has shown SPECT ⁹⁹Tcm (v) DMSA scintigraphy is as efficient as clinical examination (but more efficient than planar ⁹⁹Tcm (v) DMSA scintigraphy) in detecting patients overall with cancer, patients with primary tumours and patients with metastatic neck carcinoma. One clinically occult primary tumour was detected
Using both planar and SPECT and only 6% of clinically N\textsubscript{0} lateral compartment necks were correctly upstaged by SPECT (4% for planar imaging).

Although SPECT improved the image quality, sensitivity and spatial resolution of the investigation, $^{99}$Tc\textsuperscript{m} (v) DMSA SPECT scintigraphy has no role to play in the routine evaluation of patients with head and neck SCC (to include the clinically N\textsubscript{0} neck).

Further developments in SPECT, PET and fine resolution CT can only lead to an increase in diagnostic sensitivity and specificity and subsequently an overall improvement in the diagnosis and treatment of head and neck cancer.

Acknowledgements

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99Tc\textsuperscript{m}(v)-DMSA planar scintigraphy: does it have a role in the management of patients with head and neck squamous carcinoma?

J.C. WATKINSON\textsuperscript{1,2}, C.R. LAZARUS\textsuperscript{2}, M.N. MAISEY\textsuperscript{2} and S.E.M. CLARKE\textsuperscript{2}

Departments of \textsuperscript{1}Otolaryngology and \textsuperscript{2}Nuclear Medicine, Guy's Hospital, St Thomas' Street, London SE1 9RT, UK

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Summary

99Tc\textsuperscript{m}(v)-DMSA is a new tumour-imaging agent which has recently been proposed as a scintigraphic marker for head and neck squamous cell carcinoma (SCC). Seventy-seven patients were studied prospectively, of whom 58 had a history and diagnosis of head and neck SCC. All patients were examined, imaged using 99Tc\textsuperscript{m}(v)-DMSA planar scintigraphy and then followed up clinically. In addition, 35 patients were followed up with scintigraphy (81 studies). Scintigraphy was less sensitive and less accurate than clinical examination for the overall detection of patients with SCC, for the detection of patients with SCC at presentation and for the detection of patients with primary tumours, possible nodal disease and with residual and recurrent disease following surgery and irradiation. Approximately 50% of patients exhibited positive uptake of 99Tc\textsuperscript{m}(v)-DMSA in the salivary glands following radiotherapy. Although 99Tc\textsuperscript{m}(v)-DMSA is accumulated at sites of head and neck SCC, its inability to detect low volume disease and apparent low specificity following surgery and irradiation means it has no role to play in the routine evaluation of patients with head and neck SCC.

Introduction

Head and neck cancer remains a significant disease with considerable morbidity and mortality in the adult population. Squamous cell carcinoma (SCC) is the commonest histological subtype which is associated not only with the continued consumption of alcohol and tobacco but also with the emergence of new predisposing factors such as HIV infection [1]. One of the greatest prognostic factors in head and neck SCC is the presence or absence, level and size of metastatic cervical lymphadenopathy [2-4]. Therefore, accurate staging of both the primary lesion and the neck are crucial to subsequent management and prognosis.

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Over the past 15 years, head and neck surgeons have been attracted by scintigraphic techniques in an attempt to identify primary and occult primary tumours with cervical metastases together with residual and recurrent disease following surgery and irradiation. They have, however, been frustrated in their efforts using mercury-197 dichloride [5], $^{99}$Tc$^{m}$-pertechnetate [6], $^{99}$Tc$^{m}$-bleomycin [7], indium-111 bleomycin [8], cobalt-57 bleomycin [9], $^{99}$Tc$^{m}$-MDP [10] and gallium-67 ($^{67}$Ga) citrate [11] due to both a low sensitivity and specificity, considerable cost and prolonged blood clearance which delays the scanning time for up to 48 h. Similar disappointing results have been obtained using both radiolabelled monoclonal antibodies [12] and lymphoscintigraphy [13].

Although $^{67}$Ga-citrate remains the radiopharmaceutical most extensively evaluated, it has no role to play in the management of head and neck SCC although it may be of value in patients with either occult infection or lymphoma. Despite this, reports continue to be published describing its uptake in head and neck SCC [14].

Consequently, most head and neck surgeons stage their patients by clinical examination coupled with computerized tomography (CT) as appropriate. $^{99}$Tc$^{m}$ (v) dimercaptosuccinic acid (pentavalent DMSA) is a new tumour imaging agent which has been used to image head and neck tumours and, in particular, medullary carcinoma of the thyroid (MCT) and SCC [15]. Recent reports have confirmed pentavalent DMSA uptake at sites of known head and neck SCC and concluded that this radiopharmaceutical may be of value in the management of patients with this disease [16]. The aim of this study was to evaluate the role $^{99}$Tc$^{m}$ (v)-DMSA planar scintigraphy may play in the evaluation and subsequent management of patients with head and neck SCC.

**Patients and methods**

Between 1 January 1986 and 1 March 1989, 77 patients were studied prospectively (47 male, 30 female; age range 19 to 82 years, mean 58 years). There were 67 patients with malignancy (Fig. 1) and 10 patients with benign lesions (Table 1). Eight patients had had previous surgery, 12 had had previous radiotherapy and five had had combined treatment. All patients were examined clinically, staged using current UICC criteria and a head and neck data sheet was completed. Ethical committee approval was obtained to use the radiopharmaceutical $^{99}$Tc$^{m}$ (v)-DMSA which was prepared using an in-house method [17]. The purity of the complex was analysed by thin-layer chromatography (Merck silica gel, developed with n-butanol/acetic acid/H$_{2}$O (3:2:3), and no free pertechnetate or other $^{99}$Tc$^{m}$ derivative was detected. Patients were injected with $^{99}$Tc$^{m}$ (v)-DMSA (370 MBq) and then imaged at the optimal imaging time, i.e. between 2 and 4 h [18]. Anterior, left and right lateral planar head and neck images were acquired in all patients at presentation using a Scintronix Digicamera interfaced to a Scintronix Data Processor. In addition, five patients had anterior and posterior thoracic images and four had whole body images.

All patients were followed up after primary evaluation and imaging to 1 March 1989. In addition, after initial treatment, 35 patients were followed up using $^{99}$Tc$^{m}$ (v)-DMSA planar scintigraphy (81 studies). Within this group, ten had surgery, eight had radiotherapy and 16 had combined treatment. One patient had chemotherapy followed by radiotherapy. All
Fig. 1. Details of the patients studied with regard to tumour histology and staging.

Table 1. Data from 27 patients (ten with benign lesions, 17 with malignancy).

<table>
<thead>
<tr>
<th>Benign lesions</th>
<th>No. of lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomus tumours</td>
<td>2</td>
</tr>
<tr>
<td>Inflammatory neck nodes or masses</td>
<td>12</td>
</tr>
<tr>
<td>Acromegaly (post-surgery and DXT)</td>
<td>1</td>
</tr>
<tr>
<td>Nasopharyngeal abscess</td>
<td>1</td>
</tr>
<tr>
<td>Chronic laryngitis</td>
<td>1</td>
</tr>
<tr>
<td>Squamous papilloma of the oral cavity</td>
<td>1</td>
</tr>
<tr>
<td>Parotid pleomorphic adenoma</td>
<td>1</td>
</tr>
<tr>
<td>Fibrous dysplasia of the maxilla</td>
<td>1</td>
</tr>
<tr>
<td>Periodontal disease</td>
<td>6</td>
</tr>
<tr>
<td>Postoperative haematoma</td>
<td>1</td>
</tr>
<tr>
<td>Inflamed biopsy or operation sites</td>
<td>3</td>
</tr>
<tr>
<td>Occipital arteriovenous malformation</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>31</strong></td>
</tr>
</tbody>
</table>

Images were reported blind by two of the authors (JCW and SEMC) without prior knowledge (where possible) of the patient's condition, histology or previous treatment. Uptake was reported as being strongly positive, positive or negative. Each lateral compartment of the neck was reported separately.
Results

There were no adverse reactions to $^{99}$Tc$^{m}(v)$-DMSA. The results for imaging patients are shown in Table 2. Clinical examination was not only a more efficient method of detecting which patients had cancer, but also in detecting both primary tumours and

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall ($n = 77$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(35 patients followed up with 81 studies; Total = 158 studies)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clin. exam.</td>
<td>92</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>Scintigraphy</td>
<td>80</td>
<td>42</td>
<td>61</td>
</tr>
<tr>
<td>Overall at presentation ($n = 77$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clin. exam.</td>
<td>94</td>
<td>85</td>
<td>97</td>
</tr>
<tr>
<td>Scintigraphy</td>
<td>79</td>
<td>57</td>
<td>89</td>
</tr>
<tr>
<td>Primary tumour ($n = 63, 71 lesions$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clin. exam.</td>
<td>90</td>
<td>83</td>
<td>96</td>
</tr>
<tr>
<td>Scintigraphy</td>
<td>71</td>
<td>50</td>
<td>88</td>
</tr>
<tr>
<td>Possible neck disease ($n = 134, each lateral neck compartment reported as one site)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clin. exam.</td>
<td>76</td>
<td>95</td>
<td>85</td>
</tr>
<tr>
<td>Scintigraphy</td>
<td>43</td>
<td>91</td>
<td>64</td>
</tr>
<tr>
<td>Possible nodal disease ($91 lesions; 83 malignant, 8 benign, in 134 lateral neck compartments)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clin. exam.</td>
<td>39</td>
<td>94</td>
<td>84</td>
</tr>
<tr>
<td>Scintigraphy</td>
<td>19</td>
<td>91</td>
<td>64</td>
</tr>
<tr>
<td>Residual and recurrent disease ($n = 35, 81 studies$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clin. exam.</td>
<td>87</td>
<td>98</td>
<td>95</td>
</tr>
<tr>
<td>Scintigraphy</td>
<td>82</td>
<td>37</td>
<td>33</td>
</tr>
</tbody>
</table>

Clin. exam., clinical examination.
metastatic cervical lymphadenopathy. Overall, for detecting patients with cancer there were 68 true positives, 30 true negatives, 43 false positives and 17 false negatives. In those patients with no cancer, the normal biodistribution of $^{99m}$Tc(v)-DMSA was confirmed with radioactivity observed in the lacrimal glands, nasal mucosa, blood pool, kidneys and the bladder [16].

For patient planar imaging of the 77 patients at presentation, there were 50 true positives (eight strongly positive), eight true negatives, six false positives and 13 false negatives. In 63 patients with 59 primary tumours and 14 benign lesions, there were 40 true positives, six true negatives, eight false positives and 17 false negatives. In these patients, staging of the primary tumour was possible in 49 (51 tumours, 48 SCC, two embryonal rhabdomyosarcoma and one adenocarcinoma). For $^{99m}$Tc(v)-DMSA planar scintigraphy in this group there were 37 true positives and 14 false negatives and size was an important factor in tumour detection (Table 3). Approximately 50% of T$_1$, T$_2$ and T$_3$ tumours were detected scintigraphically and this increased to 100% for T$_4$ tumours (Fig. 2). On the basis of scintigraphy, no tumours would have been upstaged using current UICC criteria.

<table>
<thead>
<tr>
<th>Table 3. $^{99m}$Tc(v)-DMSA planar scintigraphy in 49 patients with 51 tumours 'T'-staged using current UICC criteria.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Scintigraphy</td>
</tr>
<tr>
<td>True positive</td>
</tr>
<tr>
<td>False negative</td>
</tr>
<tr>
<td>Clinical examination</td>
</tr>
<tr>
<td>True positive</td>
</tr>
<tr>
<td>False negative</td>
</tr>
</tbody>
</table>

All tumours were SCC except three (two embryonal rhabdomyosarcoma and one adenocarcinoma).

For patient lateral neck compartment imaging in the 67 patients at risk for neck disease (Table 2), there were 16 true positives, 88 true negatives, nine false positives and 21 false negatives. Each side of the neck was reported as being scintigraphically positive or negative. No attempt was made to ascribe a level or size to areas of increased radioactivity within the neck, and it was never possible to discern more than one positive area of nodal uptake within an increased area of radioactivity on the scan. Details such as normal capsular outline and extracapsular spread were never identified but in one patient with a fixed N$_3$ nodal mass with a proved necrotic centre, a central ‘cold’ scintigraphic area compatible with central necrosis was clearly observed (Fig. 3).
Fig. 2. (Left) $^{99}$Tc$^m$-DMSA planar image (right lateral) of a patient with a T$_4$N$_0$ squamous carcinoma of the right lower buccal alveolus. Accumulation of radioactivity (strongly positive) is seen at the site of known primary disease (A). (Right) $^{99}$Tc$^m$-DMSA planar image (left lateral) in a patient with a recurrent T$_2$ squamous carcinoma of the larynx following radiotherapy. Positive accumulation of radioactivity is seen at the site of known tumour (B) and in a benign occipital arteriovenous malformation (C).

Fig. 3. (Left) $^{99}$Tc$^m$-DMSA image (right lateral) in a patient with a T$_2$N$_3$ squamous carcinoma of the tongue. Positive accumulation of radioactivity is seen at the site of both primary disease (A) and a fixed necrotic metastatic cervical lymph node (B). (Right) Anterior $^{99}$Tc$^m$-DMSA image in a patient with a T$_4$N$_0$ floor of mouth squamous carcinoma. Accumulation of radioactivity (strongly positive) is seen at the site of known primary disease (C). Note the false-positive uptake in the contralateral (pathologically N$_0$) neck (D).
Within the 134 lateral neck compartments, there were 91 cervical lesions (83 malignant, eight benign). For detecting malignant nodes in any one lateral neck compartment there were 16 true positives, 89 true negatives, nine false positives and 67 false negatives (Table 2). Size was an important factor for the scintigraphic detection of neck nodes and, compared to $^{99}$Tc$^{m}(v)$-DMSA planar scintigraphy, palpation was a more efficient method of detecting lateral neck compartments with, and nodes within those compartments with, metastatic carcinoma (Table 4).

Table 4: $^{99}$Tc$^{m}(v)$-DMSA scintigraphy v. palpation related to the relative size of node(s), or nodal masses, in 29 patients (37 lateral neck compartments) who had cervical metastatic carcinoma.*.

<table>
<thead>
<tr>
<th>Relative size (cm)</th>
<th>0–3</th>
<th>3–6</th>
<th>&gt;6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scintigraphy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>True positive</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>False negative</td>
<td>11</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td><strong>Palpation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>True positive</td>
<td>8</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>False negative</td>
<td>9</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Information based on findings at palpation, CT and pathology.
*All patients had SCC except one who had adenocarcinoma.

Compared with information available from clinical examination, supplemented by CT (and MRI and ultrasound in one patient) and by information from pathological specimens, four clinically N0 (no palpable lymphadenopathy) lateral neck compartments would have been upstaged by scintigraphy. One patient was correctly upstaged from N0 to N1, while two other patients (three lateral neck compartments) were upstaged from N0 to N1 when the correct pathological neck status was N2b.

In the 35 patients followed up after treatment to include clinical examination and $^{99}$Tc$^{m}(v)$-DMSA planar scintigraphy (81 studies, Table 2) there were 18 true positives (four strongly positive), 22 true negatives, 37 false positives (Fig. 4) and four false negatives. Of the 35 patients scanned, 19 (54%) exhibited uptake in the salivary glands following treatment and, of these, 18 had received radiotherapy.

Four patients had distant metastases (15 lesions: ten malignant, five benign). For identifying patients with cancer using planar scintigraphy, there were two true positives, one false negative and one false positive. For tumour lesion scintigraphy, there were five true positives.
Discussion

One of the main problems for the surgeon in the evaluation of patients with head and neck SCC is the detection at presentation of occult metastatic cervical lymphadenopathy and the detection of residual and recurrent disease following surgery and irradiation. In the United Kingdom, all patients with head and neck SCC are staged initially by clinical examination. Further investigations to include CT can only be justified if they radically alter treatment strategy, i.e. from radiotherapy to surgery or from surgery to no treatment at all [19], or if the extent of the tumour is required for radiotherapy planning. At present, scintigraphic techniques to include the use of $^{67}$Ga-citrate, $^{99}$Tc$^m$(v)-MDP and radiolabelled monoclonal antibodies have no role to play in the routine evaluation of patients with head and neck SCC.

Any new tumour imaging modality to evaluate head and neck cancer must be judged against clinical examination and CT. Since CT provides anatomical information, direct comparisons with scintigraphic physiological imaging are not entirely valid although certain comparisons may be justified if the information required is similar and if $^{99}$Tc$^m$(v)-DMSA scintigraphy is to have any role in the management of patients with head and neck SCC.

This study has shown that $^{99}$Tc$^m$(v)-DMSA is taken up at sites of head and neck
cancer and, in particular, SCC. However, scintigraphy provided little anatomical information when compared to clinical examination and CT, and was inferior to both these investigations in detecting which patients had cancer. One occult primary tumour and one second occult primary tumour were identified by scintigraphy but since no upstaging of a clinically apparent primary tumour occurred, $^{99}$Tcm(v)-DMSA scintigraphy (like $^{67}$Ga) has no role to play in the routine evaluation of patients with a primary or occult primary head and neck cancer.

For imaging primary tumours, size was an important factor in detection, and visualization was best for those tumours situated away from the nasopharynx, maxillary sinus, the floor of the mouth and for those tumours with any bony involvement. Of the seven false negatives, two patients had an occult primary (presumed head and neck), another had a lymphoma of the cervical oesophagus and 14 had head and neck SCC tumours (one T1, five T2, eight T3). Of the eight false positives, two had received previous radiotherapy to laryngeal tumours (and therefore the laryngeal skeleton), one had a vascular occipital arteriovenous malformation diagnosed on CT and four patients had uptake in five proved areas of periodontal disease.

Similar arguments apply to those patients with distant metastases. Of the four patients with distant metastases (15 lesions: ten malignant, five benign), the five positive results were all observed in bony metastases (four from metastatic breast carcinoma, one from SCC) and all were positive on $^{99}$Tcm-MDP imaging. Of the two false-positive results, one occurred in a patient with fibrous dysplasia of the maxilla, while another was observed in a patient with an inflamed biopsy site (but no residual disease) in the right axilla. Distant metastases are uncommon in head and neck cancer and since $^{99}$Tcm(v)-DMSA scintigraphy, like $^{99}$Tcm-MDP [10], cannot reliably distinguish benign from malignant disease, $^{99}$Tcm(v)-DMSA planar scintigraphy plays no role in the routine evaluation of patients with distant metastases from head and neck cancer.

Of those patients imaged with benign lesions, there were 16 false positives. Of these, there were four presumed inflammatory neck nodes, one stage IV functional glomus jugulare tumour, one area of fibrous dysplasia of the maxilla, six areas of proved periodontal disease, one inflamed operation site and one occipital arteriovenous malformation. In addition, one patient with a squamous papilloma of the oral cavity had unexplained uptake in the region of the larynx on both the pre- and postoperative images. The explanation for this is unclear, although uptake into the thyroid cartilage is recognized with $^{57}$Co-bleomycin [9], and many metal chelates are taken up into immature bone [20] which may be present in varying amounts in the adult larynx during the normal process of ossification that occurs with ageing. Apart from the four inflammatory neck nodes and the one inflamed operation site, all the other false-positive benign lesions were either highly vascular or associated with bone or possible cartilaginous pathology.

It has been previously stated that one of the biggest prognostic factors in head and neck SCC is the presence or absence, level and size of metastatic cervical
lymphadenopathy so that accurate staging of the neck is crucial to subsequent management. At present, an imaging agent is required which can detect occult disease in the clinically $N_0$ neck, since palpable malignant nodes are usually managed by surgery (preceded by CT where appropriate). In the past, it has been the practice of head and neck surgeons to operate on the clinically $N_0$ neck on the basis that there is retrospective evidence of a high incidence of occult disease. Recent prospective studies, however, have shown no evidence to support operating on the clinically $N_0$ neck [21].

There is now ample evidence that elective neck irradiation can sterilize occult neck disease. Therefore, most surgeons adopt a policy of 'wait and see', irradiating 'at risk' $N_0$ necks and operating on all necks that subsequently become clinically positive.

Overall, in this study $^{99}$Tc$^m$(v)-DMSA scintigraphy upstaged approximately 4% of clinically $N_0$ lateral neck compartments and, therefore, it would have been much cheaper and as effective to adopt a policy of 'wait and see' and operate on four lateral neck compartments than perform 100 $^{99}$Tc$^m$(v)-DMSA scans. This, coupled with a low sensitivity and positive predictive accuracy, means that $^{99}$Tc$^m$(v)-DMSA has no role to play in the management of the clinically $N_0$ neck.

For the detection of residual and recurrent disease following surgery and irradiation, although $^{99}$Tc$^m$(v)-DMSA planar scintigraphy had a sensitivity of 82% (37% specificity), these values were inferior to clinical examination (87% sensitivity, 98% specificity). Of the 37 false positives, all had received previous surgery and/or irradiation (Fig. 4) and these results show that $^{99}$Tc$^m$(v)-DMSA planar scintigraphy has no role to play in the detection of residual and recurrent head and neck SCC following surgery and irradiation.

Uptake in the salivary glands of $^{67}$Ga-citrate following radiotherapy is well recognized and is thought to be due to interstitial oedema, perivascular inflammation and subsequent interstitial fibrosis [22]. We have confirmed uptake of $^{99}$Tc$^m$(v)-DMSA in the salivary glands of approximately 50% of our patients having radiotherapy, a finding not observed by other workers [15]. Similar mechanisms probably operate for both $^{67}$Ga-citrate and $^{99}$Tc$^m$(v)-DMSA to explain this phenomenon.

The biological tumour uptake of $^{99}$Tc$^m$(v)-DMSA varies from moderate through to intense. The uptake mechanism is poorly understood although it has been suggested that it is due, in part, to the similarity of the TcO$_5^-$ pentavalent core to the phosphate molecule which is avidly taken up by some tumour cells [23]. However, this cannot be the only mode of uptake since bony accumulation would be more prominent than is currently seen with $^{99}$Tc$^m$(v)-DMSA, although high bone uptake has been demonstrated in both rodents and rabbits, species characterized by incomplete bone maturation. Recent human in vitro studies have shown the mean tumour : blood ratio to be 1.7 : 1, a mean tumour uptake of 0.0043% injected dose per gram, with the majority of radioactivity being associated with the cell membrane (34%) and cytosolic (47%) fractions [24]. Although these results show that there is uptake of radioactivity into SCC, the lack of any evidence of any specific intracellular localization mechanism in the presence of good clinical images may mean that the majority of $^{99}$Tc$^m$(v)-DMSA is located in tumour extracellular fluid.
Conclusion

This study has confirmed that $^{99}$Tc$^{m}$-DMSA is accumulated at sites of known head and neck SCC with sensitivity (71%) and specificity (50%) rates for the detection of primary tumours slightly less than those observed by others: 80% and 75% respectively [15]. $^{99}$Tc$^{m}$-DMSA has many attractive advantages as a tumour imaging agent. It is ideally suited for imaging with the gamma camera: it is a cheap safe radiopharmaceutical with minimal patient irradiation, which is ideally suited for SPECT.

However, its inability to detect low volume disease and the fact that it is inferior to clinical examination means $^{99}$Tc$^{m}$-DMSA planar imaging has no role to play in the management of patients with head and neck SCC. However, its future in the evaluation and subsequent management of patients with MCT is assured so that follow-up scans in those patients with MCT who have received radiotherapy should be interpreted with caution.

In the future, the further evaluation of both non-specific and specific radiopharmaceuticals, fine resolution and 3-D CT, SPECT and PET (to include image superimposition) can only increase diagnostic sensitivity and specificity and ultimately improve the way head and neck cancer is diagnosed, staged and treated.

References


What is the optimal imaging time for $^{99}$Tcm-(v)-DMSA planar scintigraphy in the detection of squamous carcinoma? A comparative study in humans and in an animal tumour model

J.C. WATKINSON$^1$, $^2$, *, S. ALLEN$^2$, C.R. LAZARUS$^2$, M.N. MAISEY$^2$ and S.E.M. CLARKE$^2$

$^1$Department of Otolaryngology and $^2$Department of Nuclear Medicine, Guy's Hospital, St Thomas Street, London, UK

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Summary

$^{99}$Tcm-(v)-DMSA is a new tumour imaging agent which has been used to image squamous cell carcinoma (SCC) of the head and neck. There have been, however, no studies to date evaluating its optimal imaging time for SCC. Seven patients were studied (six SCC; one nontumour) and seven rabbits (six with SCC, (17 tumours); one nontumour). For the human qualitative studies there was a 67% sensitivity at 2, 4 and 6 h with image quality being optimum at 4 h. Maximum quantitative uptake occurred between 2 and 4 h. For the rabbit qualitative studies the optimum imaging time was 4 h (92% sensitivity, 100% specificity) and maximum quantitative uptake occurred at between 1.5 and 5 h. Taking into account the human and rabbit qualitative and quantitative studies combined with the pharmacokinetics and biodistribution of $^{99}$Tcm-(v)-DMSA, the optimum imaging time of $^{99}$Tcm-(v)-DMSA in humans with SCC was between 2 and 4 h.

Introduction

$^{99}$Tcm-(v)-DMSA is a new head and neck tumour imaging agent which has been used to image medullary carcinoma of the thyroid (MCT) and squamous cell carcinoma (SCC) [1-5]. There is, however, no published quantitative data and very little published qualitative data on the optimum imaging time for $^{99}$Tcm-(v)-DMSA in

* Author to whom correspondence should be addressed.

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humans. Ohta in his original article [1] imaged patients with MCT at 2 h but gave no reasons for choosing such a time. He and others have since followed a similar protocol [1-8]. Indeed, Ohta states [4] that although good quality pictures can be obtained as early as 30 min, images are best acquired at 1 to 2 h post-injection but this is again based on qualitative rather than quantitative data. Aw and co-workers imaged two patients with nasopharyngeal carcinoma at 1, 2, 4 and 24 h and stated that all the images were essentially similar [9]. Recent reports [10] suggest an imaging time of between 3 to 6 h for patients with MCT although again no reasons are given for choosing such times.

The aim of this study was to evaluate the optimal qualitative and quantitative imaging time for $^{99}$Tc$^{m}$-(v)-DMSA in humans with SCC and in an animal SCC tumour model.

Methods

This study was conducted as part of a larger study to evaluate the role of $^{99}$Tc$^{m}$-(v)-DMSA in the management of patients with head and neck SCC [11]. Ethical committee approval was obtained to use $^{99}$Tc$^{m}$-(v)-DMSA which was prepared using an in-house method [12]. The purity of the complex was analysed by thin layer chromatography (Merck silica gel, developed with n-butanol/acidic acid/H$_2$O (3:2:3)) and no free pertechnetate or other $^{99}$Tc$^{m}$ derivative was detected. Seven patients were studied (four male, three female; age range 44 to 80 years, mean 63). There were six tumour and one nontumour patients and, of these, three had received radiotherapy. All tumours were confirmed histologically and patients staged using UICC criteria [13]. Five patients had $T_4$ primary growths and one had an 8 cm tumour of the pinna. Two patients had cervical metastases. The one nontumour patient had post-irradiation change in the larynx following radiotherapy to a $T_2$ laryngeal tumour.

Patients were injected i.v. with $^{99}$Tc$^{m}$-(v)-DMSA (370 MBq) and anterior, right and left lateral head and neck planar images acquired at 2, 4 and 6 h p.i. using a Scintronix Digicamera interfaced to a Scintronix Data Processor. Patients were allowed to eat and drink normally. Images were reported by two of the authors (JCW and SEMC) without prior knowledge, where possible, of the patient’s condition, histology or previous treatment. Uptake was reported as being positive or negative and each lateral compartment of the neck was reported separately.

For each patient, the primary tumour:background (soft tissue) ratios were calculated at each consecutive imaging time.

Seven New Zealand white (NZW) male rabbits were studied (six tumour; one nontumour). The tumour studied was the transplantable rabbit VX2 SCC which was prepared and injected into the shoulder and/or the loin regions of the rabbit using a previously described technique [14, 15]. Six tumour rabbits with 17 tumours were studied (range 0.5–10 cm, mean 3.6 ± 4.30 cm). All tumours were confirmed histologically and their size measured in three dimensions at subsequent post-mortem using callipers. $^{99}$Tc$^{m}$-(v)-DMSA was prepared in an identical manner to that described for the human studies and 200–240 MBq was injected i.v. via the marginal ear vein. Under general anaesthesia [14, 15] anterior thoraco–abdominal planar images (10$^6$ counts) were acquired sequentially to 6 h p.i. using an Ohio-Nuclear Series 120 mobile gamma camera linked to an Ohio-Nuclear data logger. Tumours were confirmed to be in the field of view using a $^{99}$Tc$^{m}$ marker and the bladder was shielded using conventional 2 mm lead shielding. Hydration was maintained by administration of 15 ml normal saline given hourly, subcutaneously, into the nape of the neck. All images were reported blind by two of the authors (JCW
Results

For the evaluation of the qualitative optimal imaging time in humans, \(^{99}\text{Tc}^m\text{(v)}\)-DMSA planar imaging detected all primary tumours at 2, 4 and 6 h p.i. (six true positives, Fig. 1 and Table 1). The patient planar sensitivity for primary lesions at each consecutive imaging time was therefore 100%. For nodal metastases (12 lateral neck compartments), there were no true positives. There were 10 true negatives and two false negatives at 2 and 4 h with no false positives (Table 1). The patient nodal planar sensitivity at these two times was 0%. At 6 h there was one false positive, 10 true

Fig. 1. Sequential anterior \(^{99}\text{Tc}^m\text{(v)}\)-DMSA planar head and neck images in patient 4 with an 8 cm squamous carcinoma of the pinna taken at 2, 4 and 6 h p.i. The tumour is visualized at 2(A), 4(B) and 6(C) h. Image quality is best at 4 h.
Table 1. The qualitative planar imaging results for six patients with head and neck squamous carcinoma at 2, 4 and 6 h following an i.v. injection of $^{99}$Tc$^{m}$-(v)-DMSA.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Primary site and TNM stage*</th>
<th>Time (hours)</th>
<th>Neck compartment</th>
<th>Number of neck nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1+ Supraglottic</td>
<td>larynx $T_4N_0$</td>
<td>2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2 Tongue base</td>
<td>$T_4N_1$</td>
<td>4</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3 Tonsil</td>
<td>$T_4N_0$</td>
<td>4</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4 Pinna</td>
<td>$T_4N_0$</td>
<td>4</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5 Supraglottic</td>
<td>Larynx $T_4N_2^b$</td>
<td>2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6+ Supraglottic</td>
<td>Larynx $T_4N_0$</td>
<td>6</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* UICC, 1987 [12].
+ Previous radiotherapy.

negatives and one false negative. The overall (primary and nodal) lesion sensitivity was 67% at 2, 4 and 6 h.

For the evaluation of the quantitative optimal imaging time, the primary tumour:background (soft tissue) ratios were calculated for each tumour at each consecutive imaging time (Table 2). In patients 2, 3, 5 and 6, maximum uptake was observed at 2 h. In patients 1 and 4 maximum uptake occurred at 4 h. Taking into account the qualitative and quantitative data, previously reported pharmacokinetic and biodistribution data [16], together with the half-life of $^{99}$Tc$^{m}$ and the busy schedule of any nuclear medicine department, the human optimal imaging time was taken to be between 2 and 4 h.

One patient (7) who had post-irradiation change in the larynx following radiotherapy to a $T_2$ tumour, and who had no evidence of residual and recurrent disease, was also imaged at 2, 4 and 6 h p.i. The inflammation:background (soft tissue) ratios in this patient were the highest observed for all the patients studied (except patient 1 at 4 h) and maximal uptake in patient 7 occurred at 6 h (Table 2).
Table 2. The primary tumour:background (soft tissue) quantitative ratios at 2, 4 and 6 h p.i. of $^{99}$Tc$^{m}$-(v)-DMSA in seven patients who had, or had had, a head and neck squamous carcinoma.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Primary site and TNM stage*</th>
<th>Time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>1+</td>
<td>Supraglottis $T_4N_0$</td>
<td>1.46</td>
</tr>
<tr>
<td>2</td>
<td>Tongue base $T_4N_1$</td>
<td>0.97</td>
</tr>
<tr>
<td>3</td>
<td>Tonsil $T_4N_0$</td>
<td>1.47</td>
</tr>
<tr>
<td>4</td>
<td>Pinna $T_xN_0$</td>
<td>0.81</td>
</tr>
<tr>
<td>5</td>
<td>Supraglottis $T_4N_0$</td>
<td>0.68</td>
</tr>
<tr>
<td>6+</td>
<td>Supraglottis $T_4N_0$</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>0.55-1.47</td>
</tr>
<tr>
<td>7+</td>
<td>Larynx $T_0N_0$</td>
<td>1.55</td>
</tr>
</tbody>
</table>

* UICC, 1987 [12].
† Previous radiotherapy.

Table 3. The sequential qualitative planar scintigraphic results for detecting 17 superficially transplanted squamous cell carcinomas in six NZW rabbits following an injection of $^{99}$Tc$^{m}$-(v)-DMSA.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>0.5</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>71</td>
<td>76</td>
<td>73</td>
<td>76</td>
<td>87</td>
<td>92</td>
<td>85</td>
<td>92</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>67</td>
<td>67</td>
</tr>
</tbody>
</table>

For rabbit planar imaging using $^{99}$Tc$^{m}$-(v)-DMSA, one nontumour rabbit was imaged sequentially to 6 h and normal biodistribution confirmed in the bone, kidneys and bladder [14, 17]. In the six rabbits with 17 tumours, the sensitivity and specificity for tumour detection at each imaging time is shown in Table 3. Normal biodistribution of $^{99}$Tc$^{m}$-(v)-DMSA was observed in bone, kidneys and bladder. The optimal qualitative imaging time was 4 h (92% sensitivity, 100% specificity). There were nine true positives (Fig. 2) and eight false negatives and of these, five occurred at 30 min when the tumours ranged in size from 0.5-3.0 cm (mean 1.9 ± 1.6 cm). By 3 h, only
Fig. 2. Sequential $^{99}\text{Tc}^m$-(v)-DMSA thoraco-abdominal planar images in a tumour bearing rabbit taken at approximately 2, 3, 4 and 5 h p.i. There are three transplanted tumours in the right shoulder (A), right loin (B) and left loin which measured $4.5 \times 3.5$, $3.5 \times 2.5$ and $0.5 \times 0.5$ cm respectively. Both the larger tumours are visualized at all times but, although there is very little difference in image quality between 2 and 4 h, tumour visualization is less apparent at 5 h. The smaller left tumour is a false negative.

two tumours remained undetected ($2 \times 1$ and $0.5 \times 0.5$ cm) and, of these, only the smaller one was not visible scintigraphically at 5 and 6 h (Fig. 2). There were two false positives observed at the same site in one rabbit at 5 and 6 h.

The tumour:soft tissue (background) quantitative ratios are shown in Table 4 and Fig. 3. Maximum quantitative uptake occurred between 1.5 and 5 h. Taking into consideration the qualitative and quantitative rabbit tumour results, together with pharmacokinetic and biodistribution data [14, 17, 18] and the half-life of $^{99}\text{Tc}^m$ the overall optimal imaging time for rabbit SCC was taken to be 4 h.
Table 4. The primary tumour:background (soft tissue) sequential quantitative ratios 6 h p.i. of $^{99}$Tcm-(v)-DMSA in two NZW rabbits with four transplanted squamous cell carcinomas.

<table>
<thead>
<tr>
<th>Time after injection (hours)</th>
<th>Tumours</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>0.71</td>
<td>0.53</td>
</tr>
<tr>
<td>1.5</td>
<td>1.41</td>
<td>0.75</td>
</tr>
<tr>
<td>2</td>
<td>1.30</td>
<td>0.71</td>
</tr>
<tr>
<td>3</td>
<td>1.30</td>
<td>0.75</td>
</tr>
<tr>
<td>4</td>
<td>1.10</td>
<td>0.63</td>
</tr>
<tr>
<td>5</td>
<td>1.14</td>
<td>0.68</td>
</tr>
<tr>
<td>6</td>
<td>0.46</td>
<td>0.46</td>
</tr>
</tbody>
</table>

— no tumours detected at 30 min.

Fig. 3. The $^{99}$Tcm-(v)-DMSA sequential quantitative tumour:background ratios in rabbits.

Discussion

The optimal imaging time for any tumour imaging radiopharmaceutical is governed by a combination of factors depending on blood clearance, normal biodistribution and the sequential tumour uptake or accumulation of radioactivity.

For the human qualitative optimal imaging time, six patients with primary tumours all measuring greater than 4 cm were studied and there were six true positives at 2, 4
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and 6 h. Image quality was optimum at 4 h. Two patients had cervical node metastases which measured 3 x 3, 1.5 x 1.5 and 2 x 1.5 cm on computerized tomography (CT) and none of these were detected by scintigraphy. Obviously, size is an important factor for tumour detection since all the primary tumours which measured greater than 4 cm were detected while cervical node metastases which measured 3 cm or less were not. Other factors, however, may be important. Two primary laryngeal tumours had been irradiated and radiotherapy may have affected uptake of radioactivity into the laryngeal skeleton, and one tumour of the pinna had obvious bony involvement. Cervical node metastases may be difficult to detect scintigraphically due to the close proximity of vascular structures (i.e., the great vessels) and gamma ray attenuation by overlying tissues.

The scintigraphic appearances in the nontumour and tumour rabbits confirm previously reported biodistribution results [14] with a distribution to include bone, kidneys and bladder. The rabbit qualitative imaging time of 4 h is a function of the biodistribution and pharmacokinetics results, together with the half-life of 99Tcm. 99Tcm-(v)-DMSA has a bi-exponential blood clearance in both rabbits and humans with mean clearance times of approximately 28 and 340 min and 30 and 392 min respectively, and about 50% of radioactivity is excreted by 4 h p.i. [14, 16]. At 30 min, the eight false negatives were probably due to minimal tumour uptake together with high blood levels, both of which would have contributed to observed low tumour: blood (0.26% g⁻¹ at 2 h, [18]) and tumour:background ratios. By 4 h, optimum conditions with appreciable blood clearance [14, 17, 18] allowed visualization of all but the smallest tumour (0.5 x 0.5 cm), and the tumour:background (soft tissue) ratios confirmed maximum quantitative uptake at between 1.5 and 5 h. This may indicate a prolonged tumour washout phase and such a phenomenon has been observed in vitro (mean tumour:blood ratios were 0.6:1.0 at 2 h, 1.1:1.0 at 4 h, 2.0:1.0 at 6 h, 3.8:1.0 at 24 h [16, 18]).

The cause of the two rabbit false positive results is unclear. Uptake may have occurred in nonmalignant lymph nodes or nodes with microscopic disease although this is unlikely. The most likely reason is uptake in the tip of the scapula although the rabbits’ forearms were always extended to try and exclude this structure from the field of view. The one false positive human result occurred in a lateral neck compartment. This could have been due to uptake in a palpable inflammatory lymph node or could have been a true positive with a false negative CT and fine needle aspiration biopsy result.

For the human quantitative optimal imaging time, maximum uptake occurred in all tumours at between 2 and 4 h, and in only two tumours did the tumour:background (soft tissue) ratios exceed 1:1. These results suggest there is probably very little active tumour accumulation of 99Tcm-(v)-DMSA and this agrees with recently published data showing that the mean in vitro tumour:blood uptake 3–5 h p.i. is 1.7:1.0 with no evidence of any specific intracellular localization mechanism [17]. The range of tumour:background (soft tissue) ratios were similar in both rabbits and humans at 2 h (0.71:1.00–1.30:1.00 and 0.55:1.00–1.47:1.00 respectively).
Optimal imaging time for $^{99}$Tcm($v$)-DMSA planar scintigraphy

At 4 and 6 h there was a wider range of tumour:background (soft tissue) ratios observed in humans when compared to rabbits (0.40:1.00–1.86:1.00 and 0.63:1.00–1.10:1.00 respectively at 4 h, 0.48:1.00–1.73:1.00 and 0.42:1.00–0.65:1.00 respectively at 6 h). However, the rabbit and human tumours were of different sizes and the human results were affected by the two patients whose ratios exceeded 1:1, and one of these had received radiotherapy. Previous irradiation may affect $^{99}$Tcm($v$)-DMSA uptake since patient 7 who had received radiotherapy to a $T_2$ laryngeal carcinoma, and who was diagnosed subsequently as having post-irradiation oedema and inflammation, had the highest observed ratio (inflammation:soft tissue; 1.89:1.00 at 6 h).

Taking into account the difference in sizes between the rabbit and human tumours and the fact two patients with positive disease had received previous irradiation, the optimal imaging times in both groups are comparable and occur approximately between 2 and 4 h. Such congruous findings may indicate that similar mechanisms exist for $^{99}$Tcm($v$)-DMSA tumour uptake in both rabbits and humans with squamous cell carcinoma.

Conclusion

This study has evaluated the optimal imaging time for $^{99}$Tcm($v$)-DMSA planar scintigraphy in the detection of squamous carcinoma both in patients with head and neck cancer and in an established animal tumour model system. The optimal imaging time in both rabbits and humans was between 2 and 4 h.

Acknowledgements

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An evaluation of the uptake of Technetium-99m (v) Dimercaptosuccinic acid in patients with squamous carcinoma of the head and neck

J. C. WATKINSON, S. E. M. CLARKE
AND O. H. SHAHEEN

Departments of Nuclear Medicine and Otolaryngology, Guy's Hospital, London

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Over the last 15 years head and neck surgeons have been attracted by the use of nuclear medicine scanning techniques in patients with squamous carcinoma of the head and neck, in an attempt to identify occult primary tumours and cervical metastases together with residual or recurrent disease following surgery or irradiation. They have, however, been frustrated in their efforts using gallium67 citrate1 or cobalt57 bleomycin2 due to low sensitivity and specificity, considerable cost and slow blood clearance which prolongs the scanning time for up to 48 h.

A new imaging agent Te99m (v) DMSA has recently been developed3,4,5 with the same ligand but different characteristics from the well-established renal imaging agent Te99m (III) DMSA. Recent reports have described its use in the detection of head and neck tumours6 and in particular SCC and embryonal rhabdomyosarcoma. The aim of this study was to assess further the uptake of Te99m (v) DMSA in patients with primary and metastatic SCC of the head and neck using both planar imaging and SPECT.

Materials and methods

DMSA is a low molecular weight organic acid which forms the ligand for the well-recognized static renal imaging agent
Tc\textsuperscript{99m} (III) DMSA. Under alkaline conditions with a low stannous chloride concentration, DMSA forms polymeric complexes with Tc\textsuperscript{99m} to form a pentavalent core. For this study a standard DMSA kit (Amersham International plc) was used with a modification\textsuperscript{7} of the technique described by Ohta \textit{et al.}\textsuperscript{6}; 370 MBq (10 mCi) of the prepared radiopharmaceutical Tc\textsuperscript{99m} (v) DMSA was injected intravenously and patients imaged at 2 h using a Scintronix large field of view gamma camera interfaced to a Scintronix data processor to obtain standard planar views.

Twenty-four patients were imaged and of these 19 had positive primary pathology, the distribution of which is depicted in Table 1. Two patients had occult primaries and 13 had palpable neck nodes. There were 3 patients with benign lesions. One had an inflammatory neck mass, 1 had a squamous oropharyngeal papilloma and 1 had chronic laryngitis.

In 7 cases SPECT imaging was also performed using a rotating gamma camera fitted with a parzen filter 1.5, with elliptical orbits (where possible) using 64 projections over 360°, each view for a 5.6° rotation of the camera. This information was combined with data already available from conventional planar views and, using the data processor, mathematically reconstructed tomographic images were obtained in the coronal, sagittal and transaxial planes by utilizing a filtered back-projection technique.\textsuperscript{8} The elliptical orbit allows close camera approximation to the patient in the anterior and posterior projections, but where patients were unable to lie completely flat, circular orbits were performed.

All images were reviewed blind by one of us (S.E.M.C.) without prior knowledge of the patient’s history, histology or surgical management.

\section*{Results}

Twenty-four patients were imaged (16 male, 8 female), age range 28–75 years (mean 60), of whom 21 had a histologically proven head and neck SCC. Of those patients with primary disease there were 15 true positives and 3 true negatives, of whom 2 were imaged following surgery and 1 had a normal laryngoscopy for hoarseness. There were 3 false negatives, all of whom had mucosal exophytic lesions, of the tonsil, floor of mouth and supraglottis respectively with a high surface area to bulk ratio. The 1 false positive patient had equivocal uptake noted in the larynx which disappeared following removal of the squamous papilloma from his oropharynx. In 1 patient an occult primary SCC was discovered in the right lung, despite a normal chest radiograph, using planar Tc\textsuperscript{99m} (v) DMSA imaging (Figure 1).

\begin{table}[h]
\centering
\begin{tabular}{l|c}
\hline
Larynx & 4 \\
Hypopharynx & 6 \\
Tongue & 4 \\
Floor of mouth & 2 \\
Others & 3 \\
\hline
Total & 19 \\
\hline
\end{tabular}
\caption{Tc\textsuperscript{99m} DMSA (v) imaging—primary site}
\end{table}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{Figure1.png}
\caption{Planar Tc\textsuperscript{99m} (v) DMSA image showing a $T_2N_3$ tongue SCC. Uptake is seen at the sites of the primary lesion (A) and a fixed cervical node with a necrotic centre (B). Uptake is noted also at the site of a histologically proven occult second primary in the right lung (C).}
\end{figure}
other occult primary remained undetected despite a CAT scan and SPECT evaluation. The sensitivity of imaging patients using Tc$^{99m}$ (v) DMSA represents the number of patients correctly identified with SCC. This figure was 83%. The percentage number of patients correctly identified without SCC relates to the specificity of the investigation and this was 75%.

Fourteen patients with cervical neck nodes were imaged and of these there were 12 true positives which were all proven histologically. There were 2 true negatives, one of whom had an obstructed submandibular gland consequent upon a floor of mouth tumour and the other had an inflammatory neck mass. All these patients had palpable disease, except 1 who had a T3 laryngeal tumour with occult metastases undetected by either CAT or planar Tc$^{99m}$ (v) DMSA imaging. The results of planar imaging patients with cervical metastases yielded a 92% sensitivity and 100% specificity. The sensitivity increased to 100% with SPECT.

Seven patients had SPECT imaging performed which improved not only the image quality and spatial resolution (Figures 3–5) but also the sensitivity of the

![Figure 2. Transverse axial CAT scan showing an extensive SCC of the left external auditory meatus (A). Tumour had extended to involve the squamous temporal bone, parotid gland, middle cranial fossa floor and the infratemporal fossa.](image)

![Figure 3. Coronal SPECT Tc$^{99m}$ (v) DMSA scan in the same patient as in Figure 2. Note uptake in the squamous temporal bone (A), parotid gland (B) and the middle cranial fossa floor extending into the infratemporal fossa (C), which corresponded to the sites of disease on CT scanning.](image)
Figure 4. Skull radiograph in the same patient as in Figure 2 following sub-total petrosectomy. A liqaclip (arrowed) marks confirmed residual disease on the middle cranial fossa floor which extended into the infratemporal fossa.

Figure 5. Postoperative coronal SPECT Tc$^{99m}$ (v) DMSA in the same patient as in Figure 2. Note uptake in the middle cranial fossa floor and infratemporal fossa region (A) which corresponds to the site of the ligaclip marking residual tumour (Figure 4).

Investigation (Figures 6 and 7). All patients with no histological evidence of disease were negative on scanning.

Discussion

Fifteen years ago a new imaging agent, gallium$^{67}$ citrate, was introduced which, at the time, was enthusiastically described as 'tumour seeking' not only for head and neck malignancy but for tumours in general. Such claims have since been disregarded and its current clinical use is confined to the investigation of some tumours, notably lymphoma, bronchial carcinoma and hepatoma, in the investigation of sarcoidosis and to assist in abscess localization.$^9$

Tc$^{99m}$ (v) DMSA is a new imaging agent which has been used to evaluate head and neck malignancy and, in particular, SCC
and embryonal rhabdomyosarcoma. It is more sensitive and specific than gallium\(^5\) and has the distinct advantage that patients can be imaged 2 h after injection. Our accumulative data shows that \(\text{Tc}^{99m}\) (v) DMSA is undoubtedly taken up by SCC of the head and neck. The sensitivity of the investigation correlates well with other series\(^6\) but the high specificity may reflect the low number of patients scanned with benign lesions. \(\text{Tc}^{99m}\) (v) DMSA is not 100% sensitive or specific for head and neck SCC and uptake has been reported in inflammatory masses, soft tissue and benign tumours.\(^5\) \(\text{Tc}^{99m}\) (v) DMSA uptake has also been evaluated in medullary carcinoma of the thyroid.\(^10\)

Using a standard large field of view gamma camera to obtain planar AP and lateral views, a 2-dimensional image is obtained of a 3-dimensional object. This is often of great diagnostic value but has a number of distinct disadvantages. It provides poor depth information, allows tissue superimposition and makes little allowance for gamma ray attenuation by the tissues. Using SPECT, since 1-dimensional views are taken of a 2-dimensional transverse axial slice, the algebraic sum of the activity within that slice can be mathematically
reconstructed. This greatly improves depth interpretation and reduces tissue superimposition artefacts. In our results 1 patient had a T3 laryngeal tumour with no detectable lymphadenopathy on clinical examination, CAT scan or planar Tc99m (v) DMSA imaging. Evaluation with SPECT revealed cervical lymphadenopathy, which was confirmed at operation and 1 node less than 2 cm in size was subsequently found to contain metastatic tumour on histological examination. Other series have confirmed increased sensitivity, image quality and spatial resolution with SPECT.6 These results are encouraging since an early criticism of both gallium67 citrate and cobalt57 bleomycin imaging of cervical nodes was their inability to detect lesions less than 2 cm in size (by which time they were usually clinically palpable).2

In those patients scanned with primary disease there were 3 false negatives. These were all superficial exophytic lesions and it may well be that the tumour bulk to surface area ratio is an important factor in controlling Tc99m (v) DMSA uptake. None of these patients had SPECT and had this been performed the sensitivity of the investigation might have been improved. In our series the sensitivity improved 100% for solid infiltrative lesions. The 1 false positive was a patient with a squamous papilloma of the anterior faucial pillar. There was unexplained equivocal uptake in the larynx which was not present on the postoperative image following surgical removal of the papilloma. The reason for this remains unclear.

The biological tumour uptake of Tc99m (v) DMSA varies considerably from moderate through to intense. The uptake mechanism is poorly understood although it has been suggested it is due, in part, to the similarity of the TC04- pentavalent core to the phosphate molecule which is avidly taken up by some tumour cells.5 This cannot be the only mode of uptake since bony accumulation would be more prominent than is currently seen with Tc99m (v) DMSA. Further work is necessary to investigate this mechanism and to assess the uptake, if any, in the inflammatory tissue which surrounds tumour cells. Other factors which may influence Tc99m (v) DMSA uptake including surgery, chemotherapy and radiotherapy also require further evaluation.

As a tumour imaging agent in head and neck SCC, Tc99m (v) DMSA has many attractive advantages. It is a cheap radiopharmaceutical, ideally suited for imaging with the gamma camera with a high sensitivity and specificity, minimal patient irradiation and a short imaging time. It is also suitable for SPECT.

Tc99m (v) DMSA provides a rapid means of investigating patients with head and neck SCC and further studies are necessary to evaluate its role in the detection of the occult primary and cervical metastases together with residual or recurrent disease following surgery or irradiation.

Acknowledgements

We are grateful to the staff and patients of both the Head and Neck Oncology Unit and Nuclear Medicine Department at Guy’s Hospital.

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Uptake of Te-99m (v) DMSA

Imaging head and neck cancer using radioisotopes: a review

J C Watkinson MSc FRCS Department of Otolaryngology, Guy's Hospital, London SE1 9RT
M N Maisey FRCP FRCR Department of Nuclear Medicine, United Medical and Dental Schools, Guy's Hospital, London SE1 9RT

Keywords: imaging; head and neck cancer; radioisotopes

Introduction
The accumulation of radioactive iodine in metastatic thyroid cancer was reported in 1942, and iodine isotope (131I) thyroid scanning was introduced into clinical practice 9 years later. The discovery that the isotope of technetium (99mTc) pertechnetate (TcO4⁻) was trapped by the thyroid gland led to its routine use in imaging. Together with 123I and 131I, it is the current agent of choice to diagnose and treat differentiated thyroid malignancy. Other radiopharmaceuticals may be used to image both thyroid cancer and other head and neck malignancies, and with the wider availability of radioisotopes, together with improved technology, there has been a renewed interest in imaging head and neck cancers. This paper reviews the clinical use of radiopharmaceuticals currently available to image head and neck cancer.

Tumour localizing radiopharmaceuticals for imaging head and neck cancer may be divided into specific and non-specific agents. Tumour-specific agents localize only within one specific tumour, or follow one specific metabolic pathway. Examples include 131I for differentiated thyroid cancer, 99mTc(V) dimeracpsosuccinic acid for medullary thyroid cancer, and indium isotope (111In) monoclonal antibodies for squamous cell carcinoma (SCC). Tumour-nonspecific agents localize not only within a number of histologically different malignant tumours, but also in benign tumours and inflammatory lesions. Examples within the head and neck include gallium (67Ga)-citrate for lymphoma, and thallium (201Tl)-chloride for differentiated thyroid cancer.

Thyroid Cancer
The investigation of differentiated (papillary, follicular and medullary) thyroid cancer involves preoperative diagnosis, usually the investigation of a solitary thyroid nodule, and the postoperative assessment of residual thyroid tissue together with the detection of residual recurrent tumour.

Clinical situations in which the possibility of differentiated thyroid malignancy arises include a solitary thyroid nodule found on palpation; a multinodular goitre with suspicious features; local symptoms such as hoarseness, dysphagia and pain, with or without a thyroid gland on palpation; exposure of the head, neck and upper thorax to previous irradiation; and, lastly, cervical lymphadenopathy or distant metastases with an unknown primary. By far the commonest way for thyroid cancer to present is as a solitary nodule when the incidence of malignancy is between 5 and 10%.

The choice of radiopharmaceuticals for imaging differentiated thyroid cancer has been reviewed elsewhere. 99mTc-TcO4⁻ is the agent used most often for routine thyroid imaging, although 123I is now being used more widely. However, availability and expense remain limiting factors for the more widespread use of 123I. With either 99mTc or 123I the majority of nodules greater than 5 mm in diameter can be identified and the use of oblique views greatly increases accuracy. False-negative results are often associated with smaller lesions in the isthmus, but these are usually easy to palpate and therefore do not cause a real problem. The function of all preoperative imaging of the thyroid gland is to increase the likelihood of malignancy at operation by improving the positive predictive accuracy without any loss in sensitivity.

When a clinically solitary thyroid nodule is investigated the scan may show a solitary non-functioning or hypofunctioning area (i.e. a 'cold' nodule); a functioning area (i.e. a 'hot' nodule) or a multinodular goitre. The probability of malignancy is increased if the scan demonstrates a solitary 'cold' nodule, but decreased to less than 1% if it shows a 'hot' nodule or a multinodular goitre. A solitary 'cold' nodule should be investigated further by ultrasound and by fine needle aspiration cytology. There are occasional nodules which function on the pertechnetate scan but are non-functioning on the iodine scan. These probably reflect an ability to trap iodine but not to organise it, and problems from such rare discrepancies between pertechnetate and iodine studies can be avoided by performing 123I scans on any nodule which concentrates pertechnetate or, if 123I is not available, a TRH test should be carried out since an absent TSH response to TRH confirms a truly functioning autonomous nodule.

In an attempt to increase the pathological specificity without any loss in sensitivity, many other radiopharmaceuticals have been used to investigate the solitary thyroid nodule. 67Ga-citrate, 99mTc-bleomycin, 131I[25], 131I[19] and more recently 201Tl-chloride have been evaluated and all exhibit variable uptake in malignant lesions. At the present time (for malignancy) the false-negative rate is too high for them to have a place in the routine investigation of thyroid nodules. The 67Ga-citrate scan may be of diagnostic value in patients with longstanding Hashimoto's disease who develop a solitary thyroid nodule since this may be a lymphoma which shows avid accumulation of 67Ga.

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Figure 1. Anterior head and neck $^{67}$Ga-citrate image in a patient with longstanding Hashimoto's disease. Acid uptake of $^{67}$Ga is seen at the site of known lymphoma within the thyroid gland.

Imaging undifferentiated thyroid cancer is unrewarding since it does not usually accumulate iodine. Some studies have described the uptake of $^{67}$Ga-citrate within such tumours and observed an increase in sensitivity when a positive $^{67}$Ga scan is obtained with a negative $^{201}$Tl-chloride scan.

Radionuclide imaging techniques for the postoperative assessment of differentiated thyroid cancers serve to establish the completeness or otherwise of the initial surgical treatment and to detect residual or recurrent tumour. Currently the most widely used and accepted method of follow-up is regular total-body scanning using $^{131}$I combined with serial serum thyroglobulin measurements. This is because although differentiated thyroid cancers show little or no iodine uptake in the presence of normal thyroid tissue, after ablation of normal thyroid tissue which results in high serum TSH levels, $^{131}$I will accumulate in residual or metastatic tumour which can subsequently be detected as hot lesions on a whole-body scan (Figure 2). This method permits the sensitive detection and localization of residual tumour in the neck or distant metastases and assesses the potential for radioiodine treatment.

The localization of thyroid metastases in thyroidec-tomized patients using whole body $^{131}$I scans has the disadvantage of requiring the patient to be rendered hypothyroid and not all differentiated metastatic thyroid carcinomas take up $^{131}$I. Attempts to circumvent these problems have included the use of $^{123}$I-anti-human thyroglobulin monoclonal antibody and recently, $^{201}$Tl-chloride scintigraphy has been used to detect such deposits (Figure 3) which in combination with $^{131}$I, has an increased sensitivity for the detection of metastatic disease.

Patients with medullary carcinoma of the thyroid (MCT) have been investigated with a variety of radionuclide techniques. Primary tumours can be identified as cold areas on $^{99m}$Tc-$\text{TcO}_4^-$ or $^{123}$I thyroid scans with the classical pattern of bilateral symmetrical non-functioning nodules occurring in the familial type. Since these tumours commonly metastasize to bone, $^{99m}$Tc-methylene diphosphonate (MDP) bone scanning in combination with serum thyroglobulin measurements has the advantage of identifying bone metastases in a high proportion of patients. Figure 4 shows a $^{99m}$Tc(V) DMSA scan in a patient with medullary carcinoma of the thyroid. Uptake is seen at the site of known disease.
Calcitonin levels has been used for the routine follow up of patients with MCT and two new radiopharmaceuticals have been developed recently which localize in MCT tumours. 131I-metaiodobenzyl guanidine (MIBG) was developed for imaging phaeochromocytoma and subsequently shown to be taken up into primary and metastatic MCT. More recently, pentavalent 99mTc (V) dimercaptosuccinic acid (DMSA) has been developed which is related to the well established renal imaging agent 99mTc (III) DMSA and its uptake has been confirmed in MCT (Figure 4). Recent reports have confirmed uptake of both 131I-MIBG and 99mTc (V) DMSA in both primary and recurrent MCT but have shown 99mTc (V) DMSA to have distinct advantages over 131I-MIBG. It is an easily prepared, low cost radiopharmaceutical with a short imaging time, and as a technetium labelled compound, the whole body radiation dose is markedly less than a 131I MIBG scan. The main role of 99mTc (V) DMSA is in the investigation of primary and recurrent MCT and the possible advantages of 131I MIBG scanning is to assess its therapeutic potential in an individual patient.

Parathyroid tumours
Carcinoma of the parathyroid gland is uncommon. The majority of patients present with severe hyperparathyroidism and markedly elevated serum calcium and parathormone (PTH) levels and approximately 50% have a palpable neck mass. Non-invasive pre-operative localization may be facilitated by a positive 67Ga-citrate scan although, usually, the diagnosis is made at operation. Recurrent disease should be suspected when the serum calcium or PTH levels remain elevated; localization should begin with cervical examination since 45% of recurrences are palpable. 201Tl-chloride subtraction scanning for the localization of parathyroid adenomas has been successfully used to locate recurrent tumour within the neck and mediastinum. However, the thyroid lobe is usually totally excised along with the lymph glands of a patient with Hodgkin's disease. 67Ga was added a new dimension to the evaluation of SCC, it is expensive. In addition, nodes detected less than 15 mm in size are regarded as clinically non-salvageable, and groupings of three or more 8–15 mm contiguous nodes contribute to a possible source of false-positive results.

The accumulation of 197Hg chloromerodrin at sites of head and neck SCC was first reported over 30 years ago. Since then physicians and surgeons have used a variety of radiopharmaceuticals in head and neck SCC in an attempt to identify primary and occult tumour with cervical metastases together with residual or recurrent disease following surgery and irradiation. 67Ga-citrate, cobalt (57Co)-bleomycin, 11In-bleomycin, 99mTc-bleomycin, 99mTc-TcO4 and many of the radiolanthanides have all been tried, but with limited success due to low sensitivity and specificity, considerable cost, and prolonged blood clearance which may delay the scanning time up to 48 h.

A criticism of both 67Ga-citrate and 57Co-bleomycin imaging of cervical nodes is their inability to detect lesions less than 20 mm in size, by which time they were usually clinically palpable. Recent attempts to image cervical nodes using 99mTc-sulphur colloid lymphoscintigraphy and 111In monoclonal antibody against the epidermal growth factor receptor, have proved similarly unsuccessful due to an unacceptable false-negative rate and the inability to detect nodes less than 20 mm in size. Encouraging reports have described the uptake of 99mTc (V) DMSA within primary and metastatic head and neck SCC (Figure 5). It is as sensitive, but more specific than 67Ga-citrate, and being a technetium labelled compound is more suitable for single photon emission computed tomography (SPECT). The use of SPECT improves the sensitivity of the investigation, so that it is now possible to detect nodes less than 20 mm which were neither palpable nor visible on CT evaluation.

Lymph glands
Almost 20 years ago Edwards and Hayes investigated the potential of 67Ga-citrate as a bone scanning agent and noted its concentration in the cervical glands of a patient with Hodgkin's disease. 67Ga was subsequently described as 'tumour seeking', not only for head and neck malignancy, but for tumours in general. Its current clinical use in head and neck tumour imaging is now largely confined to the evaluation of lymphoma. 67Ga-citrate scanning may be applied to the evaluation of patients with head and neck lymphoma before a histological diagnosis is obtained, and during initial staging. It is, however, of particular value in the evaluation and restaging of residual and recurrent disease, although bilateral symmetrical accumulation within the salivary glands following radiotherapy is a normal phenomenon which may cause difficulty when interpreting images.

Bone
The clinical use of bone scanning using 99mTc-MDP in head and neck tumours has been reported to be of value in the pretreatment evaluation of bony
involvement from primary carcinoma, in the diagnosis of residual and recurrent disease, and to detect bony metastases to, and from, the head and neck65. Distant metastases from head and neck carcinoma (excluding the thyroid) are uncommon, and although bone scanning is highly sensitive, it is non-specific so that bony extension within the mandible from oral cancer may not be reliably distinguished from benign dental disease66. Primary bone tumours of the head and neck all accumulate 99mTc-MDP but these are all much better evaluated using other imaging modalities such as CT scanning.

Miscellaneous

Many other head and neck tumours have been evaluated using radioisotopes. Both primary and metastatic melanoma have been visualized using 131I-monoclonal antibodies and early reports are encouraging67. Similarly, the localization of 131I-MIBG within thymoma and malignant paragangliomata68 has recently been described, and further studies are underway to assess both the diagnostic and therapeutic implications of such uptake within these tumours.

Conclusion

The role of radioisotopes in the management of both differentiated and medullary carcinoma of the thyroid is now well established. Although there are many other radiopharmaceuticals available to image head and neck cancer, few can actually achieve the required diagnostic sensitivity and specificity. The search is for more sensitive and specific diagnostic and therapeutic agents. Although the introduction of monoclonal antibodies into routine imaging has been hampered by distinct practical problems69, it is now possible to properly assess the use of monoclonal antibodies, their F(ab) fragments and to evaluate new 99mTc-labelled tumour imaging agents, using animal tumour model systems60. The continued use of SPECT together with the introduction of positron emission tomography (PET) can only lead to an increase in diagnostic sensitivity and specificity and subsequently to an overall improvement in the way we diagnose, stage and treat head and neck cancer.

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(Accepted 4 May 1988)
Clinical Records

Scintigraphic evaluation of glomus tumours


Abstract

The current investigations of choice for a suspected glomus tumour are either direct or indirect angiography to include digital subtraction followed by computerized tomography (CT) or magnetic resonance imaging (MRI) or, if available, CT and MRI with gadolinium alone. Although these modalities confirm the diagnosis and give anatomical information to facilitate accurate staging, they do not provide functional data. The use of radionuclide scintigraphy can add an extra physiological dimension to glomus tumour imaging.

Iodine-131/123 metaiodobenzylguanidine (MIBG) is a tumour imaging agent which has been used to diagnose head and neck neuroendocrine tumours to include paragangliomata and medullary carcinoma of the thyroid (MCT). However, it is expensive and the new head and neck tumour imaging agent technetium-99m (Tc99m) (v) dimercaptosuccinic acid (DMSA) has superseded it as the imaging agent of choice to evaluate MCT.

We report a patient with a glomus jugulare tumour which was evaluated with I131/I123-MIBG and Tc99m (v) DMSA. The tumour was functional and is the first reported case exhibiting positive accumulation of both I131-MIBG and Tc99m (v) DMSA. The patient was subsequently treated with a therapeutic dose of I131-MIBG. The significance of these results is discussed.

Introduction

Glomus tumours of the temporal region and skull base range in size from microscopic lesions confined to the promontory to large, destructive, neurologically aggressive lesions which involve the petrous bone and skull base. They arise from small ovoid structures which lie along Jacobson’s nerve on the promontory, Arnold’s nerve or, in 50 per cent of cases, around the jugular bulb. They were discovered by Guild in 1941, and recognized as a clinical entity four years later (Rosenwasser, 1945). They are usually benign, but malignant cases have been described (Taylor et al., 1965; Baulieu et al., 1988). They are more frequent in females (5:1), may be familial, bilateral, associated with multiple paragangliomata (Spector et al., 1974) and may secrete catecholamines.

Traditionally, the clinicians ability to diagnose these tumours has far exceeded his ability to adequately treat them. Until recently, from a practical point of view, no further classification was necessary (Alford and Guilford, 1962), but due to surgical advances, a more elaborate staging system has been proposed (Jackson et al. 1982).

Any patient with a suspected glomus tumour should have a full history and examination, followed by pure tone audiometry, impedance tympanometry, caloric tests, and investigation of tumour functional status as appropriate. Further radiological investigation should confirm the diagnosis without the need for biopsy and accurately stage the disease.

Radionuclide scintigraphy has been used to evaluate head and neck paragangliomata. Early studies used technetium-99m methylene diphosphonate (MDP), and showed it was possible to detect asymptomatic familial chemodectomas (Veldman et al., 1980). More recently, radiolabelled MIBG has been introduced as a functional scintigraphic marker of monoamine uptake and which has been used to image phaeochromocytoma, neuroblastosomas, carcinoïd tumours and medullary carcinoma of the thyroid (MCT) (Sisson et al., 1981; Fischer et al., 1984; Munkner, 1985; Sone et al., 1985). Initial studies were performed with iodine-131 (I131), but I125-MIBG is now available which has superior imaging characteristics and dosimetry. More recently, I131-MIBG has been used to treat malignant phaeochromocytoma, neuroblastosoma, MCT and chemodectoma (Khafagi et al. 1987).

Tc99m (v) DMSA is a new radiopharmaceutical used to image head and neck tumours, and in particular, MCT and squamous carcinoma (Watkinson et al., 1987; Ohta et al., 1988; Watkinson et al., 1989a). It has replaced I131- and I125-MIBG in the primary evaluation of MCT, but its uptake has not been described in glomus tumours.

We describe the investigation and management of a patient with a glomus jugular tumour who was evaluated not only with arteriography, CT and MRI but also with radiolabelled iodine-MIBG, and Tc99m (v) DMSA.

Materials and methods

The CT, MRI and arteriographic studies were all performed in the department of diagnostic radiology at Guys Hospital. I125- and I131-MIBG was obtained from Amersham International plc and Tc99m (v) DMSA was prepared using an in-house method (Sampson, 1987).

The scintigraphic images were obtained using a scintronix large field-of-view gamma camera interfaced to a scintronix data processor with a rotating SPECT (single photon emission computerized tomography) facility. Following intravenous radiopharmaceutical injection (185 MBq for I131-MIBG. Thirty-seven MBq for I125-MIBG. Three hundred and seventy MBq for Tc99m (v) DMSA) planar whole body and SPECT head and neck images were acquired (2, 4, 6 and 24 hrs for I125-MIBG; 24 and 48 hrs for I131-MIBG; 2 hrs for Tc99m (v) DMSA).

From the Departments of *Otolaryngology and †Nuclear Medicine, Guy’s Hospital, London.

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Single vessel indirect angiogram of the left carotid system showing a vascular stage IV glomus jugulare (arrow), supplied principally by the ascending pharyngeal and occipital arteries.

**Case report**

A 60-year-old lady presented with a ten-year history of hoarseness and a nine-month history of progressive deafness in the left ear with pulsatile tinnitus for six months, occasional dizziness and intermittent headaches and facial flushing. On examination, the patient appeared acromegalic. There was a red mass behind the left tympanic membrane and there was a left conductive deafness confirmed by pure tone audiometry. She had a left vocal cord palsy which had been documented ten years previously, but all her other cranial nerves were intact. Clinically, the findings were those of a glomus jugulare tumour.

She was investigated using CT and MRI together with arteriography to include DSA. DSA confirmed the lesion to be highly vascular, with a small branch from the pre-cavernous segment of the left internal carotid supplying the mass, but a predominant blood supply from the ascending pharyngeal and occipital branches of the external carotid (Fig. 1). The venous phase showed obstruction to the left jugular vein with extensive collateral drainage. The patient had plasma amine and urinary vanillylmandelic acid (VMA) levels measured, and these, together with other sequential blood tests, are shown in Table I. In addition, she was imaged with $^{131}$-MIBG and $^{131}$-MIBG (planar and SPECT) and both studies showed positive accumulation of radioactivity at the site of known tumour (Fig. 2). The percentage of injected dose of $^{131}$-MIBG in the tumour was approximately one per cent. A psychiatric assessment diagnosed an anxiety neurosis.

Both CT and MRI showed a lesion destroying the left petrous apex, extending intracranially into the left cerebellopontine angle, distorting the pons with some extension around the internal carotid (Fig. 3). The empty sella syndrome was also noted.

On the basis that the disease was stage IV and functional, together with an anxiety neurosis possibly caused by increased tumour dopamine (Azzarelli *et al.*, 1988), primary treatment was radiotherapy. Based on previous reports using $^{131}$-MIBG to treat paragangliomata together with the positive uptake of $^{131}$-MIBG in this patient, it was decided to administer a therapeutic dose of $^{131}$-MIBG (11000 MBq) with potassium iodide cover to block uptake by the thyroid gland.

Following treatment, there was a subjective elevation of mood, a marked improvement in her tinnitus and a return of the supine plasma noradrenaline to within normal levels (Table I). However urinary vanillylmandelic acid (VMA) remained elevated and post-treatment MRI and $^{131}$-MIBG (planar and SPECT) scans showed no change in either tumour anatomy or physiology. The patient remains alive and well eight months later.

**Discussion**

Although the management of glomus tumours remains controversial, the current consensus in the literature appears to be that glomus tympanicum tumours should be treated primarily by surgery (with or without post-operative radiotherapy). Glomus jugulare tumours present more of a problem. Small lesions confined to the middle ear and possibly some cases with minimal involvement of the petrous pyramid respond well to radiotherapy alone, and this may be curative. More extensive disease requires surgical intervention by mastoidectomy or petrosectomy depending on the size and direction of spread of the tumour.

### TABLE I

**SEQUENTIAL URINE AND BLOOD TESTS**

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* = Raised; † = Supine; ‡ = Standing.

VMA — Vanillyl mandelic acid
VMA/Creatinine ratio — VMA Creatinine ratio
L-D — L-Dopa
DA — Dopamine
NA — Noradrenaline
A — Adrenaline
Cal — Calcitonin

$^{131}$-MIBG over two hours IV
$^{131}$-MIBG over two hours IV

* = Raised; † = Supine; ‡ = Standing.

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<td></td>
<td></td>
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</tr>
<tr>
<td>6/12:</td>
<td>34*</td>
<td>3.4*</td>
<td>0.06</td>
<td>3.18*</td>
<td>0.22*</td>
<td></td>
<td>0.05</td>
</tr>
</tbody>
</table>

* = Raised; † = Supine; ‡ = Standing.

VMA — Vanillyl mandelic acid
VMA/Creatinine ratio — VMA Creatinine ratio
L-D — L-Dopa
DA — Dopamine
NA — Noradrenaline
A — Adrenaline
Cal — Calcitonin

$^{131}$-MIBG over two hours IV
$^{131}$-MIBG over two hours IV

* = Raised; † = Supine; ‡ = Standing.
the lesion, followed by radiotherapy, although the radiotherapy in these cases is probably biostatic rather than curative (Shaheen, 1983).

Accurate diagnosis is crucial to correct management. Although CT, MRI and angiography confirm the diagnosis and provide anatomical information for staging, they do not give any detail with regard to the functional status of the tumour. Radionuclide scintigraphy cannot replace arteriography, since although it is often diagnostic, it has an inherent false negative rate, probably related primarily to tumour size.

However, there are obvious advantages to $^{113m}$I$^{131}$-MIBG scintigraphy. It can demonstrate bilateral tumours in the head and neck and both localization and accuracy can be enhanced by SPECT.

Intense focal uptake may indicate functional activity and whole body scans may be performed to identify distant metastases and incidental paragangliomata in the thyroid, retroperitoneum or adrenal gland. In addition, intense focal uptake may indicate the potential for $^{131}$I-MIBG therapy, particularly in metastatic disease when there may be more than one lesion.

This paper has outlined the advantages of radionuclide scintigraphy.

Fig. 2
$I^{131}$-MIBG planar scan (a) and Tc$^{99m}$ (v) DMSA coronal SPECT scan (b) demonstrating accumulation of radioactivity (arrowed) at the site of the known glomus jugulare tumour.

Fig. 3
Coronal MRI (a) and oblique CT (b) showing the glomus tumour arising from the jugular bulb, with intracranial extension. Note brain stem compression clearly demonstrated with MRI (arrowed).
tigraphy of glomus tumours using radiolabelled iodine-MIBG. 

$^{99m}$Tc (v)-DMSA is a new tumour-imaging agent which is of proven value in the diagnosis and management of MCT (Clarke et al., 1987). The uptake of this agent at the site of the glomus jugulare in this study may reflect tumour uptake, tumour functional activity or blood pooling.

$^{99m}$Tc (v)-DMSA is a cheap radiopharmaceutical compared to I$^{131}$I-MIBG with a low radiation dose (Watkinson et al., 1989b) which may not only be of diagnostic value in the management of glomus tumours, but labelling the sulphhydryl groups with sulphur-35 could have important therapeutic implications.

References


Address for correspondence:
Dr T. A. Rockall,
Department of Otolaryngology,
Guys Hospital,
St Thomas' Street,
London SE1 9RT.
$^{99}$Tc$^m$ (v) DMSA: the pituitary sign

J.C. WATKINSON1, 2* C.R. LAZARUS2, M.N. MAISEY2 and S.E.M. CLARKE2

The Departments of 1 Otolaryngology and 2 Nuclear Medicine, Guy’s Hospital, St Thomas Street, London, SE1 9RT, UK

Received 19 October 1989

Summary

Technetium-99m ($^{99}$Tc$^m$) DMSA is a new tumour imaging agent which has been used to evaluate head and neck tumours. It has a normal head and neck biodistribution to include the lacrimal glands, nasal mucosa and the blood-pool.

Seventy-seven patients were studied of whom 63 had a head and neck malignancy. Of these patients, 19 (25%) exhibited positive accumulation of radioactivity in the region of the pituitary gland and this was a constant finding in those followed-up after treatment. Biodistribution studies in forty New Zealand white rabbits confirmed pituitary accumulation of $^{99}$Tc$^m$ (v) DMSA.

The pituitary gland region should be included in the normal biodistribution of $^{99}$Tc$^m$ (v) DMSA.

Introduction

$^{99}$Tc$^m$ (v) DMSA is a new tumour imaging agent which has been used to evaluate head and neck tumours [1] and, in particular, medullary carcinoma of the thyroid [2] and squamous cell carcinoma (SCC) [3]. It has a normal head and neck biodistribution to include the lacrimal glands, the nasal mucosa and the blood-pool. In a recent prospective study to evaluate the role of $^{99}$Tc$^m$ (v) DMSA in the management of patients with head and neck SCC [4], it was noted that some patients exhibited positive accumulation of radioactivity in the region of the pituitary gland. The aim of this study was to evaluate retrospectively the incidence of the pituitary sign in those patients studied previously with head and neck SCC [4] and to quantify $^{99}$Tc$^m$ (v) DMSA pituitary uptake in a rabbit tumour model with SCC.
Fig. 1. Uptake of $^{99m}$Tc (v) DMSA in the region of the pituitary gland (arrowed) in a patient with a $T_4$ floor of mouth squamous carcinoma. The primary tumour is not visualized on these views. Positive accumulation of radioactivity in the region of the pituitary is seen pre-operatively (A) and immediately post-operatively (B).

Materials and methods

Between January 1, 1986 and March 1, 1989 77 patients were studied (47 male, 30 female; age range 19–82, mean 58), using $^{99m}$Tc (v) DMSA planar scintigraphy [4]. There were 67 patients with malignancy and 10 with benign lesions. Eight patients had had previous surgery, 12 had had previous radiotherapy and five had had combined treatment. $^{99m}$Tc (v) DMSA was prepared using an in-house method [5]. The radiopharmaceutical was injected intravenously (370 MBq) and patients imaged at the optimal imaging time (that is between 2 and 4 h [6]). Anterior, left and right lateral planar head and neck images were acquired in all patients at

<table>
<thead>
<tr>
<th></th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pituitary</td>
<td>0.015</td>
<td>0.0087</td>
<td>0.0064</td>
<td>0.010</td>
</tr>
<tr>
<td>Blood</td>
<td>0.031</td>
<td>0.014</td>
<td>0.0079</td>
<td>0.0021</td>
</tr>
<tr>
<td>Pituitary: blood ratio</td>
<td>0.48</td>
<td>0.62</td>
<td>0.81</td>
<td>4.76</td>
</tr>
</tbody>
</table>

Mean data from 10 rabbits (5 non-tumour; 5 tumour).

Fig. 2. SPECT $^{99m}$Tc (v) DMSA study in a patient with a $T_3$ squamous carcinoma of the tongue (not visualized). Positive accumulation of radioactivity (A) in the region of the pituitary gland is seen in the transaxial and sagittal images. Note the normal biodistribution of $^{99m}$Tc (v) DMSA in the nasal mucosa (B).
All patients were followed-up clinically after primary evaluation and imaging to March 1, 1989. In addition, after initial treatment, 35 patients were followed-up using $^{99}$Tc$^m$ (v) DMSA planar scintigraphy (81 studies). Within this group, 10 had surgery, eight had radiotherapy and 16 had combined treatment. One patient had chemotherapy followed by radiotherapy.

All images were reported blind by two of the authors (J.C.W. and S.E.M.C.).

The rabbit biodistribution studies were carried out as part of a larger study to evaluate the pharmacokinetics and biodistribution of $^{99}$Tc$^m$ (v) DMSA [7]. Forty New Zealand white male rabbits were studied (20 non-tumour; 20 tumour). The rabbit SCC tumours were induced using a previously described technique [8]. Following administration of $^{99}$Tc$^m$ (v) DMSA (100–120 MBq i.v.), five non-tumour and tumour bearing rabbits were sacrificed at 2, 4, 6 and 24 h post-injection and the pituitary gland together with blood samples were removed. The % of the injected dose in the pituitary gland was then calculated.

Results

One hundred and fifty eight $^{99}$Tc$^m$ (v) DMSA head and neck scans were performed. In 19 patients (25%; 18 with malignancy, one with benign disease; age range 45–82 years, mean 68) positive accumulation of radioactivity was observed in the region of the pituitary gland on planar scintigraphy (Fig. 1) and the site of localization was confirmed on SPECT (Fig. 2). In 11 of these patients who were subsequently followed-up, a positive pituitary sign was a constant finding (Fig. 3). Eight of the patients were female (age range 45–82 years, mean 66) and 11 were male (age range 55–80 years, mean 69).

![Fig. 3. Uptake of $^{99}$Tc$^m$ (v) DMSA in the region of the pituitary gland (arrowed) in the same patient as Fig. 1. Positive accumulation of radioactivity is seen following post-operative radiotherapy (A) and at follow-up 15 months following the operation (B).](image-url)
Of the 19 patients studied, 17 had no known pituitary pathology. One patient was imaged after having a hypophysectomy for bone pain due to metastatic breast cancer while another had the empty sella syndrome.

For the rabbit studies, there was no significant difference ($p > 0.05$) in the pituitary biodistribution results between the non-tumour and tumour groups and combined results for the 40 rabbits are shown in Table 1.

**Discussion**

Reasons for the accumulation of $^{99}$Tc$^m$ (v) DMSA at the site of the human pituitary are unclear. Variations in hormonal activity are unlikely since all patients (except two) were over the age of 60 and there were approximately equal numbers of male and female patients with positive $^{99}$Tc$^m$ (v) DMSA pituitary accumulation. The pituitary gland has a high blood flow and this phenomenon is reflected in the rabbit biodistribution studies. However, it should be noted that the rabbit pituitary was washed and blotted dry (but not perfused) so it is possible that such results reflect, in part, actual blood content although the pituitary:blood ratios suggest some active $^{99}$Tc$^m$ (v) DMSA pituitary accumulation.

The positive accumulation of radioactivity at the site of the human pituitary could reflect a blood-pool effect and although no pituitary uptake has been visualized on rabbit scintigrams [6, 9] this can be explained by the small size of the rabbit pituitary (approximately 1 mm$^2$) and the significant bone accumulation of $^{99}$Tc$^m$ (v) DMSA in the surrounding immature calvarium. Another possible explanation for the accumulation of radioactivity observed in the region of the pituitary could be uptake in a calcified pineal gland. Although only one of the patients had a skull X-ray (which showed no pineal calcification) the position of the uptake on the planar and SPECT studies suggest that the accumulation of radioactivity is in the region of the pituitary and not the pineal gland (Fig. 2).

The accumulation of $^{99}$Tc$^m$ (v) DMSA in the patient who has had a hypophysectomy could be explained by uptake in the surrounding resultant inflammatory tissue, although the patient was not imaged pre-operatively, while in another patient no uptake of $^{99}$Tc$^m$ (v) DMSA was observed in the region of a pituitary eosinophil adenoma which had recently been irradiated. What is interesting is that another patient with a stage IV Glomus Jugulare and the empty sella syndrome exhibited positive uptake of radioactivity in the region of the pituitary gland. The reason for this is unclear.

**Conclusion**

This study has shown that in approximately 25% of patients, $^{99}$Tc$^m$ (v) DMSA is accumulated at the site of the pituitary gland and that this is a constant phenomenon in patients followed-up after treatment. The reasons for such findings are unclear. The rabbit biodistribution studies confirm similar accumulation of radioactivity in the
rabbit pituitary but it is not clear whether or not this is blood-pool, active accumulation or both.

The pituitary gland region should be included in the normal biodistribution of $^{99}\text{Tc}m$ (v) DMSA.

References

The following table was missing from the published version of the paper:

**Table 4.** Spearman rank order correlation coefficients between DV-index and status at admission, at SPECT and outcome.

<table>
<thead>
<tr>
<th>Vascular area</th>
<th>Number of patients</th>
<th>DV-index vs status at admission</th>
<th>p</th>
<th>DV-index vs status at SPECT</th>
<th>p</th>
<th>DV-index vs outcome</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACA</td>
<td>5</td>
<td>0.67</td>
<td>0.22</td>
<td>0.97</td>
<td>&lt;0.001</td>
<td>0.87</td>
<td>0.055</td>
</tr>
<tr>
<td>MCA</td>
<td>41</td>
<td>0.59</td>
<td>&lt;0.001</td>
<td>0.59</td>
<td>&lt;0.001</td>
<td>0.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PCA</td>
<td>6</td>
<td>0.14</td>
<td>0.78</td>
<td>0.14</td>
<td>0.78</td>
<td>-0.13</td>
<td>0.79</td>
</tr>
<tr>
<td>All</td>
<td>64</td>
<td>0.49</td>
<td>&lt;0.001</td>
<td>0.57</td>
<td>&lt;0.001</td>
<td>0.75</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>


The third sentence of the abstract should read: It can be shown that, as tissue blood flow (Q) increases, J rises to reach a plateau, i.e. becomes independent of Q.
Pharmacokinetics, biodistribution and dosimetry of $^{99}$Tc$^{m}$(V)DMSA in humans with squamous cell carcinoma

J.C. WATKINSON\textsuperscript{1*}, S. ALLEN\textsuperscript{2}, C.R. LAZARUS\textsuperscript{2}, J. SINCLAIR\textsuperscript{2}, G.M. BLAKE\textsuperscript{2} and S.E.M. CLARKE\textsuperscript{2}

Departments of \textsuperscript{1}Otolaryngology and \textsuperscript{2}Nuclear Medicine, Guy's Hospital, St Thomas' Street, London SE1 9RT

Received 18 January 1990

Summary

Technetium-$^{99}$m ($^{99}$Tc$^{m}$)(V) dimercaptosuccinic acid (DMSA) is a new tumour imaging agent which has been used to evaluate squamous carcinoma (SCC) of the head and neck. This study evaluated the pharmacokinetics and biodistribution of $^{99}$Tc$^{m}$(V)DMSA in patients with SCC and calculated the bone mass of a New Zealand White (NZW) rabbit. This data was then used to calculate the effective dose equivalent in man.

A total of 16 patients were studied (5 with no tumour, 11 with tumour). $^{99}$Tc$^{m}$(V)DMSA had a fast bi-exponential blood clearance in patients with no tumour (30 and 401 min) and patients with tumour (30 and 387 min) with no significant difference ($p > 0.05$) between the two groups. $^{99}$Tc$^{m}$(V)DMSA had a fast cumulative urine excretion with mean half-times in non-tumour and tumour patients of 183 min and 244 min respectively. There was no significant difference ($p > 0.05$) between these two latter groups.

The effective dose equivalent of $^{99}$Tc$^{m}$(V)DMSA in man is 5.1$\mu$Sv/MBq.

Introduction

$^{99}$Tc$^{m}$(V)DMSA is a new tumour imaging agent which has been used to evaluate head and neck SCC [1]. Recent studies in humans [2,3] and in NZW rabbits with transplanted SCC [4] have shown that $^{99}$Tc$^{m}$(V)DMSA has a bi-exponential blood clearance and cumulative urine excretion. The former study [2,3] evaluated 10 patients of whom some had benign disease, some had medullary carcinoma of the thyroid and some had SCC. The latter study [4] compared bi-exponential blood clearance in non-tumour and tumour rabbits with SCC showing that there was no

*Author to whom correspondence should be addressed.

0143-3636/90 $03.00+ .12 \copyright$ 1990 Chapman and Hall Ltd.
significant difference ($p > 0.05$) in mean half-times between the two groups and that clearance appeared unaffected by tumour mass.

The aim of this study was to evaluate the pharmacokinetics of $^{99m}$Tc(V)DMSA in patients with no tumour and in patients with SCC, to assess the biodistribution of $^{99m}$Tc(V)DMSA in human SCC and to establish the bone mass of a NZW rabbit. By combining this data with rabbit data [4] the effective dose equivalent of $^{99m}$Tc(V)DMSA in man will be calculated.

**Materials and methods**

This study was carried out as part of a larger one to evaluate the role of $^{99m}$Tc(V)DMSA imaging in the management of patients with head and neck SCC [5]. Ethical committee approval was obtained to use $^{99m}$Tc(V)DMSA.

Sixteen patients were studied (5 with no tumour, 11 with tumour; 12 male, 4 female; age range 44-70 years, mean 59). Eleven had proven head and neck SCC and the other five had had head and neck SCC successfully treated and were free of disease at the time of investigation. All these five remain alive and well.

$^{99m}$Tc(V)DMSA was prepared using an in-house method [6]. The purity of the complex was analysed by thin-layer chromatography (Merck silica gel, developed with n-butanol/acetic acid/H$_2$O (3:2:3)) and no free pertechnetate or other $^{99m}$Tc derivative was detected.

For the pharmacokinetic studies, 10 patients were studied (5 with no tumour; 5 with tumour). All patients were allowed to eat and drink normally. As part of the routine imaging protocol, all patients were injected intravenously with $^{99m}$Tc(V)DMSA (370MBq) having previously obtained blood and urine samples for background estimation. The syringe was weighed before and after injection and sequential 1ml venous whole blood samples obtained from the other arm at 5, 15, 30, 45 and 60min and then 1.5, 2, 3, 4, 6 and 24h post-injection. Patients were asked to collect all urine passed over the 24h post-injection period. At each voiding, the sample was placed into a separate numbered container and the time of voiding noted. In three patients with tumour, this protocol was carried out under observation as part of in-patient investigations. All blood and urine samples were weighed and then counted for 10s along with standards of the injection (1:10-1:10$^5$ dilution). The percentage of the injected dose per gram for both blood and urine samples was then calculated. Using regression analysis, the best fitting slow exponential was fitted to the blood samples obtained at, and after, 120min. This best fitting curve was subtracted from the data samples obtained before 120min to obtain the fast exponential component. A correlation coefficient for each best-fit was calculated and from these two lines, the half-time blood clearance values ($t_{1/2}$) for each patient were calculated. The percentage of the injected dose in urine was plotted graphically against time and cumulative excretion half-times obtained. A mean value ($t_{50\%}$) was then calculated. The whole body (WB) retention half-time (i.e. $WB = 100-\Sigma \%$ (injected dose excreted)) values were calculated by using a least squares fit on the points obtained and a mean value then calculated.

Estimation of whole body retention assumes the only route of excretion to be via the kidneys, although it has been shown that some biliary excretion of $^{99m}$Tc(V)DMSA does occur in rabbits [2, 4].

For the biodistribution studies, six patients with proven head and neck SCC undergoing primary surgery were studied. $^{99m}$Tc(V)DMSA (150MBq) was given intravenously 1h prior to surgery at the time of premedication and the syringe weighed before and after injection. On removal of the surgical specimens 3-5h post-injection, samples of SCC and normal muscle were obtained, washed and blotted dry and these, together with samples of whole blood (1ml), were weighed and the percentage of the injected dose/g calculated. Samples of SCC and
normal muscle were confirmed histologically. During surgery, one of the surgeons wore a finger thermoluminescent dose monitor to estimate the dose to the fingers during the course of the study.

The bone mass of a NZW rabbit was calculated by feeding the carcass to African flesh-eating beetles (*Domesticus lardius*) and weighing the bones three weeks later. For the dosimetric calculations, a combination of both animal [4] and human data was used and the method is shown in the appendix.

**Results**

The blood clearance of $^{99}$Tc$_m$(V)DMSA in patients with no tumour was bi-exponential (Fig. 1). The half-times for each patient were calculated (range 27–33 and 363–426 min,

![Graph](image1.png)

**Fig. 1.** The blood clearance of $^{99}$Tc$_m$(V)DMSA in non-tumour and tumour patients.
Table 1. The bi-exponential half-time blood clearance values* of $^{99}$Tc$^{m}(V)$DMSA in five non-tumour and five tumour patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>First phase $R^+$</th>
<th>Second phase $R^+$</th>
<th>Patient</th>
<th>First phase $R^+$</th>
<th>Second phase $R^+$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27±7</td>
<td>0.97</td>
<td>6</td>
<td>25±5</td>
<td>0.98</td>
</tr>
<tr>
<td>2</td>
<td>29±8</td>
<td>0.96</td>
<td>7</td>
<td>27±4</td>
<td>0.98</td>
</tr>
<tr>
<td>3</td>
<td>31±7</td>
<td>0.97</td>
<td>8</td>
<td>34±7</td>
<td>0.98</td>
</tr>
<tr>
<td>4</td>
<td>31±9</td>
<td>0.96</td>
<td>9</td>
<td>34±9</td>
<td>0.97</td>
</tr>
<tr>
<td>5</td>
<td>33±15</td>
<td>0.91</td>
<td>10</td>
<td>35±14</td>
<td>0.93</td>
</tr>
<tr>
<td>Range</td>
<td>27–33</td>
<td>363–426</td>
<td>Range</td>
<td>25–35</td>
<td>344–548</td>
</tr>
<tr>
<td>Mean</td>
<td>30</td>
<td>401</td>
<td>Mean</td>
<td>30</td>
<td>387</td>
</tr>
<tr>
<td>Standard error of the mean</td>
<td>10</td>
<td>37</td>
<td>Standard error of the mean</td>
<td>9</td>
<td>40</td>
</tr>
</tbody>
</table>

*±2 standard deviations.

$^+$R = correlation coefficient.

Table 1) and from these, the mean half-times obtained (30 and 401 min). The blood clearance of $^{99}$Tc$^{m}(V)$DMSA in patients with tumours was bi-exponential (Fig. 1). All tumours were confirmed histologically. The half-times for each patient were calculated (range 25–35 and 344–548 min, Table 1) and, from these, the mean half-times obtained (30 and 387 mins). Using a student $t$-test, there was no significant difference ($p > 0.05$) between the mean half-time for non-tumour and tumour patients.

The cumulative urine excretion of $^{99}$Tc$^{m}(V)$DMSA was bi-exponential in non-tumour and tumour patients (Fig. 2) and the $t_{50\%}$ values are shown in Table 2. The mean cumulative urine excretion $t_{50\%}$ values in non-tumour and tumour patients were 183 and 244 min respectively (range 70–270 and 200–350 min, Table 2). Using a student $t$-test there was no significant difference ($p > 0.05$) between the $t_{50\%}$ values in the two groups (including and excluding patient 2; see whole body retention data and discussion) although there appeared a wider scatter of cumulative urine excretion values in the non-tumour patients.

The whole body retention half-time values in non-tumour and tumour patients are shown in Fig. 3 and Table 3. The mean whole body retention half-time values in non-tumour and tumour patients were 778 and 375 min (range 278–12,144 and 163–884 min) and, using a student $t$-test, there was a significant difference between these two values ($p < 0.005$). However, the whole body retention half-time in patient 2 was 12,144 min (202 h, correlation coefficient 0.60) which was radically different from all the other non-tumour patients. Excluding this patient from the non-tumour group, the mean whole body retention half-time was 631 min and, using a student $t$-test,
there was no significant difference between this value and the value for the tumour group (375 min, $p > 0.05$).

The biodistribution results are given in Table 4. Two patients had hypopharyngeal tumours, two had laryngeal tumours and the remaining two had tumours of the tongue and maxillary sinus respectively. There was a wide range observed in the tumour:blood radioactivity ratios (0.3–4.1:1) and in the tumour:muscle ratios (0.6–4.0:1). The two patients with hypopharyngeal lesions had the highest
Table 2. The cumulative urine excretion half-time ($T_{50\%}$) values of $^{99m}$Tc(V)DMSA in five non-tumour and five tumour patients.

<table>
<thead>
<tr>
<th>Non-tumour</th>
<th>Tumour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Cumulative half-time (min)</td>
</tr>
<tr>
<td>1</td>
<td>125</td>
</tr>
<tr>
<td>2</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>270</td>
</tr>
<tr>
<td>4</td>
<td>250</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
</tr>
<tr>
<td>Range</td>
<td>70-270</td>
</tr>
<tr>
<td>Mean*</td>
<td>183</td>
</tr>
<tr>
<td>Standard error of the mean</td>
<td>38</td>
</tr>
</tbody>
</table>

*Mean for four patients (excluding patient 2; see whole body retention and discussion) = 211 min (standard error, 32).

Discussion

$^{99m}$Tc(V)DMSA has a fast bi-exponential blood clearance and cumulative urine excretion in humans. These results are not significantly different to those observed in rabbits [4] but are faster than those observed in rabbits and humans using $^{99m}$Tc(III)DMSA or $^{99m}$Tc-O$_4^-$ [2]. Blood clearance is dependent upon the amount of red cell and plasma protein binding, the volume of distribution and on the tumour:blood ratios (4.1:1 and 2.5:1). The ratios in the other four patients showed no apparent uptake in the tumour when compared with blood and muscle. With one hypopharyngeal tumour, the tumour:blood and tumour:muscle ratios were approximately the same (4.0:1 and 4.1:1) but the tumour:blood ratio (2.5:1) was greater than the tumour:muscle ratio (0.6:1) in the second patient with a hypopharyngeal tumour. With all the other tumours studied, the tumour:muscle ratios were greater than the tumour:blood ratios. There was no apparent relationship between tumour:blood and tumour:muscle ratios and tumour histology.

The finger dose to one surgeon (first assistant) during two biodistribution studies (total operating time 9h) was less than 0.3mSv (legal limit = 12.5mSv per month). The maximum finger exposure time for one surgeon during the biodistribution studies was 12h.

The bone mass of a NZW rabbit was 137g kg$^{-1}$ body weight and the effective dose equivalent in man for $^{99m}$Tc(V)DMSA was 5.1μSv MBq$^{-1}$.
WHOLE BODY RETENTION OF $^{99}$Tc$^m$-V-DMSA IN NON-TUMOUR PATIENTS.

Mean $T_{1/2} = 778$ mins
Data from 5 patients
(Data from 4 patients
Mean $T_{1/2} = 631$ mins; see discussion)

WHOLE BODY RETENTION OF $^{99}$Tc$^m$-V-DMSA IN TUMOUR PATIENTS.

Mean $T_{1/2} = 375$ mins
Data from 5 patients

Fig. 3. The whole body retention of $^{99}$Tc$^m$(V)DMSA in non-tumour and tumour patients.

mechanisms of renal clearance. $^{99}$Tc$^m$(V)DMSA has a large blood-pool component in rabbits and humans with the majority of activity probably loosely bound to plasma proteins with minimal activity in, or on, red blood cells [2]. Renal clearance is dependent, not only on the volume of distribution, but also molecular size, lipid solubility and plasma protein binding. $^{99}$Tc$^m$(V)DMSA is a larger molecule than either $^{99}$Tc$^m$(III)DMSA or $^{99}$Tc$^m$.TcO$_4^-$ . It has a low lipid solubility, a negative charge and a pentavalent core in which the sulfhydryl groups of separate dimercaptosuccinic molecules possibly bind to each other. This prevents them binding to the
Table 3. The whole body retention half-time values of \(^{99}\text{Tc}^{m}\text{(V)}\text{DMSA}\) in five non-tumour and five tumour patients.

<table>
<thead>
<tr>
<th>Non-tumour</th>
<th>Tumour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Whole body retention half-time (min)*</td>
</tr>
<tr>
<td>1</td>
<td>2036±422</td>
</tr>
<tr>
<td>2</td>
<td>12144±14593</td>
</tr>
<tr>
<td>3</td>
<td>1699±424</td>
</tr>
<tr>
<td>4</td>
<td>598±78</td>
</tr>
<tr>
<td>5</td>
<td>278±12</td>
</tr>
<tr>
<td>Range</td>
<td>278–12144</td>
</tr>
<tr>
<td>Mean R+</td>
<td>778</td>
</tr>
<tr>
<td>Standard error of the mean</td>
<td>46</td>
</tr>
</tbody>
</table>

+±2 standard deviations.
+R = correlation coefficient.
+Mean half-time = \(\ln 2/\text{mean} \).
+Mean (patients 1, 3, 4, 5) = 631 (standard error = 29; see discussion).

The finding that there was no significant difference between the first and second phase mean blood clearance half-times in the patients with no tumours and patients with tumour groups is to be expected since the tumours were relatively small in relation to body weight (in contrast to rabbit tumours) and so would have little effect on pharmacokinetic data. There was little inter-human variation in the first phase (range 6 min) and second phase (range 63 min) clearance results in patients with no tumours, and these findings are similar to those observed in rabbits [4].

There was no significant difference \((p > 0.05)\) in the mean blood clearance half-times between the human group with no tumour and rabbits with no tumours [4]. In the absence of any tumour uptake, the wider range observed in the first phase (range 10 min) and second phase (range 204 min) blood clearance times in humans with tumour is likely to be due to variations in the patient’s clinical condition which could affect renal function. For example, non-metastatic manifestations of bronchial squamous carcinoma are well recognized and hypercalcaemia due to the secretion of a parathormone-like substance and other substances such as Vitamin D (or its metabolites) or prostaglandins has been described [7]. There was no significant difference \((p > 0.05)\) in the mean blood clearance half-times between the human group with tumour and a rabbit group with tumours [4] suggesting, in conjunction...
Table 4. The human biodistribution results 3–5 h post-injection of $^{99}$Tc$^{m}$-DMSA (in percentage injected dose per gram of tissue).

<table>
<thead>
<tr>
<th>Primary site</th>
<th>Hypopharynx</th>
<th>Hypopharynx</th>
<th>Larynx</th>
<th>Larynx</th>
<th>Tongue</th>
<th>Maxilla</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient number</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Time post-injection (min)</td>
<td>240</td>
<td>252</td>
<td>192</td>
<td>290</td>
<td>175</td>
<td>195</td>
<td>2.15 x 10⁻³</td>
</tr>
<tr>
<td>Blood</td>
<td>2.40 x 10⁻³</td>
<td>2.15 x 10⁻³</td>
<td>2.25 x 10⁻³</td>
<td>3.13 x 10⁻³</td>
<td>5.5 x 10⁻³</td>
<td>3.45 x 10⁻³</td>
<td>5.5 x 10⁻³</td>
</tr>
<tr>
<td>Muscle</td>
<td>2.50 x 10⁻³</td>
<td>9.59 x 10⁻³</td>
<td>1.06 x 10⁻³</td>
<td>7.90 x 10⁻⁴</td>
<td>2.1 x 10⁻³</td>
<td>1.87 x 10⁻³</td>
<td>7.90 x 10⁻⁴</td>
</tr>
<tr>
<td>Tumour</td>
<td>9.90 x 10⁻³</td>
<td>5.46 x 10⁻³</td>
<td>2.38 x 10⁻³</td>
<td>7.98 x 10⁻⁴</td>
<td>3.1 x 10⁻³</td>
<td>4.41 x 10⁻³</td>
<td>9.90 x 10⁻⁴</td>
</tr>
<tr>
<td>Tumour:blood ratio</td>
<td>4.1:1</td>
<td>2.5:1</td>
<td>1.1:1</td>
<td>0.3:1</td>
<td>0.6:1</td>
<td>1.3:1</td>
<td>0.3–4.1:1</td>
</tr>
<tr>
<td>Tumour:muscle ratio</td>
<td>4:1</td>
<td>0.6:1</td>
<td>2.3:1</td>
<td>1:1</td>
<td>1.5:1</td>
<td>2.4:1</td>
<td>0.6–4.0:1</td>
</tr>
</tbody>
</table>

Mean tumour:blood ratio = 1.7:1.
Mean tumour:muscle ratio = 2:1.
with the results from similar groups with no tumour that mechanisms of clearance may be similar in rabbits and humans.

The pharmacokinetic blood results in this study are different from those quoted previously [2,3]. However, in this earlier work patients were not divided into tumour and non-tumour groups, the humans studied included SCC and medullary carcinoma of the thyroid and mean clearance times were calculated graphically. In this study, patients were divided into non-tumour and SCC tumour groups and as a first and second phase blood clearance was calculated for each individual patient the results in this study must be considered more accurate.

For the human studies it was possible to collect all the urine passed in the 24h following radiopharmaceutical injection (in contrast to rabbit results [4] so that the mean cumulative urine excretion half-times could be analysed statistically. Cumulative urine excretion was bi-exponential and (as for the rabbits), approximately 50% of radioactivity was excreted within 4h post-injection. The finding that there was no significant difference in mean cumulative urine excretion between the patients with no tumour and patients with tumour groups is to be expected since there was no significant difference in blood clearance between the two groups and tumour mass was relatively small in relation to body weight.

There was quite an unexpectedly large range in the cumulative urine excretion half-times in both the human group with no tumours (range 200min) and the human group with tumours (range 150min). One would anticipate a narrower range in the former relative to the latter. However, as some urine samples were collected at home by patients, inevitable errors in collection can be expected and could possibly account for variations in results leading to the unexpected large range.

The whole body retention in patients with no tumour and patients with tumour were calculated assuming whole body retention (percentage) was given by the expression '100 - urinary excretion (percentage)'. This may not be absolutely true since traces of radioactivity in the bile of rabbits injected with $^{99m}$Tc$^{m}$V)DMSA have been demonstrated [2, 4] (but these were insignificant when compared with the urinary route) and similar mechanisms may operate in man. Therefore errors which occurred in the urinary excretion results are reflected in the whole body retention results.

Initially, there appeared a significant difference in the whole body retention between the two groups. However one of the patients with no tumour (patient 2), who had collected his own urine overnight, had the shortest cumulative urine excretion half-time (70min) and a whole body retention value which was radically different from any of the other patients. It is probable that this difference is due to a collection error by the patient. Excluding this whole body retention value from the rest of the results, there was no significant difference between the two groups. This is to be expected based on the absence of tumour uptake together with previous blood and urine data. The cumulative urine excretion and whole body retention results in this study are different to those quoted previously [2,3]. However, in this earlier work, the mean urine cumulative half-time values were obtained graphically with
urine collected only up to 6 h, with no distinction made between patients with and without tumours. Therefore, the quoted results in this paper must be considered more accurate.

The disappointing lack of uptake of $^{99}$Tc$^{m}$(V)DMSA in human tumours (mean tumour: blood ratio 1.7:1; 1.1:1 in rabbits with SCC [4]) probably reflects the non-specific nature of the $^{99}$Tc$^{m}$(V)DMSA for squamous cell carcinoma and the poor vascularity of the tumours. The wide ranges in the tumour: blood and tumour: muscle ratios reflect the different natures of the tumours since each tumour has its own intrinsic biological behaviour. Narrower ranges with similar ratios for each of the tumours would have been surprising. Although the tumour: blood ratios for the laryngeal, tongue and maxilla tumours indicated very little uptake of radioactivity, the two patients with hypopharyngeal tumours were biopsied approximately 4 h post-injection. The former tumour was moderately differentiated while the latter was well differentiated squamous carcinoma. The reason for this difference in the ratios between the hypopharyngeal tumours and those in the laryngeal, tongue and maxilla tumours is unclear.

The mechanism of uptake for $^{99}$Tc$^{m}$(V)DMSA is poorly understood although it had been suggested it is due, in part, to the similarity of the TcO$_{5}^{3-}$ pentavalent core to the phosphate molecule which is avidly taken up by some tumour cells [8]. However, this cannot be the only mode of uptake since bony accumulation would be more prominent than is currently seen with $^{99}$Tc$^{m}$(V)DMSA and human imaging, although high bone uptake has been demonstrated in both rodents and rabbits [4], which are species characterized by incomplete bone maturation.

Like all dosimetric calculations, a number of distinct assumptions had to be made in this study because of the incomplete and sparse biological results which were obtained by combining animal and human data. Human clearance from the kidney and bladder was derived from results which made the assumption that whole body retention is '100 − urinary excretion (percentage)', a statement which may not be entirely true since biliary excretion of radioactivity does occur in rabbits [4]. The cumulative activity in the human kidney was estimated from rabbit biodistribution data [4], corrections being made for both kidney mass and minimal bony uptake in humans. Errors in estimating the bone mass of a rabbit are inevitable and the bone mass of a NZW rabbit (137 g kg$^{-1}$ body weight) estimated in this study is less than previously reported ([2], 171 g kg$^{-1}$), although in this earlier work the carcass of the rabbit was probably removed prior to complete removal of flesh by the African flesh-eating beetles. In this study, complete removal of flesh was achieved and, as such, the figure of 13.7% of body weight for rabbit bone mass must be considered more accurate and one which is similar, not only to the expected value in rabbits (10–11% body weight, [9]), but also to that observed in humans (10% body weight, [10]).

The human pharmacokinetic, rabbit biodistribution and bone mass results used for the dosimetry calculations in this study are, in theory, more accurate than those used previously [2,3] which resulted in estimations of the effective dose equivalent of $^{99}$Tc$^{m}$(V)DMSA of 14 and 8.2 $\mu$Sv/MBq respectively. However, the pharmacokinetic
data used by Clarke et al. [3] was derived from a mixture of patients with and without tumours, no urine was collected after 6 h post-injection, and the kidney was not used as a source organ. Watkinson [2] used a compartmental model similar to that used in this study to calculate the effective dose equivalent in man. However, he used the same pharmacokinetic data used by Clarke et al. [3] to estimate cumulative blood-pool activity and whole body retention. In addition, the rabbit kidney biodistribution results represented mean data from two rabbits and the rabbit bone mass was less accurate since all the flesh on the carcass was not completely removed.

Therefore, the value of 5.1 \( \mu \text{Sv/MBq} \) may be a more valid estimation of the effective dose equivalent for \(^{99}\text{Tc}^{m}(V)\text{DMSA}\) in man and, as such, it is less than those figures quoted for other technetium labelled compounds \(^{99}\text{Tc}^{m}\text{DTPA}, 10 \mu \text{Sv/MBq}; \) \(^{99}\text{Tc}^{m}(\text{III})\text{DMSA}, 12.5 \mu \text{Sv/MBq}; [11])\). This may reflect either a true lower radiation dose or an underestimation resulting from the assumptions previously described. Despite this, an adult dose of \(^{99}\text{Tc}^{m}(V)\text{DMSA} (370\text{MBq})\) would result in an effective dose equivalent of 1.9 mSv per scan which is still considerably less than the effective dose equivalent for \(^{67}\text{Ga} (18\text{mSv/150MBq}, [11]).\)

**Conclusion**

This study has confirmed that \(^{99}\text{Tc}^{m}(V)\text{DMSA}\) has a fast bi-exponential blood clearance and cumulative urine excretion in humans and that there was no significant difference in clearance times between non-tumour and tumour patients.

Overall, there was little evidence of active tumour uptake of \(^{99}\text{Tc}^{m}(V)\text{DMSA}\) (mean tumour:blood ratio = 1.7:1) and this reflects the non-specific nature of the radiopharmaceutical for SCC and the poor vascularity of the tumours.

The effective dose equivalent for \(^{99}\text{Tc}^{m}(V)\text{DMSA}\) is 5.1 \( \mu \text{Sv/MBq} \).
Appendix

A compartmental model was constructed (Figs 4a and b) using the whole body (WB), kidney and bladder as source organs. Data from six patients was used. The whole body cumulative activity (A) was calculated using the human whole body retention results (WB = 100 − Σ% (injected dose excreted), Fig. 2a). Kidney A (as a fraction of the injected dose) was estimated using the rabbit kidney biodistribution data ([4] and Fig. 5) correcting for bone uptake and kidney mass in rabbits (assuming minimal bone uptake in humans). The bone mass of a NZW rabbit carcass was estimated by allowing African flesh-eating beetles (*Domesticus lardius*) to devour the flesh on the carcass and the bones then weighed dry 3 weeks later.

Estimation of kidney uptake assumes all radioactivity detected was due to initial

\[
% \text{injected dose in kidney (Rabbit, 0.92%, (4))}
\]

Correct for mass of human kidney

\[
\frac{0.92 \times 4.4}{5.2} = 0.78\%
\]

And for rabbit bone uptake

\[
\frac{0.78}{100-28} \times 100 = 1.08\%
\]

\* RABBIT KIDNEY = 5.2g/kg Body weight (4)

\* HUMAN KIDNEY = 4.4g/kg body weight (10)

Fig. 4 (a) Compartmental dosimetry model.
uptake with no washout occurring during the study. Bladder $\tilde{A}$ was calculated from the area under a plot of the radioactivity in the bladder (expressed as a fraction of the injected dose) against time (Fig. 5).

The cumulative activities for whole body, kidney and bladder are shown in Fig. 5. Using the 'S' values for $^{99}$Tc for whole body, kidney and bladder from the MIRD Tables [12] the absorbed dose [D (i.e. $\tilde{A}$S)] for each of the organs in Fig. 6 was calculated.

**KIDNEY**

\[
\tilde{A}_{\text{Kidney}} = \int_{0}^{\infty} (Ce^{-\lambda_{\text{Tc}}t}) \, dt = \frac{C}{\lambda_{\text{Tc}}} \tag{C=1.08\% \text{ (Figure 1A)}}
\]

\[
\tilde{A}_{\text{Kidney}} = 0.0108 \frac{0.415}{0.415} = 0.094 \text{ hrs}
\]

**BLADDER**

$\tilde{A}_{\text{bladder}} = \text{Area under urine excretion plot}$

Assumes complete bladder emptying and linear rise in radioactivity from one bladder emptying to another

% injected dose in the bladder

\[
\begin{array}{c|c}
\text{patient} & \text{Bladder } \tilde{A} \text{ (hrs)} \\
4 & 0.260 \\
5 & 0.848 \\
7 & 0.905 \\
8 & 0.345 \\
9 & 0.365 \\
10 & 0.521 \\
\end{array}
\]

Fig. 4 (b) Compartmental dosimetry model.
**CUMULATIVE ACTIVITIES (\(\tilde{A}\)) IN SOURCE ORGANS**

**WHOLE BODY (WB)**

\[
A_{WB} = \left( e^{-\lambda_A t} + \frac{e^{-\lambda_B t}}{\lambda_B / \lambda_Tc} \right) e^{-\lambda_Tc t}
\]

\[
\tilde{A}_{WB} = \int_{0}^{\infty} A \text{ blood dt} = \left[ \frac{A}{\lambda_A / \lambda_Tc} + \frac{B}{\lambda_B / \lambda_Tc} \right] e^{-(\lambda_A + \lambda_Tc) t}
\]

\[
\tilde{A}_{WB} = \left[ \frac{A}{(\lambda_A + \lambda_Tc) / \lambda_A} + \frac{B}{(\lambda_B + \lambda_Tc) / \lambda_B} \right] \text{ hrs}
\]

\(\lambda = \text{ Decay Constant}\)

\[
\lambda = 0.693 \frac{\lambda_Tc}{1.155 \text{ hr}^{-1}}
\]

The curves produced by Log Linear plots of whole body retention vs time were fitted to monoexponential (patients 4, 5 and 9) or biexponential (patients 7, 8 and 10) terms. Similar plots for patients 1, 2, and 3 appeared to indicate incomplete urine collection, and more than 100% excretion in patient 6, and were therefore excluded.

**WHOLE BODY RETENTION CURVES** i.e., **WHOLE BODY (1) =**

\[
A_{WB} = e^{-\lambda_A t} + B e^{-\lambda_B t}
\]

**PATIENT NUMBER**

<table>
<thead>
<tr>
<th>Patient</th>
<th>WB (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Incomplete urine Collection</td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>A = 85.9%, (\lambda_A = 1.157 \times 10^{-3})</td>
</tr>
<tr>
<td>5</td>
<td>A = 99.0%, (\lambda_A = 2.488 \times 10^{-3})</td>
</tr>
<tr>
<td>6</td>
<td>More than 100% excretion</td>
</tr>
<tr>
<td>7</td>
<td>A = 62%, (\lambda_A = 3.379 \times 10^{-3}), B = 29.9%, (\lambda_B = 5.479 \times 10^{-4})</td>
</tr>
<tr>
<td>8</td>
<td>A = 62%, (\lambda_A = 3.229 \times 10^{-3}), B = 35.3%, (\lambda_B = 1.097 \times 10^{-3})</td>
</tr>
<tr>
<td>9</td>
<td>A = 108.1%, (\lambda_A = 1.918 \times 10^{-3})</td>
</tr>
<tr>
<td>10</td>
<td>A = 61.2%, (\lambda_A = 3.235 \times 10^{-3}), B = 33.3%, (\lambda_B = 3.679 \times 10^{-4})</td>
</tr>
</tbody>
</table>

**Fig. 5. Cumulative activities in source organs.**

The effective dose equivalent was then calculated (Fig. 6) from the sum of the products of the weighting factors \(W_T\) for each target organ and the absorbed dose \(\tilde{A}\) to that target organ from each of the three source organs i.e. effective dose equivalent =

\[
(W_{\text{Target organ}} \times S_{WB} \rightarrow \text{Target organ}) \tilde{A}_{WB}
\]

\[
+(W_{\text{Target organ}} \times S_{\text{Bladder}} \rightarrow \text{Target organ}) \tilde{A}_{\text{Bladder}}
\]

\[
+(W_{\text{Target organ}} \times S_{\text{kidney}} \rightarrow \text{Target organ}) \tilde{A}_{\text{kidney}}
\]
DOSIMETRIC CALCULATIONS IN SIX PATIENTS using The MIRD* S* values, weighting factors (W_T) for the relevant Target organs and the Whole Body, Bladder and kidney as Source organs.

<table>
<thead>
<tr>
<th>MIRD * S* Factors rads / μ Ci hrs</th>
<th>W_T</th>
<th>Whole Body</th>
<th>Bladder</th>
<th>kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>GONAD</td>
<td>0.25</td>
<td>2.4 E - 6</td>
<td>7.3 E - 6</td>
<td>1.1 E - 6</td>
</tr>
<tr>
<td>BREAST</td>
<td>0.15</td>
<td>2.0 E - 6</td>
<td>2.4 E - 8</td>
<td>8.5 E - 7</td>
</tr>
<tr>
<td>LUNG</td>
<td>0.12</td>
<td>2.0 E - 6</td>
<td>2.4 E - 8</td>
<td>8.5 E - 7</td>
</tr>
<tr>
<td>MARROW</td>
<td>0.12</td>
<td>2.9 E - 6</td>
<td>2.2 E - 6</td>
<td>3.8 E - 6</td>
</tr>
<tr>
<td>THYROID</td>
<td>0.03</td>
<td>1.5 E - 6</td>
<td>2.1 E - 9</td>
<td>4.8 E - 8</td>
</tr>
<tr>
<td>BONE</td>
<td>0.03</td>
<td>2.5 E - 6</td>
<td>9.2 E - 7</td>
<td>1.4 E - 6</td>
</tr>
<tr>
<td>BLADDER</td>
<td>0.06</td>
<td>2.3 E - 6</td>
<td>1.6 E - 4</td>
<td>2.8 E - 7</td>
</tr>
<tr>
<td>KIDNEY</td>
<td>0.06</td>
<td>2.2 E - 6</td>
<td>2.6 E - 7</td>
<td>1.9 E - 4</td>
</tr>
<tr>
<td>UTERUS</td>
<td>0.06</td>
<td>2.6 E - 6</td>
<td>1.6 E - 5</td>
<td>9.4 E - 7</td>
</tr>
<tr>
<td>LARGE INTESTINE</td>
<td>0.06</td>
<td>2.3 E - 6</td>
<td>7.4 E - 6</td>
<td>7.2 E - 7</td>
</tr>
<tr>
<td>SMALL INTESTINE</td>
<td>0.06</td>
<td>2.5 E - 6</td>
<td>3.0 E - 6</td>
<td>2.9 E - 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.322 E - 6</td>
<td>13.323 E - 6</td>
<td>12.694 E - 6</td>
</tr>
</tbody>
</table>

\( \sum W_T S_T \)

\[ \mu \text{Sv}/\text{MBq hrs} = 2.7 \times 10^5 \times \frac{1 \text{ rad}}{\mu \text{ Ci hrs}} \]

\[
\text{Whole Body } \sum W_T S_T = 6.276 \times 10^{-1} \mu \text{Sv}/\text{MBq hrs} \\
\text{Bladder } \sum W_T S_T = 3.6008 \mu \text{Sv}/\text{MBq hrs} \\
\text{Kidney } \sum W_T S_T = 3.4308 \mu \text{Sv}/\text{MBq hrs} \\

\text{Effective Dose equivalent} = \left\{ \begin{array}{l}
\text{Whole Body Activity + 3.6008 Bladder Activity} \\
+ 3.4308 \end{array} \right\} \\
\text{(μ Sv/MBq)}

\text{Patient 4, 4.19} \\
\text{Patient 5, 5.79} \\
\text{Patient 7, 6.08} \\
\text{Patient 8, 4.66} \\
\text{Patient 9, 4.59} \\
\text{Patient 10, 4.98}

**Fig. 6.** Dosimetric calculations.

**Acknowledgements**

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Technetium-99m (v) Dimercaptosuccinic Acid Uptake in Patients with Head and Neck Squamous Carcinoma: Experience in Imaging

John C. Watkinson, Colin R. Lazarus, Raman Mistry, Omar H. Shaheen, Michael N. Maisey, and Susan E. Clarke

Departments of Nuclear Medicine and Otolaryngology, Guy's Hospital, London, UK

A recently developed imaging agent, technetium-99m (v) dimercaptosuccinic acid (99mTc (v) DMSA), has been used to assess head and neck squamous carcinoma (SCC). We have prospectively studied 62 patients of whom 53 had a histologically proven head and neck SCC. The remaining nine had benign lesions. The results of planar imaging in patients with primary disease yielded an 85% sensitivity and 78% specificity. Planar imaging in patients with cervical lymphadenopathy revealed a 59% sensitivity. Nineteen patients also had single photon emission computed tomography imaging which improved the image quality, spatial resolution and sensitivity of the investigation. Twenty-seven patients were scanned before and after radiotherapy and, of these, 96% showed positive uptake in the salivary glands with no evidence of tumor recurrence. This study has shown 99mTc (v) DMSA imaging provides a cheap and rapid method of investigating head and neck SCC and further studies are necessary to evaluate its role in the management of patients with this disease.


In 1965, Johnston, Larson, and McCurdy (1) reported the accumulation of mercury-197 chloromerodrin at sites of head and neck squamous cell carcinoma (SCC). Since then, both physicians and surgeons have been attracted by the use of radionuclide scanning techniques in head and neck SCC in an attempt to identify primary and occult tumor with cervical metastases together with residual or recurrent disease following surgery and irradiation. They have, however, been frustrated in their efforts using gallium-67 (67Ga) citrate (2-12), cobalt-57 (57Co) bleomycin (13-15), indium-111 (111In) bleomycin (16-17), and technetium-99m (99mTc) bleomycin (18-19) due to low sensitivity and specificity, considerable cost, and prolonged blood clearance which delays the scanning time up to 48 hr. Indium-111 transferrin (20), [99mTc]sodium pertechnetate (21), [99mTc]sulfur colloid (22-23), many of the radiolanthanides (24), and radiolabeled monoclonal antibodies (25-26) have also been evaluated with similar limited success.

Recently, a new imaging agent technetium-99m (v) dimercaptosuccinic acid (99mTc (v) DMSA) has been developed (27-28) with the same ligand but different characteristics to the well-established renal imaging agent 99mTc (III) DMSA and which now occupies a distinct role in the management of patients with medullary carcinoma of the thyroid (MCT) (29-31). Recent reports have described its use in the detection of head and neck tumors and, in particular, SCC and rhabdomyosarcoma (32-34). The aim of this study was to evaluate the uptake of 99mTc (v) DMSA in patients with head and neck SCC using both planar imaging and single photon emission computed tomography (SPECT) and to assess salivary gland uptake before and after radiotherapy.

MATERIALS AND METHODS

DMSA is a low molecular weight organic acid which forms the ligand for the static renal imaging agent 99mTc (III) DMSA. Under alkaline conditions with a low stannous chloride concentration, DMSA forms polymeric complexes with 99mTc to form a pentavalent core. In this study a standard DMSA kit (Amersham International plc, Buckinghamshire, UK) was employed using a modification (35) of the technique described by Ohta et al. (32). This modification method was chosen since it produces pentavalent DMSA which is identical to that produced using the technique described by Ohta et al. (32).
TABLE 1

<table>
<thead>
<tr>
<th>Primary Site</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tongue</td>
<td>9</td>
</tr>
<tr>
<td>Floor of mouth</td>
<td>4</td>
</tr>
<tr>
<td>Larynx</td>
<td>11</td>
</tr>
<tr>
<td>Hypopharynx</td>
<td>13</td>
</tr>
<tr>
<td>Ear</td>
<td>3</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>3</td>
</tr>
<tr>
<td>Others</td>
<td>8</td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>53 (80 scans)</td>
</tr>
</tbody>
</table>

The purity of the complex was analyzed by thin layer chromatography (TLC) [Merck silica gel, developed with n-butanol/acetic acid/H₂O (3:2:3)], and no free pertechnetate or other ⁹⁹mTc derivative was detected; 10 mCi (370 MBq) of the prepared radiopharmaceutical was injected intravenously and patients imaged at 2 hr using a large field-of-view gamma camera interfaced to a data processor to obtain standard planar views.

A prospective study was carried out from December 1985 to September 1987 on patients with suspected head and neck cancer referred to a "tertiary referral" specialist head and neck unit. Sixty-two patients were imaged (89 scans), age range 28-82 yr (mean 60 yr), 15 females and 47 males, and of these, 53 had a histologically proven head and neck SCC (Table 1), the remaining nine having benign lesions (Table 2). Of the 53, two patients had an occult primary, one had a second occult primary, 24 patients had cervical lymphadenopathy and of these, 22 had palpable disease. Twenty-seven patients were imaged before and after radiotherapy and, of these, four were followed up with scans at 6 mo and 1 yr.

In 19 patients, SPECT imaging was also performed using elliptical orbits (where possible) and a parzen filter 1.5 to perform the tomographic reconstructions. All scans were reviewed blind by two of the authors (SEC and JCW) without prior knowledge (where possible) of the patient's history, histology, or surgical management.

RESULTS

The results of planar imaging patients with primary disease yielded an 85% sensitivity and 78% specificity. The positive and negative predictive accuracies were 79% and 84%, respectively, (Table 3). There were 33 true positives (Fig. 1), all proven histologically, and 32 true negatives. There were six false negatives, all of whom had mucosal exophytic lesions of the tonsil (1), floor of mouth (2), and supraglottis (3), respectively, with a high surface area-to-bulk ratio which was assessed visually and at subsequent surgery. There were nine false positives, who had all had recent surgery or radiotherapy. Two patients had occult primaries which remained undetected despite ⁹⁹mTc (v) DMSA planar whole-body imaging, head and neck SPECT evaluation, and computerized axial tomography (CAT). In one patient a histologically proven occult second primary was discovered in the apex of the right lung using planar ⁹⁹mTc (v) DMSA imaging despite a normal chest radiograph.

TABLE 2

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<th>Benign Lesions</th>
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<tr>
<td>Branchial cysts</td>
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</tr>
<tr>
<td>Inflammatory neck masses</td>
<td>1</td>
</tr>
<tr>
<td>Chronic laryngitis</td>
<td>1</td>
</tr>
<tr>
<td>Squamous papilloma of the oropharynx (pre- and post-operation)</td>
<td>2</td>
</tr>
<tr>
<td>Nasopharyngeal abscess</td>
<td>1</td>
</tr>
<tr>
<td>Hypophysectomy (pre- and post-operation)</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>9 scans</td>
</tr>
</tbody>
</table>

FIGURE 1
Two-hour right lateral planar ⁹⁹mTc (v) DMSA head and neck image in a patient with a T4 squamous carcinoma of the right retromolar trigone. Uptake is seen at the primary site (A).
The results of planar imaging patients with cervical metastases yielded a 59% sensitivity and 100% specificity. Twenty-four patients were imaged and, of these, there were 13 true positives, all of whom were proven histologically, and had palpable disease with nodes measuring more than 2 cm in size. There were two true negatives, one of whom had an obstructed submandibular gland due to a floor of mouth tumor; the other had reactive lymphadenopathy secondary to oral sepsis. The 100% specificity reflects the small number of patients scanned with benign disease. There were nine false negatives with a lesion size ranging from 1.5 cm to 6 cm and of these, seven had palpable disease.

In ten patients with no tumor, the normal biodistribution at 2 hr was defined with tracer visualized in the nasal mucosa, lacrimal glands, blood pool, breast tissue, testes, kidney, and bladder. All patients with benign lesions were negative on imaging except one, who had equivocal uptake in the larynx which disappeared following removal of a squamous papilloma from his oropharynx.

In addition to planar scintigraphy, 19 patients also had SPECT imaging performed which improved not only the image quality and spatial resolution (Figs. 2 and 3) but also the sensitivity of the investigation (Fig. 4 and Table 4). The observed sensitivity for planar imaging in Table 4 is lower than that previously described for primary disease and metastatic lymphadenopathy since this table contains a subgroup with false negatives from both those groups.

In 27 patients treated primarily by radiotherapy, images were obtained in all patients before and after treatment and, of these, 26 (96%) showed positive uptake in the salivary glands with no sign of tumor recurrence (Fig. 5). All patients were followed up clinically to 1 yr and, of these, four were followed up sequentially with planar $^{99m}$Tc (v) DMSA imaging. All four showed a marked reduction in salivary gland uptake at 6 mo which had completely disappeared by 1 yr (Fig. 6).

There was 95% agreement between the authors (SEC and JCW) on reporting the scintigraphic studies. A decision on the remaining 5% was reached by joint discussion.

DISCUSSION

Although many radiopharmaceuticals have been investigated as possible tumor imaging agents in head and neck SCC, $^{67}$Ga citrate remains the one extensively evaluated. It is now 19 yr since Edwards and Hayes (36) investigated its potential as a bone scanning agent and noted its concentration in the cervical lymph nodes of a patient with Hodgkins disease. Following this early work $^{67}$Ga citrate was enthusiastically described as “tumor-seeking” and a number of early reports evaluated its uptake, not only for head and neck malignancy but
Two-hour anterior head and neck coronal SPECT $^{99m}$Tc (v) DMSA image in a patient with a laryngeal SCC. No cervical lymphadenopathy was demonstrated, either by palpation or CAT evaluation. Uptake is seen at the primary site (A) and at the site of a left cervical lymph node (B) which contained histologically proven metastatic SCC and which measured < 2 cm.

for tumors in general (37–39). However, it is expensive, images are acquired at 48 hr, and its well-recognized distribution in bone, liver, bowel, and inflammatory tissue contributes to both a low sensitivity and specificity. Such “tumor-seeking” claims have since been disregarded and although its current clinical use in tumor imaging is confined to the evaluation of lymphoma, bronchial carcinoma, hepatoma, and seminoma (40–41), reports continue to be published describing its value in the investigation of patients with head and neck SCC (12). Technetium-$^{99m}$ (v) DMSA is a new imaging agent which has been used to evaluate head and neck malignancy and, in particular, SCC. It is as sensitive and more specific than $^{67}$Ga citrate (28) and has the distinct advantage that patients can be imaged 2 hr after injection. We have shown that $^{99m}$Tc (v) DMSA is undoubtedly taken up at the sites of head and neck SCC. The sensitivity of the investigation correlates well with other series (28) but the high specificity for primary and secondary cervical disease reflects the small number of patients scanned with benign conditions. The 100% specificity for planar imaging cervical nodes reflects the highly selective nature of the group. Patients were only imaged prior to treatment and that following surgery and/or radiotherapy, the false-positive rate would have been increased. Patients with head and neck cancer have a high incidence of inflammatory neck nodes and the high specificity in this group suggests $^{99m}$Tc (v) DMSA is not avidly accumulated by inflammatory tissue. Technetium-$^{99m}$ (v) DMSA is not 100% sensitive for head and neck SCC and uptake has been reported in inflammatory masses, soft-tissue, and benign tumors (42), together with MCT (28–31).

Using planar imaging in patients with primary disease there were six false negatives, all of whom had superficial mucosal exophytic lesions with a large surface area-to-bulk ratio and this may well be an important factor in controlling $^{99m}$Tc (v) DMSA uptake. In this series, the sensitivity increased to 100% for solid infil-
Two-hour anterior planar head and neck $^{99m}$Tc (v) DMSA image in the same patient as in Figure 5, performed 1 yr later. Uptake is seen at the site of clinically recurrent SCC (A). The previously noted accumulation of $^{99m}$Tc (v) DMSA within the submandibular salivary glands has now disappeared.

FIGURE 6

There were nine false positives, all of whom had recently undergone surgery and radiotherapy and both these modalities can modify the uptake of radiopharmaceuticals into human tissue (5,43). Of those patients with benign disease, there was one false positive who had a squamous papilloma of the anterior faucial pilliar. There was unexplained equivocal uptake in the larynx which was not present on the postoperative scan following surgical removal of the lesion. The explanation for this is unclear although uptake into the thyroid cartilage is recognized with $^{111}$In-bleomycin (15) and many metal chelates are taken up into immature bone (44) which may be present in varying amounts in the adult larynx during the normal process of ossification that occurs with aging. Similar mechanisms may affect $^{99m}$Tc (v) DMSA uptake. The increased sensitivity with SPECT is encouraging. Of the four patients imaged that were converted from false negatives to true positives, two had mucosal exophytic lesions of the supraglottic larynx and two had cervical lymphadenopathy. While the increased sensitivity for primary lesions may be of interest, it is of little value to the surgeon since most head and neck SCCs can be diagnosed under direct or indirect vision with the naked eye.

Using $^{99m}$Tc (v) DMSA planar scanning, no lymph nodes were detected that measured < 2 cm in size. One patient, however, had a laryngeal tumor with no detectable lymphadenopathy on clinical examination, CAT, or planar $^{99m}$Tc (v) DMSA imaging. SPECT imaging showed uptake in the primary tumor and also at the site of a cervical lymph node (Fig. 4), which was confirmed at operation, and one node measuring < 2 cm in size was subsequently found to contain metastatic tumor on histologic examination. Other workers have confirmed increased sensitivity, image quality, and spatial resolution with SPECT (28,32,45). These results are encouraging since an early criticism of both $^{67}$Ga-citrate and $^{57}$Co-bleomycin planar imaging of cervical nodes was their inability to detect lesions < 2 cm in size by which time they were usually clinically palpable (15).

In the management of head and neck SCC the most important prognostic factor at the time of initial presentation is the presence or absence, level, and size of metastatic cervical lymphadenopathy (46-47). There is a large observer error when palpating the neck (48) and although CAT scanning has added a new dimension to its evaluation, it is expensive, nodes detected < 1.5 cm in size are regarded as clinically nonsignificant, and groupings of three or more 8-15 mm contiguous nodes contribute to a possible source of false-positive results (49).

Of those patients with primary disease, all the false positives had had previous surgery or radiotherapy. While further work is underway to assess the effect these modalities have on $^{99m}$Tc (v) DMSA uptake, these preliminary results suggest SPECT imaging may be of value in the pre-operative evaluation of patients with the N0 neck (no palpable lymphadenopathy). This would be an important contribution since should tumor upstaging take place it would directly affect the way patients are initially treated and, ultimately, their prognosis.

Uptake in the salivary glands of $^{67}$Ga-citrate following radiotherapy is well recognized and is thought to be a result of interstitial edema, perivascular inflammation, and subsequent interstitial fibrosis (30). We have confirmed uptake of $^{99m}$Tc (v) DMSA in the salivary glands of 96% of patients following radiotherapy, a finding not observed, to our knowledge, by other workers (28,32). In four patients followed up to 12 mo, the uptake gradually subsided in a similar manner to that observed with $^{67}$Ga-citrate (49) and similar mechanisms probably operate with both radiopharmaceuticals to explain this phenomenon. Uptake of $^{99m}$Tc (v) DMSA in the normal breast and nasal mucosa has been confirmed by others (45), and $^{99m}$Tc (v) DMSA is currently being evaluated as an imaging agent in breast carcinoma (30). The inclusion of the nasal mucosa in the normal biodistribution of $^{99m}$Tc (v) DMSA reflects, in part, the rich blood supply which this organ receives and, because of this, it is included in the biodistribution of other radiopharmaceuticals such as $^{67}$Ga-citrate (2-5). This uptake of $^{99m}$Tc (v) DMSA in the region of the nasal mucosa in patients receiving radiotherapy, however, is less than that occurring following surgery (43).
nasopharynx and paranasal sinuses is well recognized (45) and may lead to reduced sensitivity and specificity necessitating caution in the interpretation of images of this area.

The biologic tumor uptake of $^{99m}$Tc (v) DMSA varies from moderate to intense. The uptake mechanism is poorly understood although it has been suggested that it is partly a result of the similarity of the TcO$_4$ pentavalent core to the phosphate molecule which is avidly taken up by some tumor cells (28). This cannot be the only mode of uptake, however, since bony accumulation would be more prominent than is currently seen with $^{99m}$Tc (v) DMSA, although high bone uptake has been demonstrated in both rodents and rabbits, species characterized by incomplete bone maturation (27). Further work is underway to investigate the uptake mechanism in SCC using cellular subtraction techniques in an animal model, and also to assess the uptake in inflammatory tissue which surrounds tumor cells as well as other factors such as radiotherapy and surgery, which are both known to modify radiopharmaceutical behavior.

CONCLUSION

Technetium-99m (v) DMSA has many attractive advantages as a tumor imaging agent. It is ideally suited for imaging with the gamma camera, a cheap radiopharmaceutical with an apparent high sensitivity and specificity, minimal patient irradiation (30), and is ideally suited for SPECT. Monoclonal antibodies offer a potential answer to SCC tumor imaging (25–26) but there are, however, many theoretic and practical problems associated with their use. Technetium-99m (v) DMSA provides a rapid method of investigating head and neck SCC. Further studies are underway to evaluate its role in the detection of the occult primary and cervical metastases together with late recurrence following surgery and irradiation. SPECT imaging using $^{99m}$Tc (v) DMSA may be of value in the assessment of the N$_0$ neck, but follow-up scans in patients who have had radiotherapy should be interpreted with caution.

ACKNOWLEDGMENTS

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Technetium—99m(v) Dimercaptosuccinic acid planar scintigraphy in head and neck cancer: Clinical, scintigraphic and radiological study


Abstract
Technetium-99m (Tc⁹⁹m)(v) Dimercaptosuccinic Acid (DMSA) is an imaging agent which has been proposed as a scintigraphic marker for head and neck squamous cell carcinoma. Fifty-four patients were studied of whom 51 had a head and neck tumour. All patients were examined and then imaged using Tc⁹⁹m(v) DMSA scintigraphy and computerized tomography.

Scintigraphy was less sensitive than clinical examination in the detection of patients with cancer, patients with primary tumours and patients with metastatic neck disease. CT was as sensitive and as accurate as clinical examination but more sensitive than Tc⁹⁹m(v) DMSA in detecting patients with cancer and with primary tumours. CT was more sensitive and more accurate than both clinical examination and Tc⁹⁹m(v) DMSA scintigraphy in predicting which patients had metastatic neck disease.

Although Tc⁹⁹m(v) DMSA is accumulated by squamous cell carcinoma, its inability to detect low volume disease and apparent low specificity means it has no role to play in the management of patients with head and neck squamous cell carcinoma.

Introduction
It is now twenty years since Edwards and Hayes (1969) investigated the potential of Gallium-67 (Ga⁶⁷) citrate as a bone scanning agent and noted its concentration in the cervical lymph nodes of a patient with Hodgkin's disease. Since then, both physicians and surgeons have been attracted by the use of radionuclide imaging techniques in head and neck squamous cell carcinoma (SCC) in an attempt to identify primary and occult primary tumours with cervical metastases together with residual or recurrent disease following surgery and irradiation.

They have, however, been frustrated in their efforts using not only Ga⁶⁷-citrate (Teates et al., 1980) but also Cobalt-57 bleomycin (Cummings et al., 1981), Tc⁹⁹m sulphur colloid (Blakeslee et al., 1985) and radiolabelled monoclonal antibodies (Soo et al., 1987).

Tc⁹⁹m(v) DMSA is a new imaging agent which now occupies a distinct role in the management of patients with medullary carcinoma of the thyroid (Clarke et al., 1988). Recent reports have described its use in evaluating other head and neck tumours to include SCC (Ohta et al., 1988; Watkinson et al., 1989a).

Tc⁹⁹m(v) DMSA is a new imaging agent which now occupies a distinct role in the management of patients with medullary carcinoma of the thyroid (Clarke et al., 1988). Recent reports have described its use in evaluating other head and neck tumours to include SCC (Ohta et al., 1988; Watkinson et al., 1989a).

The aim of this study was to evaluate the uptake of Tc⁹⁹m(v) DMSA in patients with head and neck SCC and compare sensitivity and accuracy with clinical examination and computerized tomography (CT).

Materials and methods
This study was conducted as part of a larger investigation to evaluate the role of Tc⁹⁹m(v) DMSA in the management of patients with head and neck cancer (Watkinson et al., 1989b).

Fifty-four patients (37 male, 17 female; age range 19–82 years, mean 59) were studied prospectively. Fifty-one patients had head and neck tumours and of these 47 had squamous cell carcinoma. One patient had a second lung primary, one had a synchronous second lung primary and three had benign lesions (glomus jugulare, glomus tympanicum and an infected branchial cyst). The primary tumour sites are shown in Table I. In 42 patients (43 tumours), primary tumour staging was possible and there were 2 T₁, 10 T₂, 13 T₃ and 18 T₄ tumours. Of those patients with nodal metastases, there were 12 N₁, 1 N₂a, 15 N₂b and 3 N₃ lateral compartment necks. Patients were then imaged within two weeks using Tc⁹⁹m(v) DMSA planar scintigraphy and computerized tomography.

Tc⁹⁹m(v) DMSA was prepared using an 'in-house' method (Sampson, 1987) and 370MBq (10mCi) was injected intravenously. Patients were imaged at the optimal imaging time, i.e. between 2 and 4 hours (Watkinson et al., 1989c). Anterior, left and right head and neck planar images were acquired in all patients. Accumulation of radioactivity was described as positive or negative and each side of the neck (lateral neck compartment) was reported separately.

The CT scans were performed using a Philips Tomoscan 350 third generation scanner. All patients (except...
one) received intravenous contrast (Iopamidol 370) and 6 mm contiguous sections of the neck were obtained. Of the 54 patients, 52 had head and/or neck scans. Two patients had chest CT alone. Each side of the neck was the 54 patients, 52 had head and/or neck scans. Two

<table>
<thead>
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The results for clinical examination, Tc<sup>99m</sup>(v) DMSA planar scintigraphy and CT in the overall detection of patients with cancer, in the detection of primary tumours and the detection of lateral neck compartments with cervical metastases are shown in Figures 1, 2, 3.

For clinical examination, two patients were thought to have cancer who were subsequently shown to be disease-free. Both patients had received radiotherapy to tumours of the tongue and larynx respectively. There were five patients with clinically occult primaries and metastatic neck disease. Of these, three were identified as having a nasopharyngeal primary on CT and, in addition, one was also identified by scintigraphy.

For identifying patients with metastatic neck disease, there were 3 false positives (13 per cent) and 4 false negatives (14 per cent). Each lateral neck compartment was reported as a separate site. For the 108 lateral neck compartments, clinical examination yielded four false positives (14 per cent) and 9 false negatives (11 per cent).

For Tc<sup>99m</sup>(v) DMSA planar imaging, clinical examination was not only a more efficient method of detecting which patients had cancer, but also in detecting both primary tumours and lateral neck compartments with metastatic disease. In those patients with no cancer, the normal biodistribution of Tc<sup>99m</sup>(v) DMSA was confirmed with radioactivity observed in the lacrimal glands, nasal mucosa, blood pool, kidneys and the bladder (Watkinson et al., 1989a).

For the imaging of primary tumours (48 tumours, 12 benign lesions) with Tc<sup>99m</sup>(v) DMSA there were 35 true positives (Fig. 4), five true negatives, eight false positives and 12 false negatives. Of the eight false positive results, one patient had received radiotherapy to the larynx (also falsely positive on CT) and another had a stage IV glomus jugulare. Of the false negative studies, there were two occult primary’s (presumed head and neck), one lymphoma of the cervical oesophagus and four T<sub>1</sub> and five T<sub>2</sub> tumours. Size was an important factor for tumour detection. In those patients where ‘T’ staging was possible, approximately 75 per cent of T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> tumours were detected scintigraphically and this increased to 100 per cent for T<sub>4</sub> tumours. On the basis of scintigraphy, one occult tumour was detected but no other tumours were upstaged using current UICC criteria. Visualization of tumours was best for those tumours situated away from the nasopharynx, maxillary sinus, the floor of the mouth and for those tumours with bony involvement.

For the 108 lateral neck compartments, there were 15 true positives (Fig. 5), 7 true negatives, 17 false positives (Fig. 5) and 16 false negatives. Size was an important factor for the detection of malignant nodes or nodal masses and approximately 43 per cent of N<sub>1</sub> and N<sub>2</sub> masses were detected scintigraphically and this increased to 100 per cent for N<sub>3</sub> tumours.

There were 77 clinically N<sub>0</sub> lateral neck compartments. One was upstaged to N<sub>1</sub> (correct status N<sub>2b</sub>) while seven were incorrectly upstaged to N<sub>3</sub> and of these, two were incorrectly upstaged to N<sub>3a</sub> on CT.

The CT features of malignant spread of all the primary tumours were confirmed and no previously undescribed findings were noted (Mancuso and Hanafie, 1985). The one false positive result occurred in a patient with a T<sub>2</sub> glottic carcinoma treated with radiotherapy. Of the five true negatives, two were glomus tumours and three had primary sites (nasopharynx 1; tongue 2) previously treated by radiotherapy. All of these, but one, were negative on clinical examination.

Of those patients with false negative CT scans for a primary head and neck tumour, two had presumed occult head and neck primary’s and the other three had T<sub>2</sub> tumours of the floor of the mouth, buccal mucosa and lateral border of the tongue that had been detected clinically. Four patients had had previous successful radiotherapy to primary sites, two had ear tumours and one patient had a lymphoma of the cervical oesophagus. In the remaining 42 patients (43 tumours) there were 2 T<sub>1</sub>, 10 T<sub>2</sub>, 13 T<sub>3</sub> and 18 T<sub>4</sub> tumours. In these 43 tumours, CT primary tumour staging correlated with the clinical findings in 21, and with the clinical and pathological findings in 11 (Fig. 6). In the remaining 11 tumours, three patients with clinically occult nasopharyngeal primary’s were staged T2(1) and T3(2). Based on subsequent path-

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There were 71 clinically N₀ lateral neck compartments and, of these, five were upstaged by CT. One patient was correctly upstaged to N₁ while another was correctly upstaged from N₀ to N₁ (i.e. 2N₀ → N₁). The other two patients were false positives. One had a floor of mouth tumour with a clinically benign obstructed submandibular gland while the other had an infected branchial cyst (Fig. 7).

There were two other false positive results. Both these patients had palpable cervical lymphadenopathy (clinically N₁) and both had positive nodes by CT criteria. Both subsequently had neck dissections which were pathologically N₀.

There were six false negative lateral neck compartments (4 N₁; 2 N₂). Of these, five were operated on immediately after CT scanning while the sixth had a second neck dissection nine months later.

Three clinically positive lateral neck compartments were downstaged by CT. One was downstaged from N₁ → N₀ and one from N₁ → N₂. The last patient was clinically N₂ (i.e. N₂; N₁) and was downstaged to N₀; N₁ by CT. He subsequently died of primary disease with metastatic disease in the left neck with no clinical evidence of disease in the right neck.

**Discussion**

Any new tumour imaging modality to evaluate head and neck cancer must be judged against clinical examination and CT. Since CT provides anatomical information, direct comparisons with scintigraphic physiological imaging are not entirely valid although certain comparisons may be justified if the information required is similar and if Tc⁹⁹m (v) DMSA planar scintigraphy is to have any role in the management of patients with head and neck squamous cell carcinoma.

The results for clinical examination highlight the well recognized difficulty associated with detecting and accu-
rately staging tumours of the nasopharynx and maxillary sinus. In addition, there is a well recognized error in neck palpation with an incidence of false positives of 19 per cent (range 4.45 per cent) and, for false negatives, an incidence of 29 per cent (range 4.60 per cent) (Watkinson et al., 1989d). The results of this study illustrate this problem with incidences of 14 per cent (false positives) and 11 per cent (false negatives) respectively for the palpation of neck disease.

This study has shown Tc\textsubscript{99m}(v) DMSA is taken up at sites of head and neck cancer and, in particular, SCC. However, scintigraphy provided little anatomical information when compared to clinical examination and CT, and was inferior to both these investigations in detecting which patients had cancer. Only one occult primary tumour was identified by scintigraphy and since no upstaging of a clinically apparent primary tumour occurred, Tc\textsubscript{99m}(v) DMSA planar scintigraphy (like...
Ga\(^{67}\)) has no role to play in the routine evaluation of patients with a primary or occult primary head and neck cancer.

For imaging primary tumours, size was an important factor in detection and visualization was best for those tumours situated away from the nasopharynx, maxillary sinus, the floor of the mouth and for those tumours with any bony involvement.

The results for evaluating both patients with cancer and primary tumours show CT to be as sensitive and as accurate as clinical examination but more sensitive and accurate than Tc\(^{99m}\) (v) DMSA scintigraphy. CT was of particular value in assessing primary tumours of the nasopharynx and maxillary sinus, tongue base and the oesophagus. Although CT correctly upstaged four primary tumours, it understaged a further four located in the floor of the mouth, tonsil and hypopharynx (2) respectively. This highlights the difficulty CT has in detecting bony and cartilaginous involvement and underlines the fact that primary tumour CT prior to treatment can only be justified if the result will radically alter treatment since, in these eight patients, treatment strategy would have been the same regardless of the CT result.

If one of the biggest prognostic factors in head and neck SCC is the presence or absence, level and size of metastatic cervical lymphadenopathy, then an imaging agent or modality capable of detecting clinically occult neck disease would facilitate more accurate staging of the neck at presentation. The results for Tc\(^{99m}\) (v) DMSA...
planar scintigraphy show that it is less sensitive and accurate than clinical examination in detecting which lateral neck compartments contained cancer. Only one clinically N0 lateral neck compartment was correctly upstaged and there was an unacceptable false negative and false positive rate for scintigraphy when compared with palpation. These results show that Te99m(v) DMSA planar scintigraphy has no role to play in the evaluation of metastatic neck carcinoma (to include the clinically N0 neck). Although every patient did not have a neck dissection, a clinical, scintigraphic, radiological and pathological study on those who did confirm these findings (Watkinson et al., 1989e).

CT has been claimed to be of value in the staging of neck nodes, being more accurate than clinical examination (Friedman et al., 1984; Stevens et al., 1985). It is also suggested that clinically normal necks with abnormal CT findings should be staged N+ positive and that the inclusion of CT should be mandatory in any tumour staging system (Som, 1987). In the past, CT has been expected to correctly increase the stage of neck disease (in the untreated neck) from N0 to N1, in approximately 5 per cent of patients although further work suggests figures of 20-30 per cent may be possible (Friedman et al., 1984; Stevens et al., 1985).

The results in this study show that CT is more sensitive but as accurate in predicting which lateral neck compartment contained cancer and similar findings have been reported by others (Feinmesser et al., 1987). It seems reasonable to suggest that neck CT may be of value if the neck is being scanned as part of primary tumour evaluation, if there is a significant incidence (>25 per cent) of occult ipsilateral or bilateral nodal disease, in the presence of an ipsilateral N2 or N3 neck and lastly in those patients who are difficult to examine or who require neck restaging.

The question whether or not to CT the clinically N0 neck remains controversial. In this study, 4 per cent of lateral neck compartments were upstaged by CT (6 per cent in the subgroup who had neck dissections (Watkinson et al., 1989e). It seems the same arguments that have been applied again elective neck dissection could be directed against elective neck CT, i.e. if only approximately 5 per cent of clinically N0 necks were correctly upstaged by CT (and if false positive results are inevitable in the presence of inflammatory neck nodes), then CT would play no role in the overall evaluation of the clinically N0 neck. Treatment should be based on a sound understanding of the natural history of the disease process in question. Surely it is cheaper and as effective to adopt a policy of 'wait and see' and perform five neck dissections rather than 100 CT scans, particularly since CT false positives may increase the number of patients having unnecessary surgery, false negatives are inevitable and the natural history of the tomographically positive node is not known. Although CT correctly downstaged 3 clinically positive lateral neck compartments this did not affect subsequent management decisions since although one patient was correctly downstaged from N2 to N0—Would not most head and neck surgeons operate on a patient with a clinically positive node and a negative CT scan?

Conclusions

This study has confirmed that Te99m(v) DMSA is accumulated at sites of known head and neck SCC but with sensitivity (75 per cent) and specificity (39 per cent) rates for the detection of primary tumours which are lower than those observed by others (Ohta et al., 1988; approximately 80 per cent respectively). In addition, its inability to detect low volume disease in the neck together with the fact that it is inferior to both clinical examination and CT means Te99m(v) DMSA planar imaging has no role to play in the management of patients with head and neck SCC.

CT was as sensitive and as accurate as clinical examination but more sensitive and accurate than Te99m(v) DMSA planar scintigraphy in predicting which patients had cancer and which patients had primary tumours. CT was of particular value in assessing and staging clinically inaccessible tumours such as those of the nasopharynx and maxillary sinus. CT was both more sensitive and more accurate than clinical examination in predicting which lateral neck compartments contained cancer. However, only 4 per cent of lateral neck compartments were correctly upstaged by CT.

In the future, the further evaluation of both non-specific and specific radiopharmaceuticals, fine resolution and 3-D CT, SPECT and Positron Emission Tomography (PET) can only lead to an overall improvement in ENT diagnostic sensitivity and specificity and ultimately improve the way we diagnose, stage and treat head and neck malignancy.

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References


Address for correspondence:
J. C. Watkinson M.Sc., F.R.C.S., Department of Otolaryngology, Guy's Hospital, St. Thomas' Street, London SE1 9RT.
The principles and practice of otolaryngology are primarily concerned with the diagnosis and treatment of diseases which affect the mucosal structures of the upper aero-digestive tract, adnexal organs such as the thyroid gland, salivary glands and cervical lymph nodes as well as the cartilaginous and bony structures of the larynx and skull. Conditions affecting these structures can often be diagnosed with ease using traditional methods of history and examination combined with conventional radiography. Recent advances in imaging techniques (computerized tomography, ultrasound and magnetic resonance imaging), increase diagnostic sensitivity and specificity but suffer from distinct disadvantages since they provide only anatomical information. The use of nuclear medicine can add a physiological dimension to diagnostic imaging within the head and neck. By using a variety of radiopharmaceuticals, the metabolic functions of a number of head and neck organs and tissues affected by a variety of disease processes can be imaged. This paper outlines the current role of nuclear medicine in otolaryngology.

Classification

Nuclear medicine tests can be classified into in-vitro and in-vivo investigations. In-vitro procedures play a small but important role in current otolaryngological practice and some of the substances which can be measured using radioimmunoassay are listed in Table 1. However, the majority of nuclear medicine tests now available to the ENT surgeon are in-vivo investigations and the organs and tissues which can be imaged are discussed below.

Thyroid

Diseases of the thyroid are common. Laboratory investigations are essential to confirm or exclude a clinical suspicion of either hyperthyroidism or hypothyroidism and a number of ‘flow channels’ exist depending which condition is suspected clinically.1 Further in-vivo investigations may be necessary to establish the clinical cause of hyperthyroidism and the most useful investigation for any goitre is a radionuclide thyroid scan with quantitative uptake using either technetium-99m (99mTc)-pertechnetate (99mTcO4-) or iodine-123 (123I-iodide)

The otolaryngologist is usually involved with the investigation of the solitary thyroid nodule and the preoperative diagnosis of malignancy, together with the post-operative assessment of residual thyroid tissue and the detection of residual or recurrent tumour. There are many clinical situations when the possibility of thyroid cancer arises but by far the commonest mode of presentation is as a palpable solitary nodule when the incidence of malignancy is 5–10%.2

The choice of agents for imaging differentiated (follicular and papillary) thyroid cancer has been reviewed elsewhere.3 With either 99mTcO4- or 123I, the majority of
nodules greater than 0.5 cm in diameter can be identified and accuracy increased by using oblique views. Smaller lesions in the isthmus may contribute to false negative results, but since they are often easier to palpate they do not constitute a significant problem. The function of all preoperative thyroid imaging is to increase the possibility of a diagnosis of malignancy by improving the predictive accuracy without any loss in sensitivity.

When a clinically solitary thyroid nodule is investigated, a $^{99m}$Tc or $^{123}$I scan may show a solitary non-functioning or hypofunctioning area (i.e. a 'cold' nodule); a functioning area (i.e. a 'hot' nodule) or a multinodular goitre, with or without retrosternal extension. The possibility of retrosternal extension should be investigated further using $^{131}$I since its high gamma photon energy makes it preferable for visualizing a retrosternal thyroid. The probability of malignancy is increased if the scan demonstrates a solitary 'cold' nodule (Figure 1), but decreased to less than 1% if it shows a 'hot' nodule or a multinodular goitre. All solitary 'cold' nodules should be investigated further by fine needle aspiration biopsy and/or ultrasound which, in expert hands, increases the diagnostic sensitivity to approximately 90%.

There are some nodules which take up tracer on the $^{99m}$Tc scan but not on an iodine scan. These discrepancies probably reflect an ability to trap but not organify iodine and such problems can usually be resolved by performing $^{123}$I scans on any

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*Table 1. Substances measured by radioimmunoassay*

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<th>Substance</th>
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<tr>
<td>$T_4$, $T_3$, $TSH$</td>
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<tr>
<td>Thyroglobulin</td>
</tr>
<tr>
<td>Parathormone</td>
</tr>
<tr>
<td>Calcitonin</td>
</tr>
<tr>
<td>Human growth hormone</td>
</tr>
<tr>
<td>Vasopressin</td>
</tr>
<tr>
<td>Hepatitis B antigen</td>
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<tr>
<td>Human immunodeficiency virus antibody</td>
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</tbody>
</table>

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*Figure 1. Anterior $^{99m}$Tc pertechnetate thyroid scan showing a solitary cold nodule in the right lobe of the thyroid. Two markers (arrowed) have been placed on the upper and lower limits of the nodule and there is a lower marker on the suprasternal notch. Histology confirmed papillary carcinoma.*
hot nodule which concentrates pertechnetate followed by a perchlorate discharge test which assesses the ability of a nodule to trap and organify iodine.

Many other radionuclides have been used to investigate the solitary thyroid nodule. Phosphorus-32, caesium-131, gallium-67 ($^{67}$Ga) citrate, $^{99m}$Tc bleomycin and thallium-201 ($^{201}$Tl) chloride have all been evaluated in an attempt to increase the diagnostic specificity without any loss in sensitivity. All these agents exhibit variable uptake in malignant lesions but at the present time, for malignancy, the false negative rate is unacceptably high for them to have a place in the routine investigation of thyroid nodules. However, patients with long-standing Hashimoto's disease sometimes develop a solitary thyroid nodule. This is usually a lymphoma and, as such, accumulates $^{67}$Ga (Figure 2).

In the postoperative assessment of differentiated thyroid cancer, radionuclide imaging techniques aim to establish the completeness of initial surgical treatment and to detect residual, recurrent or metastatic tumour. At present, the most widely used and accepted method of follow up is regular whole-body imaging using $^{131}$I combined with sequential serum thyroglobulin measurements. Differentiated thyroid malignancy exhibits minimal or no iodine uptake in the presence of normal thyroid tissue but thyroid ablation results in high serum TSH levels and subsequently $^{131}$I will localize in residual, recurrent or metastatic tumour which can then be demonstrated as hot lesions on a whole-body scan. This method permits the detection of residual and recurrent tumour in the neck together with local or distant metastases and assesses the potential for radioiodine treatment.

The use of whole-body $^{131}$I scans to detect thyroid metastases in thyroidectomized patients has distinct disadvantages. Patients have to be rendered hypothyroid by curtailing thyroxine therapy and not all differentiated thyroid cancer continues to take up $^{131}$I. Recent attempts to combat these problems have involved the use not only of $^{123}$I-anti-human thyroglobulin monoclonal antibody but also $^{201}$Tl-chloride which has been used to detect residual and recurrent disease (Figure 3), and which, in combination with $^{131}$I, has an increased sensitivity for the overall detection of metastatic disease.

Patients with medullary carcinoma of the thyroid can be imaged using a number of radiopharmaceuticals. Primary tumours appear as cold areas on $^{99m}$Tc or $^{123}$I scans with the classical pattern of bilateral symmetrical non-functioning nodules occurring in the familial type. Unlike follicular carcinoma and papillary carcinoma with
Figure 3. Anterior head and neck $^{311}$Tl scan in a patient with recurrent papillary carcinoma of the thyroid. Uptake is seen at the site of known disease (arrowed) within the neck and upper mediastinum.

Follicular elements, medullary carcinoma does not trap iodine which, therefore, plays no role in either imaging or therapy. Recently, two new radiopharmaceuticals have been developed which localize in medullary tumours. $^{131}$I-metaiodobenzylguanidine (MIBG) was developed for imaging phaeochromocytoma and has subsequently been shown to be taken up by other neuroectodermally derived tumours, including medullary carcinoma and para-gangliomata. More recently, pentavalent $^{99m}$Tc (v) dimercaptosuccinic acid (DMSA) has been developed and its uptake has been described in medullary carcinoma of the thyroid. Reports have confirmed the uptake of both $^{131}$I-MIBG and $^{99m}$Tc (v) DMSA to have distinct advantages over the $^{131}$I-MIBG. At present, the main role of pentavalent DMSA is in the investigation of primary, recurrent and metastatic medullary carcinoma (Figure 4) and $^{131}$I-MIBG scanning is reserved for use in any one individual to assess uptake and, if positive, to then use a therapeutic dose.

Parathyroid

Primary hyperparathyroidism is the commonest cause of hypercalcaemia and the introduction of the multichannel analyser for biochemical measurements has resulted in more patients being identified with elevated serum calcium levels. The accurate diagnosis of primary hyperparathyroidism is usually straightforward with precise
measurements of parathormone now possible. Preoperative localization of parathyroid adenomas is now feasible using $^{99m}$TcO$_4^{-}/^{201}$TI subtraction scanning\(^\text{18}\) (Figure 5). $^{99m}$Tc pertechnetate localizes in the thyroid gland, whereas $^{201}$TI localizes in both the thyroid and parathyroid glands. By subtracting the $^{99m}$Tc image from the $^{201}$TI image it is possible to identify enlarged parathyroids within the neck and mediastinum. The technique has an overall sensitivity of 92% for detecting parathyroid adenomas\(^\text{18}\) and is particularly useful in the assessment of abnormally situated glands or in those patients having ‘second-look’ procedures. Sensitivity is related to number, size and position\(^\text{19,20}\) and is greatest for singly involved lower pole glands which exceed 1.5 cm in size. In general, the sensitivity is poor for those patients with diffuse parathyroid hyperplasia. The uptake of $^{201}$TI is non-specific and false positives can occur with thyroid neoplasms, multinodular goitres, Hashimoto’s disease, lymphoma, sarcoidosis and parathyroid carcinoma\(^\text{20}\).

Carcinoma of the parathyroid is rare and the majority of patients present with severe hyperparathyroidism and markedly elevated serum calcium and parathormone levels\(^\text{21}\). Approximately 50% of patients have a palpable neck mass\(^\text{22}\) and non-invasive preoperative localization may be facilitated by a positive $^{67}$Ga-citrate scan\(^\text{23}\) although the diagnosis is usually made at operation. Persistently elevated serum calcium and parathormone levels should alert the surgeon to the possibility of recurrent disease, when localization should begin with cervical examination since approximately 50% of recurrences are palpable\(^\text{22}\). Recurrent tumour within the neck and mediastinum has been successfully located using $^{99m}$Tc/$^{201}$TI subtraction scanning\(^\text{23}\). However, the thyroid lobe is usually totally excised along with the parathyroid carcinoma at operation, so local recurrence can be demonstrated using $^{201}$TI chloride alone. Suspected localization should be confirmed by CT scanning\(^\text{22}\).

**Salivary glands**

CT, MRI and ultrasound all demonstrate the soft tissue structure of the salivary glands and, therefore, provide an anatomical but not a physiological assessment of the gland. Contrast sialography, with or without CT, requires cannulation of one or more salivary ducts which may be difficult and require dilation or incision of the duct orifice. This can be uncomfortable for the patient and there is often an inflammatory response consequent upon the use of contrast material.
Salivary gland scanning using $^{99m}$Tc pertechnetate provides physiological images of the glands and assesses both function and drainage.\(^{24}\) The metabolism of pertechnetate is analogous to iodide. It is trapped but not organified by the thyroid, secreted by salivary ductal epithelium and excreted in saliva.

Following i.v. injection of $^{99m}$Tc pertechnetate, rapid dynamic sequential images are obtained to demonstrate salivary gland blood flow. Serial planar images are then taken every 3 min to show the progressive symmetrical accumulation of tracer within the parotid and submandibular glands. After 15 min the patient is given a sialogogue orally which causes prompt salivation with drainage if the glands are innervated or the salivary ducts are patent. This test can be useful in the evaluation of ductal obstruction with intermittent pain or gland enlargement (Figure 6) and assesses innervation of the submandibular glands in patients with facial palsy.

The excretory function of each salivary gland as well as its relative blood flow can be assessed either visually or by using a nuclear medicine computer. Palpable lesions can be divided into vascular or non-vascular and functioning or non-functioning masses. Functioning salivary gland tumours tend to be benign with large numbers of oncocytes such as Warthin's tumours. Although malignant neoplasms are generally vascular but non-functioning on the scan, so are mixed tumours, lymphomas and abscesses. Since the $^{99m}$Tc scan cannot differentiate between benign and

Figure 5. Anterior $^{99m}$Tc/$^{201}$Tl subtraction scan in a patient with primary hyperparathyroidism. A parathyroid adenoma (arrowed) has been identified at the lower pole of the left thyroid lobe which measured approximately 2 cm in size.
malignant salivary swellings, it is of limited use in their investigation although may be of value in individual cases.

Overall salivary gland function can be useful in assessing xerostomia in patients with Sjogren’s syndrome. Gallium-67 localizes faintly in normal salivary tissue but specific focal accumulation can occur in an abscess or malignancy. Non-specific uptake in both salivary and lacrimal glands occurs in sarcoidosis and can be used both for diagnosis and to monitor therapy and disease progression.

**Lachrymal glands**

Obstruction to tear duct drainage with clinical symptoms of epiphora may be due to tumours, infection and trauma of the paranasal sinuses affecting the nasolachrymal duct. Conventional imaging with contrast dacrocystography involves intubation of the canaliculi and injection to demonstrate the tear ducts. Manipulation may be difficult or contraindicated when gross anatomical distortion or infection are present.

When $^{99m}$Tc-pertechnetate is instilled into the inferior recess of the eye, tracer spreads to label the tears and to demonstrate the drainage pathways. Sequential images using a gamma camera fitted with a pinhole collimator produce high-resolution images of the canaliculi, lachrymal sac and nasolachrymal duct. Under normal conditions, drainage into the nose is seen within 1 min. Delayed drainage with stasis is easily recognized and may persist for up to 20 min. The level of obstruction can be defined as being
proximal or distal to the nasolachrymal sac. Since this procedure is rapid and atraumatic, it provides a simple screening test for those patients requiring dacrocystography and is of particular value for pre and post-operative assessment.

Bone

Bone scanning of the head and neck provides images of altered bone physiology. Any lesion which produces an osteoblastic reaction, increased or decreased blood flow, or an increase in calcium turnover will result in altered biodistribution of the bone scanning agent, $^{99m}$Tc-methylene diphosphonate (MDP). Although anatomical resolution is poor when compared with conventional radiography, early physiological changes can be detected which may be sufficient to confirm or refute pathological bony involvement.

Following an i.v. injection of $^{99m}$Tc-MDP, static 4-h anterior, posterior, lateral and oblique projections are obtained as appropriate. Standard radiographic views ('Waters' and 'Townes') may be of value. Improved resolution is possible using either converging or pinhole collimators for smaller structures and current research suggests that diagnostic sensitivity can be increased by digitizing nuclear medicine images with conventional X-rays. In addition to the static views, rapid sequential images (anterior, 'Waters' or 'Townes') taken at 3-s intervals following injection provide a low-resolution angiogram and the activity on the scan relates to local blood flow. A 5-min picture provides a 'blood pool' image and the intensity of activity on the scan indicates the size of the vascular compartment of the lesion. Static views show the extent of any osteoblastic bone reaction but the addition of dynamic pictures can increase diagnostic sensitivity and specificity. Table 2 summarizes the findings of three-phase bone scanning in head and neck lesions.

When evaluating head and neck bone images, familiarity with normal scan appearances are essential. Increased areas of uptake in the mandible and the maxillary alveolar ridges can occur due to periodontal and periapical inflammation, recent dental extractions, multiple cementomas and malfitting dentures. Although bone scanning is extremely sensitive and will demonstrate lesions before they are visible on plain radiographs, the findings are often non-specific and uptake is seen in malignancy, benign bone cysts, osteoid osteomas, osteomyelitis, trauma, and in metabolic disorders such as Paget's disease, fibrous dysplasia and hyperparathyroidism. Increased focal uptake of $^{99m}$Tc-MDP is seen in the majority of primary head and neck bony tumours. However, these lesions are much better evaluated by other imaging modalities such as CT.

The clinical use of bone scanning using $^{99m}$Tc-MDP in head and neck malignancy has been reported to be of value in the pretreatment evaluation of bony involvement from primary carcinoma, in the diagnosis of residual and recurrent disease, and to detect bony metastases to, and from, the head and neck. However, since bone scanning is poorly specific (Table 2), bony extension within the mandible cannot be reliably distinguished from benign dental disease. Distant bony metastases from head

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Perfusion and blood pool</th>
<th>Static scan</th>
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<tbody>
<tr>
<td>Tumour (primary and secondary)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acute sinusitis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acute osteomyelitis</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Chronic osteomyelitis</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Recent fracture</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Old fracture</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Vascularized bone graft (early)</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Free bone graft (early)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Free bone graft (late)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Paget's disease</td>
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and neck carcinoma (excluding the thyroid) are uncommon and, consequently, bone scanning plays no part in either diagnosis or staging. However, metastases to the facial bones and calvarium frequently occur with carcinoma of the breast and lung and anterior and lateral skull views are recommended to exclude these lesions.30

Bone scanning within the head and neck is of value in the evaluation of facial trauma and can demonstrate fractures undetected by conventional radiography.28 It is also useful in the diagnosis and management of acute and chronic sinusitis with osteomyelitis and, using 67Ga-citrate, it is possible to demonstrate an active focus within chronic osteomyelitis and distinguish chronic sinusitis from carcinoma.31 Paget’s disease of the temporal bone producing auditory symptoms and pulsatile tinnitus can be detected with a 99mTc-MDP bone scan and photon-deficient areas further investigated with 67Ga, when positive uptake will suggest an osteogenic sarcoma.

Single photon emission computerized tomography (SPECT) demonstrates anatomy and physiology in three dimensions and can increase diagnostic sensitivity for the detection of tumours and sepsis32 and is also of value in the evaluation of temporomandibular joint dysfunction.32,33 Combined 67Ga-citrate and 99mTc-MDP scanning is more sensitive than either radiographs or CT for the early detection and follow-up of malignant otitis externa and localization is improved using SPECT.34 Both planar and SPECT 99mTc-MDP bone scans may be of value in predicting the fate of free and pedicled bone grafts.28,32

Squamous cell carcinoma

In the management of head and neck squamous carcinoma, the most important prognostic factor at the time of initial presentation is the presence or absence, level and size of metastatic cervical lymphadenopathy.35,36 There is a large observer error when palpating the neck,37 and although CT scanning has added a new dimension to the evaluation of metastatic neck disease, it is non-specific. In addition, nodes detected less than 1.5 cm in size are usually regarded as clinically non-significant and groupings of three or more 8–13 mm contiguous nodes contribute to false-positive results.38 The accumulation of mercury-197 chloromerodrin at sites of known head and neck squamous carcinoma was first reported in 1965.39 Since then, physicians and surgeons have employed a variety of radio pharmaceuticals to investigate head and neck tumours in an attempt to identify primary and occult tumour with cervical metastases together with residual or recurrent disease following surgery and irradiation. 67Ga-citrate,40 cobalt-57 (57Co)-bleomycin,41 indium-111 (111In)-bleomycin,42 99mTc-bleomycin,43 99mTcO4⁻ 44 and some of the radio-lanthanides45 have all been tried with some success. However, they suffer from low sensitivity and specificity, considerable cost, and prolonged blood clearance which may delay the scanning time for up to 48 h.

One of the criticisms of using 67Ga-citrate or 57Co-bleomycin to image cervical lymphadenopathy was the inability to detect lesions less than 2 cm in size, by which time nodes were usually clinically palpable.41 Recently, 99mTc-sulphur colloid lymphoscintigraphy46 and 111In-labelled monoclonal antibody against the epidermal growth factor receptor47 have been used to image cervical lymph nodes. However, they have proved similarly unsuccessful due to an inability to detect nodes less than 2 cm in size and an unacceptable false-negative rate.

Recent reports have described the accumulation of 99mTc (v) DMSA at known sites of primary and metastatic squamous carcinoma48,49 (Figure 7). It is as sensitive, but more specific, than 67Ga-citrate, and the use of SPECT improves the sensitivity so that it is now possible to detect cervical nodes less than 2 cm in size which were neither palpable nor visible on CT.49
Figure 7. Anterior planar $^{99m}$Tc(DMSA) head and neck scan in a patient with a T$_4$N$_2$ squamous carcinoma of the right retromolar trigone. Uptake is seen at the site of known primary disease (A) and in a 3 cm metastatic submandibular lymph node (B). A palpable upper deep cervical node which measured approximately 1.5 cm in size is not visualized. Note the normal biodistribution in the nasal mucosa (C).

Lymph nodes

In 1969, Edwards and Hayes$^{50}$ evaluated the potential of $^{67}$Ga-citrate as a bone scanning agent and reported its concentration in the cervical lymph nodes of a patient with Hodgkin’s disease. $^{67}$Ga was subsequently described as a new ‘tumour seeking’ agent, not only for head and neck malignancy, but for tumours in general.$^{51}$ Its current role in imaging head and neck cancer is now largely confined to the evaluation of lymphoma. $^{67}$Ga can be used to assess patients before positive histology is obtained and during initial staging. However, it can be of particular value in the assessment and restaging of residual and recurrent disease following surgery and irradiation.$^{52}$ However, the clinician should be aware that bilateral symmetrical accumulation of the tracer can occur within the salivary glands following irradiation and that this normal phenomenon$^{53}$ may cause some confusion when interpreting images.

Cerebrospinal fluid

CFS leaks can be demonstrated using $^{111}$In-diethylenetriaminepenta-acetic acid (DTPA). Sequential, anterior, posterior, lateral and vertex views are taken at 2, 4, 24 and 48 h following a lumbar injection of $^{111}$In-DTPA. In patients with CSF rhinorrhoea, radioactivity can be demonstrated in the nose, nasopharynx or paranasal sinuses. The location of the leak can be identified by counting the radioactivity in nasal packs placed in the anterior, middle and posterior aspects of the roof of the nose by an ENT surgeon. False positives do occur due to cross-contamination and radioactivity should be related to blood levels. In those patients with otorrhoea, radioactivity can be detected in the nasopharynx following passage of the tracer down the Eustachian tube or on a cotton ball placed in the ear canal.$^{54}$

Miscellaneous

Primary and metastatic melanoma have been demonstrated using $^{111}$In and $^{131}$I-monoclonal antibodies.$^{55}$ The uptake of $^{131}$I-MIBG in thymoma and malignant paragangliomata$^{15}$ (Figure 8) has recently been described which may have diagnostic and therapeutic implications.

Summary

Nuclear medicine has a distinct role to play in otolaryngological practice. Accurate diagnosis of endocrine conditions is now
possible using precise in-vitro hormone measurement. Specific clinical questions can be answered using in-vivo investigations. $^{99m}$TcO$_4$-$^{123}$I scintigraphy is used to evaluate thyrotoxicosis and solitary thyroid nodules. $^{99m}$Tc/$^{201}$Tl subtraction scanning is of value in the preoperative localization of parathyroid adenomas and $^{99m}$TcO$_4$-$^{123}$I is particularly useful in assessing salivary and lachrymal gland function and drainage. $^{99m}$Tc-MDP bone scanning is useful in the evaluation of osteomyelitis, temporomandibular joint dysfunction, bone graft viability and some facial fractures.

The role of radioisotopes in the management of differentiated and medullary carcinoma of the thyroid is now well established. Although there are many other agents available to image head and neck cancer, few can actually achieve the required diagnostic sensitivity and specificity. The introduction of monoclonal antibodies into routine imaging has been hampered by distinct practical problems and the search is now on for more sensitive non-specific diagnostic agents. It is now possible to evaluate new $^{99m}$Tc labelled tumour-imaging agents using animal tumour model systems and the use of radioactivity in all aspects of otolaryngological research adds an extra quantitative dimension. Together with SPECT, and the introduction of positron emission tomography (PET) to image the physiology of normal tissues and tumours, the use of radionuclide investigations can lead only to an increase in ENT diagnostic sensitivity and specificity and, subsequently, to an overall improvement in the way we diagnose, stage and treat head and neck cancer.

Acknowledgements

I am grateful to Professor Michael Maisey, Dr Susan Clarke, Dr Ignac Fogelman, Mr Omar Shaheen and Mr Ellis Douek for allowing me to study patients under their care and to the Editor of the Journal of the Royal Society of Medicine for his kind permission to reproduce Figures 2-4.

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Subcellular biodistribution of $^{99}$Tc$^m$(V) DMSA in squamous carcinoma: a comparative study in humans and in an animal tumour model

J.C. WATKINSON$^1$, S. ALLEN$^2$, M. HIGGINS$^3$, A. BHARIJ$^4$, C.R. LAZARUS$^2$, M.N. MAISEY$^2$ and S.E.M. CLARKE$^2$

Departments of $^1$Otolaryngology, $^2$Nuclear Medicine and $^3$Biochemistry, Guy's Hospital, London
$^4$Chelsea Department of Pharmacy, King's College, London

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Summary

Technetium-$^{99}$Tc$^m$(V) dimercaptosuccinic acid is a new imaging agent which has been used to evaluate head and neck squamous cell carcinoma (SCC). This study compared the subcellular biodistribution of $^{99}$Tc$^m$(V)DMSA in an established rabbit tumour SCC model and in humans with head and neck SCC.

In rabbits, approximately 17–37% of radioactivity was located on tumour cell membrane. Approximately 57–80% of radioactivity was located nonspecifically in tumour cytosol, only 2–6% was bound specifically to tumour mitochondria, and 1–4% bound specifically to microsomes.

In humans, 25–45% of radioactivity was localized on tumour cell membrane and 28–60% localized nonspecifically in tumour cytosol. There was 11–20% of radioactivity specifically bound inside the cell to the mitochondria and 1–6% specifically bound to microsomes.

These results show that although $^{99}$Tc$^m$(V)DMSA is accumulated at sites of SCC, the localization process is nonspecific.

Introduction

$^{99}$Tc$^m$(V)DMSA is a new tumour-imaging agent which has been used to evaluate medullary carcinoma of the thyroid (MCT) and head and neck SCC [1]. Recent reports have described its pharmacokinetics and biodistribution in an animal tumour SCC model [2] and in humans with SCC [3] and these studies have suggested little evidence of active tumour accumulation of $^{99}$Tc$^m$(V)DMSA with mean tumour : blood
ratios 3–5 h post-injection of 1.7 : 1 and 1.1 : 1 respectively. The aim of this study was to evaluate the subcellular biodistribution of $^{99m}$Tc(V)DMSA in an animal tumour SCC model and in humans with SCC.

Materials and methods

This work was carried out as part of a larger study to evaluate the pharmacokinetics, biodistribution and optimal imaging characteristics of $^{99m}$Tc(V)DMSA in an animal tumour SCC model and in humans with SCC [2–5].

Seven male New Zealand White (NZW) rabbits (all with tumour) were studied. The rabbit was chosen because it is large enough to permit easy manipulation for anaesthetic and imaging purposes, and transplantable rabbit SCC tumours are available. Male rabbits were preferred to facilitate bladder catheterization as this study was performed in conjunction with pharmacokinetics, biodistribution and imaging studies [2, 4]. The tumour studied was the VX2 transplantable squamous carcinoma and the cancers were induced using a previously described technique [6]. $^{99m}$Tc(V)DMSA was prepared using an in-house method [7]. The purity of the complex was analysed by thin-layer chromatography (Merck silica gel, developed with n-butanol/acetic acid/H$_2$O (3 : 2 : 3)), and no free pertechnetate or other $^{99m}$Tc$^{m}$ derivative was detected.

Four patients with head and neck SCC were studied (3 male, 1 female; age range 45–70 years, mean 56). Ethical committee approval was obtained to use the radiopharmaceutical $^{99m}$Tc(V)DMSA.

For the subcellular biodistribution method quantification, the following technique was used. Following an intravenous dose of $^{99m}$Tc(V)DMSA (150 MBq) a tumour rabbit was sacrificed and samples of viable non-necrotic tumour and macroscopically normal liver removed and washed in isotonic tris buffer. Small samples were then homogenized as indicated in Fig. 1.

![Tissue homogenization flowchart](image)

Fig. 1. A schematic diagram for the differential centrifugation procedure for the separation of the different subcellular fractions.
The amount of protein in each subcellular fraction was determined using the method of Lowry et al. [8]. Each fraction was assayed for its individual characteristic marker enzyme, i.e. the cell membrane and 5' nucleotidase [9]; the mitochondria and succinate dehydrogenase [10]; the microsomes and NADPH-cytochrome C reductase [11], and the cytosol and lactate dehydrogenase [12].

For the animal studies, the tumour rabbits were pre-medicated and $^{99}$Tcm(V)DMSA (150 MBq) injected intravenously. The animals were then allowed to recover and hydration was maintained by the hourly injection of 15 ml of dextrose saline subcutaneously into the nape of the neck. Four hours later, the animal was sacrificed at the optimal imaging time, i.e. 4 h [4], and samples of macroscopically normal liver and tumour were removed, washed in tris at 4° C, small samples of tissue being retained for histological examination. The tumour and liver samples were homogenized and subfractionated (as previously described, Fig. 1) and the relative amounts of radioactivity determined in each of the cellular fractions by counting each fraction in the automatic gamma counter.

For the human studies, four patients undergoing primary surgery were studied. All patients were injected intravenously with $^{99}$Tcm(V)DMSA (150 MBq) at the time of pre-medication. Following removal of the surgical specimen, samples of tumour were obtained, placed in isotonic tris and then analysed in an identical manner as that described for the animal studies.

Results

The methods of quantification of results are shown in Figs 2 and 3 and the subcellular biodistribution of $^{99}$Tcm(V)DMSA in rabbits with tumours (and normal liver) is shown in Table 1. All samples were confirmed histologically. There was no apparent difference between the liver and tumour subcellular biodistribution results, except that the radioactivity on the liver cell membrane appeared to decrease as a function of tumour age. There was no apparent relationship between the tumour subcellular biodistribution results and tumour age.

For the liver, 16–25% of radioactivity was located on the cell membrane (18–39 days with tumour) while 52–67% was localized nonspecifically in the cytosolic fraction. There was 10–20% of radioactivity bound specifically to mitochondria, and 6–8% to microsomes.

For squamous cell carcinoma, 17–37% of radioactivity was located on the cell membrane and this did not appear to vary as a function of tumour age. There was 57–80% of radioactivity localized nonspecifically in tumour cytosol. Only 2–6% of radioactivity was bound specifically to tumour mitochondria and 1–4% specifically bound to microsomes.

The human tumour subcellular biodistribution results are shown in Table 2. All tumours were confirmed histologically. For the four primary sites (hypopharynx, larynx, tongue and maxilla), approximately 25–45% of radioactivity was localized on the cell membrane and approximately 28–60% of radioactivity was localized nonspecifically inside the cell in the cytosol. There was 11–20% of radioactivity specifically bound inside the cell to the mitochondria and 1–6% specifically bound to microsomes.
CELLULAR SUBFRACTIONATION QUANTIFICATION: LIVER

SUCCINATE DEHYDROGENASE

LACTATE DEHYDROGENASE

5' NUCLEOTIDASE

NADPH-CYTOCHROME C REDUCTASE

Fig. 2. Cellular subfractionation quantification results: liver.
Fig. 3. Cellular subfractionation quantification results: tumour.
Table 1. Subcellular biodistribution results for $^{99}$Tc$^m$(V)DMSA in tumour-bearing NZW rabbits. (Results expressed as percentage of total radioactivity in the tumours.)

<table>
<thead>
<tr>
<th>Time with tumour (days)</th>
<th>18</th>
<th>22</th>
<th>32</th>
<th>32</th>
<th>39</th>
<th>75</th>
<th>Range</th>
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<tbody>
<tr>
<td>Cell membrane</td>
<td>25</td>
<td>23</td>
<td>20</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16-25</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>12</td>
<td>12</td>
<td>20</td>
<td>10</td>
<td>15</td>
<td>15</td>
<td>10-20</td>
</tr>
<tr>
<td>Microsomes</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6-8</td>
</tr>
<tr>
<td>Cytosol</td>
<td>57</td>
<td>56</td>
<td>52</td>
<td>67</td>
<td>63</td>
<td>63</td>
<td>52-67</td>
</tr>
</tbody>
</table>

*C*Liver

<table>
<thead>
<tr>
<th>Time with tumour (days)</th>
<th>18</th>
<th>22</th>
<th>32</th>
<th>32</th>
<th>39</th>
<th>75</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell membrane</td>
<td>23</td>
<td>17</td>
<td>24</td>
<td>37</td>
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<td>17-37</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2-6</td>
</tr>
<tr>
<td>Microsomes</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1-4</td>
</tr>
<tr>
<td>Cytosol</td>
<td>69</td>
<td>78</td>
<td>68</td>
<td>57</td>
<td>60</td>
<td>80</td>
<td>57-80</td>
</tr>
</tbody>
</table>

*Data from five rabbits.
†Data from six rabbits.

All tissue samples removed approximately 4 h post-injection.

Table 2. Subcellular biodistribution of $^{99}$Tc$^m$(V)DMSA in human tumours. (Results expressed as percentage of total radioactivity in the tumours.)

<table>
<thead>
<tr>
<th>Patient No. &amp; primary site</th>
<th>Histology</th>
<th>Cell membrane</th>
<th>Mitochondria</th>
<th>Microsomes</th>
<th>Cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>G2</td>
<td>45</td>
<td>20</td>
<td>6</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>G2</td>
<td>25</td>
<td>11</td>
<td>4</td>
<td>61</td>
</tr>
<tr>
<td>3</td>
<td>G2</td>
<td>34</td>
<td>20</td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td>4</td>
<td>G1</td>
<td>30</td>
<td>16</td>
<td>2</td>
<td>51</td>
</tr>
</tbody>
</table>

Range 25-45 11-20 1-6 29-61

All tumour samples were removed 3-5 h post-injection.
*G1 = well differentiated, G2 = moderately well differentiated.

Discussion

The methods of quantification of results for subcellular biodistribution are similar to those quoted in the literature [13]. The lower relative enzymic activity of tumour lactate dehydrogenase compared to that found in the liver reflects the characteristic anaerobic metabolism of squamous cell carcinoma. Although the cell membrane, mitochondrial, microsomal and cytosol fractions are undoubtedly contaminated, not
only with each other but also with varying amounts of other organelles such as lysosomes, peroxisomes and Golgi vesicles, the quantification results show that each of the tumour and liver fractions are enriched with the correct marker enzyme. Since the techniques were identical for the rabbit and human tumour subcellular biodistribution, certain comparisons can be made, particularly since similar methodology has been used to study the subcellular localization of radiopharmaceuticals such as $^{99}$Tc$^m$(V)DMSA and $^{201}$Tl [14].

For the animal results, the finding that radioactivity on the liver cell membrane fraction decreased sequentially with time is difficult to explain, particularly since none of the other liver or tumour fractions showed similar relationships. One possible reason would be altered biodistribution due to tumour uptake but why the cell membrane should be selectively affected is unclear. $^{99}$Tc$^m$(V)DMSA was nonspecific for the normal liver cell which explains the predominant accumulation of radioactivity within the cytosol and the localization of some radioactivity on the cell membrane. Hepatocytes are highly metabolic cells involved in drug and glucose metabolism. Dimercaptosuccinic acid is a low molecular weight organic acid and its incorporation into the glycolytic pathway would explain mitochondrial activity and its subsequent metabolism by conjugation of the sulphhydryl groups with glucuronic acid may explain microsomal radioactivity.

Variations of the biodistribution of the individual rabbit liver fractions with time (particularly the mitochondrial and microsomal fractions) can be explained by the fact that many cellular drug metabolic reactions vary with the age, environment, diet and temperature of the animal, as well as the time of day the experiment is performed [15]. Other relevant factors which have been shown to affect drug metabolism include the sex of the animal and any previous drug history [15]. This study was carried out on rabbits of the same sex, fed on similar diets, housed in a similar environment, and which had not previously received $^{99}$Tc$^m$(V)DMSA or any other drug and all experiments were performed at approximately the same time of the day.

It might be expected that the subcellular localization of $^{99}$Tc$^m$(V)DMSA in the rabbit tumours would vary as a function of tumour age. Such variations may have been observed if different viable tumour depths had been sampled since, as the centre of a squamous cell carcinoma is approached, the cells become less vascular and more anoxic and consequently vary in their rate and mode of metabolism. The range of activities in each of the individual tumour subcellular fractions can be explained by the finding that, although all the tumours were squamous carcinomas, any one tumour had its own inherent biological behaviour at any one particular time. The presence of radioactivity within the tumour cytosol, and on the cell membrane, is probably due to similar reasons which were postulated to explain the liver cytosolic and cell membrane biodistribution, and again reflect the nonspecific nature of $^{99}$Tc$^m$(V)DMSA. Squamous carcinoma cells are less vascular and less metabolically active than hepatocytes, and this may partly explain the lower radioactivity observed in the tumour mitochondrial and microsomal fractions.

It is difficult to make meaningful comments when comparing the human and rabbit
subcellular biodistribution results, particularly since subcellular drug metabolic activity can vary from species to species [15]. In addition, although all the human tumours were squamous cell carcinomas, the degree of differentiation varied and they were all of different ages from different head and neck sites.

However, since similarities have been shown between rabbit and human pharmacokinetic and biodistribution results for SCC [2, 3], certain comparisons may be valid. There was no evidence of selective specific human tumour cellular or intracellular accumulation and, although the results show there is uptake of radioactivity into squamous carcinoma, the lack of evidence of any specific intracellular localization mechanism in the presence of good clinical images [1, 4, 5] may mean the majority of $^{99}$Tc$^m$(V)DMSA is located in tumour extracellular fluid or inflammatory tissue or even in areas of microcalcification within the tumour.

The majority of the radioactivity was located either nonspecifically within the cytosol or on the cell membrane. These combined results are similar to those observed in rabbits, although there was some variation in actual proportions. There was less cytosolic radioactivity but more cell membrane radioactivity observed in the human tumours. The similar amount of radioactivity within the human and rabbit tumour microsomal fractions may reflect a similar drug metabolic pathway. However, the microsomal fractions in both rabbits and humans could have contained lysosomal contaminants which might represent the site of the intracellular $^{99}$Tc$^m$(V)DMSA tumour localization (in contrast to $^{67}$Ga [16]). Since the microsomal fractions contained the least radioactivity of all the fractions in both rabbit liver and human and rabbit tumour groups such a mechanism of localization is unlikely and may mean that $^{99}$Tc$^m$(V)DMSA is probably not localized specifically in the lysosomes. Further work to include autoradiographic studies would be necessary to evaluate the exact site of intracellular localization. The overall evidence in the literature is that $^{67}$Ga is predominantly localized within the cytoplasm and this study has shown a similar subcellular biodistribution for $^{99}$Tc$^m$(V)DMSA. Obviously an increase in capillary permeability is important for tumour uptake of both agents but the findings in this study may mean that the uptake of $^{99}$Tc$^m$(V)DMSA may be, in part, similar to $^{67}$Ga and, as such, transferrin-dependent (in contrast to other workers' findings [17]).

Conclusion

This study used an established animal tumour model to compare the subcellular biodistribution of $^{99}$Tc$^m$(V)DMSA in rabbits and humans with SCC. The results show that although $^{99}$Tc$^m$(V)DMSA is accumulated at sites of known SCC in animals and humans, the localization process appears nonspecific.

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References

The reliability of palpation in the assessment of tumours

J. C. WATKINSON*†, D. JOHNSTON*, N. JONES*, M. COADY*, D. LAWS†, S. ALLEN† AND J. HIBBERT*

Departments of *Otolaryngology and †Nuclear Medicine, Guy's Hospital, London, UK

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The reliability of palpation in the assessment of tumours

There is now a joint UICC-AJC classification for cervical lymph nodes based mainly on the size of the nodes. There is a recognized error in palpation, not only for detecting the presence of tumour but also its size. This study used an animal tumour model system to compare the ability of 6 independent observers of varying experience to detect and stage superficially transplanted growths. A preclinical medical student was as good as a Consultant ENT Surgeon in predicting the presence of tumour but the ability to stage tumours accurately was related to experience. Whilst the most experienced observers accurately estimated the size of tumours less than 2 cm, they were less accurate for larger (> 2 cm) tumours which were constantly understaged. This phenomenon may have important clinical implications particularly related to current nodal staging criteria.

Keywords palpation staging cervical lymph nodes

Until recently, the UICC and AJC classified cervical nodes differently.1,2 The UICC placed emphasis on mobility versus fixation whereas the AJC used size as the major classification determinant. Joint UICC-AJC criteria have now been published,3,4 and although there remain slight differences between the 2 classifications, size is now the major determinant for both systems.

There is a well recognized error in tumour palpation in general, with considerable interobserver variation when estimating tumour size.5-8 This is particularly true in the neck where significant variation exists between experienced observers.9-23 The incidence of false-negative results ranges from 4 to 60% with most workers reporting an incidence of 15 to 40%. False-positives occur in approximately 19% of cases.

With size now being the major UICC-AJC classification criterion for cervical lymph nodes, the aim of the study was to use an animal tumour model system24,25 to investigate the fallibility of palpation in general, and to compare interobserver variation.

Materials and methods

This study was conducted as part of a project evaluating new tumour-imaging radiopharmaceutical agents. Fifteen adult male New Zealand white rabbits were studied. Under general anaesthesia, each rabbit was shaved in 4 sites (both shoulder regions and both loins) and each site was

Correspondence: J. C. Watkinson, Department of Otolaryngology, Guy's Hospital, London SE1 9RT, UK.
Table 1. Tumour classification

<table>
<thead>
<tr>
<th>Class</th>
<th>Tumour size (cm)</th>
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<tbody>
<tr>
<td>Class I</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Class II</td>
<td>1-2</td>
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<tr>
<td>Class III</td>
<td>2-3</td>
</tr>
<tr>
<td>Class IV</td>
<td>3-4</td>
</tr>
<tr>
<td>Class V</td>
<td>&gt;4</td>
</tr>
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</table>

Table 2. Tumour palpation results

<table>
<thead>
<tr>
<th>Observer*</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>All observers</th>
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<tr>
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<td>60</td>
<td>67</td>
<td>27</td>
<td>40</td>
<td>47</td>
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<td>50</td>
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<td>96</td>
<td>77</td>
<td>81</td>
<td>85</td>
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<td>Class III</td>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>† Overall (I + II)</td>
<td>80</td>
<td>85</td>
<td>59</td>
<td>66</td>
<td>71</td>
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<td>90</td>
<td>71</td>
<td>76</td>
<td>79</td>
<td>83</td>
<td>81</td>
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<table>
<thead>
<tr>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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</thead>
<tbody>
<tr>
<td>83</td>
<td>72</td>
</tr>
<tr>
<td>89</td>
<td>83</td>
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<tr>
<td>83</td>
<td>80</td>
</tr>
<tr>
<td>69</td>
<td>79</td>
</tr>
</tbody>
</table>

*Experience: observer 1, Consultant ENT Surgeon; 2, Senior Registrar ENT; 3, Registrar ENT; 4, Registrar ENT; 5, House Surgeon (General Surgery/ENT); 6, Preclinical Medical Student.
†Less than 2 cm.

Results

Fifteen rabbits were studied (14 tumour, 1 non-tumour) with 60 sites, of which 42 contained transplanted tumours. All tumours were confirmed histologically. There were 30 sites with 1 tumour, 8 sites with 2 tumours and 4 sites with 3 tumours. Fifty-eight tumours were examined (range 0.4–7.1 cm, mean 1.9 ±2.8 cm). The overall sensitivity of tumour detection by palpation was 81% with a 79% specificity.

The individual and overall observer sensitivities for each tumour are shown in Table 2. Overall, 50% of Class I tumours were palpable and this increased to 86% for Class II tumours. Seventy-three per cent of Class I and II tumours (less than 2 cm) were palpable and this increased to 100% for Class III tumours (i.e. greater than 2 cm).

All tumours measuring 2 cm and over were detected on palpation by all observers (100% sensitivity, Table 2). For those measuring less than 2 cm, observers 2 and 1 had the highest interobserver sensitivities of 85 and 80% respectively. Observer 6 had a sensitivity of 76%. Observer 5 had a 71% sensitivity while observer 4 and 3 had sensitivities of 66 and 59% respectively.

A comparison was also made evaluating the ability of the 6 independent observers to estimate tumour size. Figure 1 shows the
error in estimation (difference) compared against actual tumour size for each observer. There was no significant difference (random block analysis) between observers 1, 2, 3 and 4 in the ability to estimate tumour size (Table 3, α>0.05). Observers 1 and 2 were the most accurate and tumour size estimation was more accurate for tumours less than 2 cm in size. Observer 3 tended to underestimate the smaller (less than 2 cm) tumours while observer 4 underestimated tumour size in a similar manner to observers 1 and 2. Observers 5 and 6 were significantly less accurate and more erratic in predicting tumour size than observers 1–4 (Table 3, α>0.05).

**Discussion**

Although comparisons between palpating superficially transplanted rabbit tumours and lymph nodes in the neck must be cautious, there are a number of important observations arising from this study. Tumours greater than 2 cm were palpated by all observers and this is a similar finding to that observed in humans for neck palpation. For tumours less than 2 cm, there was no apparent interobserver variation in the ability to detect tumour although the 2 highest sensitivities occurred with the 2 most experienced observers. What was interesting was that a preclinical medical student had a sensitivity of 83% which was greater...
than the 2 registrars and the houseman, and similar to that seen with the 2 more experienced observers. This suggests each individual has an inherent ability to palpate and detect a tumour mass and that this ability improves very little with experience. The incidence of false-negative (32%) and false-positive (12%) results is similar to the mean values from the literature for neck nodes (29% and 19% respectively) and this is surprising since the tumours were superficial and the rabbits were anaesthetized and therefore easier to examine.

Possible sources for false-positive results in the neck which simulate a lymph node include the transverse processes of the first and second cervical vertebrae, the carotid bifurcation, the superior horn of the thyroid cartilage, the tail of the parotid gland and irradiated submandibular salivary glands. In the rabbit, false-positive results could have arisen due to faeces in the colon, the kidneys, the ribs and the tip of the scapula.

It appears that the ability to estimate tumour size is a function of experience and this ability is more accurate, the smaller the tumour. There was no significant difference between the 4 otolaryngologists in their ability to estimate tumour size and all 4 were significantly more accurate than either the houseman or the medical student. The 2 most experienced observers were the most accurate, not only in detecting the presence of tumour, but also in predicting the size of tumours which measured less than 2 cm (Figure 1). However, for the larger tumours (>2 cm), the 2 more experienced observers constantly underestimated tumour size (as did observer 4, but to a lesser extent). It would appear that as an individual learns to assess tumour size, accuracy increases for smaller tumours but this is accompanied by a tendency to underestimate the larger ones.

There is a recognized error in measuring tumour size at necropsy, depending on whether measurements are based on the largest dimension \( R = 0.72 \), area \( R = 0.97 \), volume (prolate sphere, \( R = 0.98 \)) or water displacement \( R = 1.00 \). Errors occur when the largest dimension is used because some tumours contain loosely associated nodules, there is often difficulty deciding what represents the greatest longitudinal diameter, and there is interobserver variation in measurement techniques. In this study, all tumour measurements were performed by 1 observer. Size was based on the largest dimension since this allowed a simple clinicopathological classification and a correlation coefficient of 0.72 is acceptable for the purpose of this work.

One of the most important prognostic factors in head and neck cancer is the presence or absence, level and size of metastatic cervical lymphadenopathy. The surgeon must first detect the presence of any nodes and then assess their size. Individuals vary in their ability to palpate tumours. False-positive and false-negative results are inevitable and the evaluation and treatment of the clinically N\(_0\) neck remains controversial. The introduction of new joint UICC-AJC staging criteria based on nodal size means that experienced observers may understage some necks. This may result in inappropriate treatment. Otolaryngologists should be aware of this dilemma and, in selected cases, nodal measurements using either computerized axial tomography or ultrasound may supplement the clinical examination.

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measuring error on the results of therapeutic trials in advanced cancer. Cancer 38, 388–394


