PATHOPHYSIOLOGICAL ASPECTS OF

CHRONIC IDIOPATHIC OROFACIAL PAIN

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A thesis submitted to the University of London
for the degree of Doctor of Philosophy in

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1993
Dedicated to Professor Malcolm Harris
ABSTRACT

This study addresses some of the questions related to the pathophysiology of chronic idiopathic orofacial pain.

1. Chapter II describes the psychological characteristics of the patients assessed by a structured interview (SCID) according to DSM-III-R criteria. Patients had a higher lifetime prevalence of major depression than the general population. However, this did not seem to be causally related to the pain in any way. The study also revealed a significant number of patients suffering from a post-traumatic stress disorder, the onset of which coincided with the onset of the pain. The implications of this finding in diagnosis and management is discussed.

2. Chapter III aims to test the hypothesis that chronic pain and depression share a common biological pathogenesis. Tyramine conjugation deficit, an established trait marker of endogenous depression was shown to be present in pain patients independent of a co-existing history of depression. This therefore confirmed a common metabolic abnormality predisposing to both idiopathic pain and depression even if both do not occur in the same individual. However, platelet monoamine-oxidase activity level was not significantly different between patients and controls.
3. Chapter IV is concerned with the systemic role of oxygen free radicals (OFR) in chronic pain. It also investigates the influence of experimental stress on free radical metabolism. Two biochemical assays were chosen for this purpose:

The measurement of 2,3,-dihydroxybenzoic acid was found to be flawed by a number of methodological problems and was therefore abandoned. An alternative method was standardized for future investigations. Furthermore the thiobarbituric acid test did not show a significant difference between the patients and controls. Also, acute experimental stress did not affect the level of thiobarbituric acid reactive substances in the blood in either patient or control group.

4. Chapter V consists of 3 separate studies on TMJ synovial fluid. The first part deals with the measurement of hyperalgesic eicosanoids in the joint. We could not find support for the published reports of the presence of PGE\textsubscript{2} and LTD\textsubscript{4} in the joint. However, considerable levels of hyperalgesic products of 15-lipoxygenase such as 15-HETE were demonstrated. The second part, describes a method for estimation of the volume of synovial fluid of TMJ. This is based on simultaneous measurement of salicylate in plasma and saline aspirates. The third part, studies the role of OFR in the local damage in the joint. The findings imply that the local release of OFR may play a role in the pathogenesis of TMJ disorders. However, the lack of positive objective clinical or arthroscopic finding in some painful joints and the well recognized effect of predisposing life events on a vulnerable personality, emphasizes the role of central modulating factors in the pain experience.
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CHAPTER I. INTRODUCTION

PATHOPHYSIOLOGICAL ASPECTS OF

CHRONIC IDIOPATHIC OROFACIAL PAIN

We must all die, but I can save him from days of torture, that is what I feel as my
great and ever new privilege. Pain is a more common lord of mankind than even
death himself.

Albert Schweitzer, 1931
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1. GENERAL CONSIDERATIONS

Pain is the most common symptom that compels patients to seek medical and dental therapy and constitutes a serious health and economic problem. It has been estimated that in the industrialized countries 15-20% of the population have acute pain and between 25 and 30% suffer from chronic pain. This costs American society 70 billion dollars annually (Bonica, 1990).

Dentists and dental specialists are concerned with two of the most common pains. The first being acute orofacial pain arising from the teeth and associated structures and secondly chronic craniofacial pain which is believed to account for 40% of all chronic pain problems (Schiffman & Fricton, 1989). An understanding of the pathophysiology of pain is needed by all those who are involved in pain relief.

This chapter aims to highlight some of the many unanswered questions related to chronic facial pain, which we have tried to find an answer for during the course of these studies. A brief outline of some of the fundamental aspects of pain pathophysiology in general, is also presented.

2. DEFINITIONS

Traditional views of pain are reflected in definitions such as that of the Butterworth's Medical Dictionary (1978): the distressing sensation excited by noxious stimuli of sufficient intensity acting on nerve endings'. It goes on to define psychogenic pain as 'pain which occurs without any organic cause and is due to a disorder of mind'.
Although much pain results from noxious stimulation, it can also occur from non-noxious stimuli as well as spontaneously when there is no stimulus at all. Wall & Devor (1983) have shown that sensory impulses from the dorsal root ganglia are constantly being transmitted into the central nervous system (CNS) even under normal conditions. This means that we have a potential source of neural impulses at all times which can reach a conscious level as pain with any decrease in central inhibitory control.

Thus newer concepts regard pain as a subjective psychological state rather than an unpleasant sensory activity that is induced solely by noxious stimulation. The definition of pain adopted by the International Association for the Study of Pain (Merskey, 1986) defines pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage. This definition avoids linking pain to a stimulus and regards pain always as an affective state, that is an emotional experience and not merely the perception of a pure sensation. However, the affective state of pain differs from other affective states in that it is always referred or projected to some part of the body with varying degrees of precision (Wyke, 1958).

Unlike elation or sorrow, pain is always 'felt' in some part of the body, even when that part is no longer present, as in the case when pain is felt in a 'phantom limb' after its amputation. However, the qualities of 'unpleasantness' are complex. These include misery, anguish, desperation and urgency that are a part of so many pain experiences. At present we must be content with guidelines towards a definition of pain, too much remains to be learned before we can define it precisely (Melzack & Wall, 1988).
3. CLASSIFICATION (Table 1)

A classification into acute, recurrent and chronic pain is chosen here as their management, and to some extent their pathophysiology, are different (Curro, 1987). Acute nociceptive pain may be considered to be a protective mechanism for the body in response to tissue damage. By stimulating the sympathetic nervous system it is often accompanied by the autonomic signs of stress and anxiety. This is also of considerable diagnostic value to the clinician when determining the nature and site of the disturbance. The control of acute pain in most instances is accomplished by the use of non-steroidal anti-inflammatory analgesics or the opioids. Chronic pain does not serve any apparent biological function and is socially and psychologically destructive. It is not just a symptom of disease but can become a serious syndrome in itself.

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<td>rheumatoid arthritis</td>
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<td>Dental: pulpitis, cracked tooth</td>
<td>Neuropathic: trigeminal neuralgia, causalgia</td>
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<td>post-herpetic neuralgia</td>
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<td>Periodontal: gingivitis, periodontitis</td>
<td>Idiopathic:</td>
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<td>Mucosal: various causes of ulceration</td>
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<td>Atypical odontalgia</td>
</tr>
<tr>
<td>Ear: otitis externa and media</td>
<td>Oral dysaesthesia</td>
</tr>
<tr>
<td>Tonsils: tonsillitis, peritonsillar abscess</td>
<td>Tension headache</td>
</tr>
<tr>
<td>Referred: cardiac angina</td>
<td>Migraine</td>
</tr>
<tr>
<td></td>
<td>Facial Migrainous neuralgia</td>
</tr>
</tbody>
</table>
Chronic pain can be recurrent or continuous. The sympathetic and neuroendocrine responses have usually become less apparent and vegetative(somatic) features emerge which are similar to those seen in depressive syndromes (Stembach, 1981). Such pains appear to fall into three groups, (a) nociceptive pain e.g. rheumatoid arthritis, carcinoma; (b) neuropathic pain arising from damage anywhere in the nervous system e.g. trigeminal neuralgia and (c) what is currently best described as idiopathic pain. This last group describes a chronic pain condition where there is no structural abnormality, or if there is any, the severity of symptoms does not match the severity of structural abnormality present. This pain has also been called 'psychogenic pain', a term introduced to psychiatry by Sommer (1894) which remains in current use despite criticism of several authors (Feinmann, 1983). Engel (1967) suggested that the term 'psychosomatic' was more accurate as this implied only that relationships existed between mind and body. We prefer to use the term 'idiopathic' which simply refers to our lack of understanding of aetiology and pathogenesis of the condition without any implication on dichotomy of mind and body. Wall (1989) states chronic idiopathic pains 'bear testimony to our ignorance'.

4. PAIN-INJURY RELATIONSHIP

The link between stimulus and perceived sensation is not always consistent. Injury can exist without pain, as seen in cases of congenital analgesia. This is a rare condition in which a person is born without the ability to feel any pain. The nervous system appears to be normal, but these patients show pathological changes in the skeletal system, and are prone to severe injuries without being aware of them. Another, more common, example is the phenomenon of episodic analgesia. Studies on soldiers injured in battle and patients brought into casualty shows that pain is often not felt for several minutes or even hours.
after severe injuries (Melzack et al., 1982). The analgesia has no relation to the severity or site of the injury; it is instant, has a limited time-course and is localized to the injury.

Pain without injury is seen in several conditions. Tension headache and migraine are no longer thought to be due to muscle tension or vasodilatation (Oleson, 1986), and trigeminal neuralgia, atypical facial pain and up to 70% of lower-back pains are not attributable to any anatomical abnormality (Loeser, 1980).

Pain disproportionate to the injury is seen in those who have experienced the passing of a renal calculus from ureter to bladder, a mechanically trivial event occurring in a poorly innervated structure. Severe pain can be felt after the healing of an injury, spontaneous post-traumatic neurogenic limb pain, and 'phantom arm' pain following brachial plexus avulsion, have been reported (Wynn-Parry, 1980).

5. THE PSYCHOLOGY OF PAIN

It is known that the psychological state of an individual may produce pain, increase the severity with which it is felt or even diminish the severity. The amount and quality of pain felt by an individual are determined by several factors other than the specific physical injury sustained at a given time. Previous experiences and memories of them, culture and race, personality and education, anxiety and anticipation, attention and distraction, and an understanding of cause and consequences - the 'meaning' of the pain - are features unique to each individual, and play an important role in what is eventually a highly personal pain experience (Craig, 1989).
Patients who feel they have some control over their pain, or are taught how to cope with it, have been shown to experience less pain. For example, preoperative information about pain, along with instructions on relaxation and distraction, have been shown to reduce the severity of pain after surgery (Langer et al., 1975). However, Weisenburg et al. (1985) found that control that is perceived as inadequate may worsen the pain, and preoperative information about the pain alone, in the absence of any coping strategy, may only increase anxiety about the pain.

The power of suggestion on pain is demonstrated by the placebo effect. A placebo (Latin: 'I shall please') is an inactive substance administered to a patient, either to compare its effects with an active drug, or for the psychological benefit to the patient through the belief he/she is receiving treatment. It is thought that the suggestion itself, and a decrease in anxiety- both effects occurring together- play a part in relieving pain (Evans, 1985). There are individual differences in the response; placebos are more effective for severe pain than mild pain, and in patients with more stress and anxiety. Suggestion, expectation, personality and the kind of pain all play a part in producing the placebo effect; attributing it wholly to a straightforward mechanism such as an increase in endogenous opioids seems simplistic (Gracely et al., 1983).

The psychological evidence refutes a one-to-one stimulus-response relationship, rather, it points to the concept of pain as a perceptual experience whose quality and intensity are influenced by past experiences, the significance given to a pain-producing situation, and the mental state of an individual at the time. It is postulated that all these factors are important in determining the actual pattern of nerve impulses ascending from periphery
to CNS, and transmission patterns within the CNS itself (Melzack & Wall, 1988).

6. CHEMICAL BASIS OF NOCICEPTION

Pain is provoked when a variety of substances are released or injected into the tissues. These pain producing substances can be released from cell membranes, mast cells and nerve endings by trauma, infection, allergic reaction, neurogenic reflexes and central emotional changes. This leads to the excitation of free nerve endings which act as nociceptors or peripheral sense organs that respond to the noxious stimulus. This group of substances include histamine, bradykinin, potassium ion, acetylcholine, prostaglandins, leukotrienes and neuropeptides.

Among these noxious substances eicosanoids have attracted much attention in the recent past. Eicosanoids include the prostaglandins, leukotrienes and various hydroxy and hydroperoxy acids (fig.1).

Although acute and some types of chronic hyperalgesia are due to a continuous generation of pain mediators by damaged tissue, there is evidence that certain mediators such as the neuropeptide substance P (Nakamura-Craig & Smith, 1988), and PGE2 (Ferreira & Lorenzetti, 1981) as well as 15-HPETE (Follenfant et al., 1990) can induce a state of prolonged hyperalgesia without continuous tissue destruction, by sensitizing the peripheral nociceptors (Ferreira et al., 1990). These observations may also be relevant in explaining other chronic painful conditions without evident structural changes.
Figure 1. Pathways of arachidonic acid metabolism
7. PAIN TRANSMISSION

It is customary to describe somatosensory pathways proceeding from peripheral receptors to areas of the CNS. However, it is important to remember that stimulation of receptors produces neural signals which enter an active CNS that is already the substrate of previous experiences and memories, culture and personality, anxiety and anticipation, and an understanding of cause and consequences. These central processes actively participate in the selection, abstraction and synthesis of afferent information (Campbell et al., 1989). Nociception is defined as the response to noxious stimuli, it is not necessarily synonymous with feeling pain. Information about noxious events is conveyed centrally by free nerve endings which act as nociceptors and which are present in all orofacial tissues including skin, oral mucosa, the TMJ, periodontium, tooth pulp, periosteum and muscles (Sessle, 1987). These nerve endings become excited by noxious stimuli and impulses are transmitted via 3 major classes of nociceptive afferents (Fields, 1990) (Table 2). Studies of electrically stimulated teeth have found that the most sensitive fibers are activated by low stimulus intensities and evoke 'prepain' sensation whereas higher intensities cause sharp pain, and that the highest intensities cause an unpleasant ache (Azerad & Woda, 1977). Because single fibre studies in animals show that A-beta fibers are more sensitive than A-delta fibers and that C-fibers are least sensitive, it is probable that pre-pain, sharp pain and dull ache correspond to A-beta, A-delta and C-fibre activation (Narhi, 1985).
Table 2. Properties of nociceptors:

<table>
<thead>
<tr>
<th>Fibre type</th>
<th>C</th>
<th>Aδ</th>
<th>Aβ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution</td>
<td>all tissues except CNS</td>
<td>body surfaces</td>
<td>body surfaces</td>
</tr>
<tr>
<td>Size</td>
<td>Small(0.3-3μm)</td>
<td>2-5μm</td>
<td>6-22μm</td>
</tr>
<tr>
<td>myelination</td>
<td>unmyelinated</td>
<td>myelinated</td>
<td>myelated</td>
</tr>
<tr>
<td>conduction velocity</td>
<td>0.5-2 m/s</td>
<td>5-30 m/s</td>
<td>33-75 m/s</td>
</tr>
<tr>
<td>pain perception</td>
<td>dull ache</td>
<td>sharp pain</td>
<td>prepain</td>
</tr>
</tbody>
</table>

Abnormal conducting patterns may occur in nerves after experimentally induced demyelination and this has been suggested as a pathogenesis of trigeminal neuralgia a paroxysmal pain which is characteristically provoked by the stimulation of myelinated fibers by light touch (Sessle, 1987). Plaques of demyelination are frequently found in the descending trigeminal tract and in the lemniscal systems, but it is not possible to argue that such plaques are either necessary or sufficient to cause tic douloureux. Nevertheless trigeminal neuralgia is associated with plaques of demyelination in multiple sclerosis and sites of nerve compression by cerebral arteries.

Once generated the impulse in the orofacial nerves travels mainly via the trigeminal nerve but also by the sensory roots of the facial, glossopharyngeal, vagus and upper cervical nerves. Substance P (SP) is the neurotransmitter at the primary sensory synapse. This neuropeptide is synthesized by the nerve cell body and can be released antidromically. In fact it is interesting to note that approximately 4 times as much SP is transported peripherally as centrally (Brimjolin et al., 1980). SP has been measured in dentine and
dental pulp (Gores & Oehme, 1989) and is believed to play a role in the reception and transmission of pain in dentine (Akai & Wakisaka, 1990). The central process of these neurons enter the brainstem and synapse on second order neurones at various levels of the trigeminal brainstem sensory nuclear complex (fig. 2) which consists of: (i) The main sensory trigeminal nucleus which is rostrally located and receives periodontal and some pulpal afferents and (ii) the spinal tract of the trigeminal nucleus which is more caudally located. The spinal tract is divided into the subnucleus oralis, subnucleus interpolaris and the subnucleus caudalis.

The subnucleus caudalis extends into the cervical spinal cord and merges with the spinal dorsal horn. It serves as the principal relay site of orofacial nociceptive information and is similar in structure and projection to the spinal dorsal horn which is the principal component of the spinal nociceptive mechanism. Evidence suggests that the subnucleus caudalis is homologous to the substantia gelatinosa of the spinal dorsal horn and acts as a 'gating' mechanism (see pain modulation) capable of modulating sensory information (Shigenaga & Nakatani, 1982). From the brainstem, sensory information may then be relayed directly to third order neurons in the thalamus and from there to cerebral cortex.

There is some reason to believe that the sensory and affective aspects of pain are subserved in part by separate neural mechanisms (Fields, 1990). The spinothalamic projections to the ventrobasal thalamus and its projections to the somatosensory cortex are required for the discriminative sensory aspects of pain. This is the means whereby the nature and source of the pain are determined. However, projections to the medial thalamus and from the medial thalamus to the frontal cortex seem to be concerned with
the affective aspect of pain. This view is supported by observation of patients who have undergone frontal lobotomy (Barber, 1959). Interestingly, these patients usually obtained striking relief of their pain problem but with no impairment in their ability to detect and identify noxious stimuli as painful. The suffering was thus eliminated with no effect on the purely sensory aspect of their pain.

Figure 2. Sensory transmission from trigeminal nerve to cerebral cortex. The unipolar cell bodies of primary neurones are located in the trigeminal (Gasserian) ganglion. Other cranial nerves supplying the orofacial region follow a similar course. For clarity connections from the midbrain periaqueductal grey (PAG) and medullary nucleus raphe magnus (NRM) to the trigeminal sensory complex are not shown.
An interesting recent finding is that cerebral responses in patients with atypical facial pain have a significant emotional component. Devani et al.(1992) used Positron Emission Tomography(PET) to obtain functional images of regional cerebral responses to painful stimuli and non-painful stimuli in patients with chronic idiopathic facial pain and also in pain-free controls. In the patient group, there were substantial responses to pain in areas of the brain relating to the motivational affective aspects of pain, including the medial pain system and its connections to the limbic system with its projections to wide areas of cortex.

8. PAIN MODULATION

The concept of pain modulation is based on the evidence that neural impulses are altered as they travel up to the higher centres. The existence of a specific pain modulatory system was proposed in the gate control theory of pain to explain the variability of the painful experience (Melzack & Wall,1965). Supraspinal influences on the dorsal horn were postulated, although evidence for descending control of nociception was limited. Later, based on new information the theory was modified (Melzack & Wall,1988). These theories have proven to be amongst the most important developments in the field of pain research and therapy. In addition to providing a comprehensive formulation of pain mechanisms, the theory has stimulated much physiological and psychological research and provoked the development of new approaches to pain therapy.
The pain modulating network:

There appears to be a specific central nervous system network for pain control. Analgesia may be demonstrated by the stimulation of brain sites such as the periaqueductal grey (PAG) and nucleus raphe magnus (NRM) (fig. 2) in animals and man. This has provided powerful evidence for a highly selective brainstem control of nociceptive transmission (Basbaum & Fields, 1978). This system is also involved in emotional and motivational functions and other complex behaviour.

Although there has been much emphasis on the pain suppressing effect of the modulation system, the brain stem modulating neurones have a bidirectional control of transmission in that the network has both excitatory and inhibitory actions on pain conduction (Fields & Heimricher, 1985). These concepts are incorporated in the new model of the gate control theory (fig. 3). Therefore, pain can result from either the loss of inhibitory control or the activation of excitatory modulating neurones.

The anatomical, chemical and physiological bases of pain modulation are progressively being unravelled. Analgesia produced by the pain modulating network is believed to be mediated by endogenous opioid substances which are synthesized by nerve cells and have pharmacological properties nearly identical to those of narcotic analgesic drugs. The first discovered endogenous opioid peptides were leucine enkephalin and methionin enkephalin (Hughes et al., 1975).
Figure 3. The gate-control theory

The transmission of nerve impulses from afferent fibers to the central transmission cell (T) of the medullary trigeminal sensory complex is modulated by a 'gate control system'. An inhibitory effect exerted by the subnucleus caudalis (Snc) on the afferent fibre terminals is increased by large diameter fibre input and decreased by activity in small diameter fibers. The new model includes excitatory (white circle) and inhibitory (black circle) links from Snc to T as well as descending inhibitory control from brain stem system. Cognitive (conscious) influences are also possible.
Since their discovery, other opioid peptides have been identified throughout the body. One of the most potent of these is beta-endorphin ('endogenous morphine'). Its precursor pro-opiomelanocortin which originates in the infundibular nucleus of the basal hypothalamus, also gives rise to adrenocorticotropic hormone (ACTH). However, it is interesting that ACTH antagonizes the analgesic effects of beta-endorphin (Smoke & Fields, 1980). This may also be of some relevance to chronic pain conditions, as patients with chronic idiopathic pain syndromes are reported to have higher levels of cortisol in their blood (Blumer et al., 1982).

Cytochemical studies of the pain-modulating networks have also revealed that in addition to the endogenous opioid peptides, a variety of other neurotransmitters are involved in the control of pain transmission. The monoamines, serotonin and noradrenaline are present in the brain-stem neurones and both inhibit spinal cord pain transmission cells (Basbaum et al., 1983).

The endogenous opioid system and the monoaminergic network interact and recent studies have shown that morphine may activate the descending serotoninergic pathway and so modulate dental pain transmission. (Yonehara et al., 1990).

In patients with chronic pain syndromes a number of studies indicate that serotonergic systems are involved in the nociceptive responses. An increase in serotonergic activity is generally associated with a decrease in pain sensitivity. Hyperpathia and spontaneous pain can be provoked by treatment with a serotonin depleting agent, clinical pain can be relieved by treatment with the serotonin precursor tryptophan or treatment with specific
serotonin reuptake inhibitors (reviewed by von Knorring, 1989). In the early seventies the development of techniques for the measurement of the serotonin metabolite 5-hydroxyindolacetic acid (5-HIAA) in the cerebrospinal fluid (CSF) led to increasing interest in the study of the role of serotonergic system in chronic pain conditions. It has been demonstrated that patients with idiopathic pains have lower levels of 5-HIAA in the CSF compared with controls (Almay et al., 1987a). However, due to ethical problems, a study of CSF serotonin metabolites is not feasible in an out-patient population of patients with chronic facial pain. Recently, evidence has accumulated to show that blood platelets may constitute an easily accessible peripheral marker for central serotonergic activity (Oreland & Hallman, 1989). Their study in patients with chronic facial pain may be of value.

9. CHRONIC IDIOPATHIC OROFACIAL PAIN

9.1. Prevalence

As stated chronic idiopathic orofacial pain is an important health problem and poses major management difficulties for doctors and dentists alike. Bonica (1980) estimated that five to seven million Americans suffered chronic pain in the face and the mouth at a cost to Society of over $4 billion a year. Asberg & Carlsson (1972) showed that 25-45% of the general population are affected at some time in life, and there has been an increasing awareness that the prevalence of facial pain is greater than the incidence of patient referral would suggest (Kent, 1985). The disorder seems to be much more common in females. However, some authors believe that men and women are equally affected, but more women seek treatment (Gross & Gale, 1983). The age range of these patients is wide
(15-80 years), but the mean age of facial arthromyalgia (FAM) patients is about 30 years, while that of patients with atypical facial pain (AFP) and its variants is 55 years (Grushka et al., 1987). Studies on whole populations have reported that TMJ clicking occurs in 32% of subjects. Crepitus is present in 16% of the TMJ's. Pain occurrence upon TMJ palpation is 45%; whereas pain upon palpation of muscles of mastication is 66%. Daily craniofacial pains were reported in 6%, weekly in 15% and recurrent headaches in 51% of the population (Reviewed by Schiffman & Fricton, 1989). These studies have documented the epidemiology of the presence of TMJ dysfunction and facial pain without any mention of the severity and duration of the problem. Chronic pain patients presenting to the Eastman Hospital usually have chronic persistent pain (minimum of 6 months) and a considerable proportion suffer from chronic pain elsewhere in their bodies. The prevalence of chronic pain in the general population based on large community studies is estimated to be 14% (Magni et al., 1990). It may be appropriate to compare our group with other chronic pain groups in terms of epidemiology.

9.2. Clinical presentation

Chronic idiopathic facial pain problems may present individually, sequentially, or simultaneously in the same patient. In some of these patients, there is an absence of objective clinical signs, and radiographic and laboratory investigations are normal. Up to 80% of patients also complain of other recurrent pain symptoms. In childhood such patients suffer from abdominal or ear pain, in adolescence TMJ pain or dysmenorrhea and later abdominal pain (usually irritable bowel syndrome), neck pain and back pain (Engel, 1959; Berry, 1969; Feinmann et al., 1984). If this concept is not
recognized the differential diagnosis of each pain episode may be misleading and clinically unhelpful, with the tendency to attribute the disorder to purely local abnormalities rather than a generalized problem. Demographic and clinical features are insufficient to conclude that chronic idiopathic facial pain problems are separate disorders (Feinmann, 1983). However, a classification according to the site of pain is as follows:

1. Facial arthromyalgia (the temporomandibular joint pain dysfunction syndrome, myofascial pain dysfunction syndrome)

This is the second most common cause of facial pain after toothache. The pain is described as a long-standing dull ache affecting one or both joints, with occasional acute exacerbations often described as an earache. It may be associated with disturbances of joint function such as clicking, trismus, locking, sticking and deviation and occasionally aural symptoms of tinnitus and a sense of fullness in the ear. The discomfort radiates to the masseteric, temporal, occipital and mastoid areas, or down to the neck and is often worse on chewing, speaking or yawning. The disorder rarely disturbs sleep, and the patient is often troubled by the symptoms for anything from a few weeks to several years. Bruxism, and tenderness over the TMJ and masticatory muscles, are frequently evident; ridging of the buccal mucosa and tongue, and wear facets on the teeth, are helpful diagnostic feature. In some patients, marked localized pain and tenderness suggest a capsulitis; if persistent, this may lead to the development of adhesions in the joint space and subsequent internal derangement and limited opening. These features can be confirmed by arthroscopy.
2. Atypical facial pain
This is a non-joint and non-muscular variant of FAM. It is usually described as a diffuse, deep soft tissue pain than can be localized to facial and alveolar bone. The discomfort can vary from a dull ache to a sharp throbbing pain, is constantly present and often of many years duration. It can affect both sides and have a wide extrafacial distribution, and will occasionally wake the patient at night. Commonly it is provoked or potentiated by trauma or dental treatment.

3. Atypical odontalgia
It is identical to pulpal or periodontal pain, however, there is no detectable pathology. Some cases appear to have been precipitated by a dental procedure but they are invariably made worse by any further active treatment.

4. Oral dysaesthesia
The most common presentation is the burning tongue (glossodynia). Occasionally the gingiva and lips are also involved, or the denture bearing areas of the hard palate and lower alveolus, making the wearing of a denture impossible.

9.3. Aetiology & pathophysiology
The accumulation of descriptive labels and differing opinions about the appropriate treatment reflect the confusion surrounding the aetiology and pathogenesis of the condition. Costen (1934), an otolaryngologist described a group of patients complaining of multiple head and neck symptoms without identifiable disease affecting the ears, nose or throat. He observed that his patients had missing teeth or inadequate dentures causing
overclosure of the mandible. Dental treatment restoring the bite proved helpful and Costen postulated that overclosure of the mandible increased pressure on nerves passing close to the mandibular condyle. This hypothesis was eventually disproved by the anatomist, Sicher (1948). No simple explanation for the aetiology exists. Among the many proposed theories regarding the aetiology of TMJ pain, two have attracted much attention, which in turn have led to different therapeutic strategies:

9.3.1. Occlusal dysharmony

The tendency to consider occlusal dysharmony as the primary cause of TMJ pain results from the suggested relationship between dental occlusion and TMJ function. Some proponents of this concept regard the clinical symptoms as resulting from mechanical displacement of the condyle from its central position in the fossa due to occlusal interferences or instability (Weinberg, 1979), while others suggest that occlusal interference causes muscle incoordination and spasm, in turn leading to pain and dysfunction (Ramfjord & Ash, 1983).

However, despite strong traditional beliefs, there is no evidence that malocclusion will give rise to this chronic pain disorder (Yemm, 1985; Berry, 1986). The incidence of malocclusion is no higher in patients with FAM than in the general population (Thomson, 1971). Furthermore, it has been suggested that occlusal dysharmonies can arise in a functioning dentition as a result rather than a cause of the problem (Laskin, 1992). There is no published study to date, showing that occlusal correction is more effective than placebo adjustment (Goodman, 1976) or simple counselling (Kopp, 1979). Also the dominantly female susceptibility of 3-4:1 calls into question any simplistic theory of
occlusal dysharmony.

9.3.2. Psychophysiologic concept

This was first introduced by Schwartz (1959) and later modified by Laskin (1969) (fig. 4). According to this theory, psychological stress, leading to spasm and pain of the masticatory muscles is the primary aetiologic factor. However, if the condition persists, it can ultimately lead to organic changes in the dentition, muscles and TMJ (fig. 4).

**Figure 4.**

Psychophysiologic theory of myofascial pain dysfunction syndrome
Although the diagram shows that stress and dental irritation both lead to muscular hyperactivity, Yemm (1985) has shown that the central neuromuscular control is the main factor rather than the local reflex disturbances.

9.3.3. The role of stress

The psychophysiological theory implicates stress-related muscular hyperactivity as the primary cause of most cases of MPD syndrome.

These pain disorders have been shown to be associated with a high incidence of stress and adverse life events. These include school, work and marital difficulties, bereavement, chronic illness in the family and financial problems (Feinmann & Harris, 1984a,b; Speculand et al., 1984). Nevertheless, a recent study on a large number of TMJ patients and controls did not find any difference between TMJ patients and controls with regard to their desirable and undesirable life events (Marbach et al., 1988). However, it was found that these patients had fewer sources of emotional support thus indicating stress as an imbalance between the demand on the individual and coping ability. It has also been suggested that whether or not the amount of stress endured is higher than normal, chronic pain patients have personalities that are in some way more vulnerable to stress (Southwell et al., 1990). An explanation of why only certain individuals develop MPD, is that individuals with a history of MPD tend to respond to stress with greater masticatory and facial muscle activity than normal subjects. Therefore patients who are prone to develop psychophysiologic disorders will develop symptoms in their target area of response to stress (Laskin, 1992). Physiological studies support the concept that stress can induce masticatory muscle hyperactivity (Yemm, 1985). Yemm (1969, 1971) observed electro-
myographic changes in masticatory muscles in normal subjects under stress from a challenging manual task. Johnson et al. (1972) and Mercuri et al. (1979) found even greater masseter responses in patients with MPD syndrome than in normal individuals, particularly when they were subjected to psychologic stress. There is also evidence from studies of both masticatory and other body muscles that hyperactivity can produce symptoms of pain and dysfunction (Laskin, 1992). However, the underlying pathophysiologic mechanisms linking stress to pain and end-organ changes in the joint are unknown.

In a review of the literature our attention was drawn to studies claiming to demonstrate that emotional stress and pain in animals were associated with an increased generation of free radicals (Aleksandrovskii et al., 1988; Golikov et al., 1987; Vitrichenko, 1985) and stress induced damage to the gastric mucosa was related to free radical production (Itoh & Guth, 1985; Salim, 1989). Duthie et al. (1989) demonstrated in stress-susceptible pigs an elevated level of thiobarbituric acid reactive substances (TBA-RS) in plasma which is an index of increased free radical production. Furthermore, it is said that free radical related disease is significantly more common in women. Nevertheless, the underlying biochemical mechanisms linking stress, pain and free radicals are not clear.

In order to study reactions to stress, how they lead to pathological changes and how they may be antagonized, a controlled situation is needed and therefore it is necessary to induce stress experimentally. A comparison of different psychological, and biochemical reactions of patients (e.g. free radical metabolism) with stress related pain or other psychosomatic illnesses against healthy controls under such circumstances will undoubtedly increase our understanding of psychosomatic illness.
9.3.4. Psychological factors

Considerable effort has been made to test the hypothesis that psychological factors particularly anxiety and depression are involved in the aetiology, pathogenesis or maintenance of chronic pain syndromes. Although considerable evidence exists to support the role of anxiety (Fine, 1971; Gale, 1978), depression (Marbach & Dworkin, 1975; Moulton, 1966) and personality types (Southwell et al., 1990) in the condition, more recent studies (Schnurr et al., 1990) suggest that temporomandibular joint pain and dysfunction (TMJPD) patients do not appear to be significantly different from other pain patients or healthy controls. It has also been suggested that emotional disturbance in pain patients is more likely to be a consequence than a cause of chronic pain (Gamsa, 1990).

As far as the role of anxiety is concerned, some studies indicate that patients with MPD are in a state of greater anxiety than normal individuals (Laskin, 1992). However, it may be that the anxiety is a consequence of the disorder rather than a cause of it. Successful treatment outcome, usually results in significant reduction of the anxiety level. An unresolved issue is whether the sufferers have a predisposition to anxiety (high anxiety 'trait' scores) or whether the syndrome leads to concurrent feeling of anxiety in otherwise stable personalities (high 'state' anxiety). Moss & Adams (1984) used Spielberger State Trait Anxiety Inventory (STAI) and found no difference between TMJ patients and controls; whereas Southwell et al. (1990) used the same instrument and showed a higher trait anxiety scores in the patient group. They suggested that TMJ patients have personalities that are vulnerable to life stresses, but that they are not more anxious than controls at any given time. An attempt to reproduce the above findings may be of some value.
The relationship between chronic pain and depression is complex and unclear. Patients who are depressed may complain of pain, and any prolonged pain may lead to a depressed mood, but the presence of depressive symptoms is not necessarily equivalent to a depressive illness. However, it has been suggested that chronic pain without a pathophysiological basis is best conceptualized as a syndrome within the spectrum of depressive disorders; patients may fail to recognize affective distress, and instead 'somatise' their complaint (Craig, 1989).

Owing to methodological problems interpretation and generalizability of most of these studies have been limited. It remains unclear, whether the extent to which emotional states may cause or promote pain, are a consequence of pain or are simply correlates of pain. Some of the methodological problems include the use of self-reported questionnaires rather than standardized psychiatric assessments, lack of rigorous psychiatric diagnostic criteria and failure to determine rates of psychopathology preceding pain. Haythornwaite et al. (1991) after reviewing the use of self-report instruments that assess depression such as Beck Depression Inventory (Beck, 1961) expressed concern over the reliance upon self-report measures when studying depression. These authors cautioned that careful diagnostic procedures are necessary in order to identify depression. Doubts have also been expressed about the extent to which high scores on Beck Depression Inventory indicate clinical depression and not depressed mood of a transient nature or some other psychopathological state.
There is clearly a need to incorporate structured assessment and criteria into the measurement of psychopathology among chronic pain patients. These instruments allow identification of those whose complaints are clinically significant and not of a transient nature and allow comparison of findings across studies. Furthermore, by recording the age of onset of psychopathology the temporal relationship between pain and depression becomes apparent. This may help to reach conclusions about the cause and effect relationship of the two conditions.

9.3.5. Biochemical abnormalities:

9.3.5.1. Systemic biological markers

The association of different pains in an individual at different stages of life suggests the existence of a pain vulnerable person. Up to 80% of patients with chronic idiopathic orofacial pain also complain of other chronic pain symptoms. In childhood, such patients suffer from abdominal pain or dysmenorrhea and later abdominal pain (usually irritable bowel syndrome), neck pain, back pain and pruritus (Berry, 1969; Feinmann et al., 1984). It has also been suggested that this vulnerability may be genetically transmitted as children of TMJ pain patients seem to suffer from a higher incidence of illness and injury (Raphael et al., 1990).

It has been estimated that 30-60% of patients with 'non-organic' chronic pain suffer from depression. This incidence seems to be higher not only than in the general population, but also than in those with chronic nociceptive pain (Magni, 1987). It has also been reported
that a high proportion of subjects with such pains also suffer from emotional disorders. This has led to the hypothesis that chronic pain and depression may share a common biological pathogenesis. This is supported by the finding that both tricyclic antidepressants and monoamine oxidase inhibitor antidepressants have been found to be effective in the treatment of chronic pain (Feinmann, 1985). Furthermore, it has been reported that the efficacy of tricyclic antidepressants is independent of its effect on depression (Sharav et al., 1987).

The search for biological markers in depression and chronic pain has attracted considerable interest (Gjerris, 1989; von Knorring, 1989). Patients with idiopathic pain have been shown to have hypercortisolaemia and abnormal dexamethasone suppression test responses as well as low platelet 3H-imipramine binding, low cerebrospinal fluid 5-hydroxyindolacetic and low platelet monoamine oxidase activity. Patients with idiopathic pain syndromes have also been demonstrated to have low concentrations of melatonin in serum and urine (reviewed by von Knorring, 1989). These studies suggest that there may be a common pathogenesis between depression and idiopathic pain, and point particularly to the possible involvement of 5-hydroxytryptamine. However, most of these markers are state markers. A recent promising lead in this field is the tyramine conjugation test. The abnormal tyramine conjugation test is shown to be a trait marker in patients with 'endogenous' depression (Hale et al., 1986). This abnormality is independent of the severity of illness and age, persists after recovery (Bonham Carter et al., 1978) and is present in some of the patients' first degree relatives who have never been depressed (Hale et al., 1986). Furthermore, the test has also been shown to be a reliable predictor of the outcome of treatment with tricyclic antidepressants (Hale et al., 1989).
A study of biological markers of chronic facial pain, in addition to providing insight into the pathophysiology of the condition, may help in the identification of those at risk and be of assistance in patient management.

9.3.5.2. Local mediators

Neither the psychophysiologic theory nor the peripheral theories such as occlusal dysharmony adequately explain the endorgan changes which may lead to pain and internal derangement.

In some patients marked localized pain and tenderness of TMJ suggests a capsulitis; if persistent this may lead to development of adhesions in the joint space and subsequent internal derangement and limited opening. These features can be confirmed with arthroscopy. Surgical exposure also reveals a joint capsule that is invariably thickened, implying a chronic capsulitis.

Synovial fluid occupies a key position in joint physiology. Although aspiration and analysis of synovial fluid is commonly performed in other joints (Cohen, 1967), owing to difficulties associated with the sample collection and problems associated with interpretation of the results, very few methodologically sound studies of TMJ are available. Toller (1961) was unable to aspirate any free fluid from the TMJ in more than 250 attempts on human subjects. In most normal joints the aspiratable volume is very small in relation to the internal area. Furthermore, the aspiration is never total and has been estimated to constitute only 52% of the total fluid volume (Rekonen et al., 1973). Toller 'guesstimated' the total volume of TMJ synovial fluid to be no more than 50μl. This small volume does not allow the use of established methods applied to larger joints.
for accurate estimation of the TMJ synovial fluid volume.

The importance of estimation of the synovial fluid volume is that it provides an estimate of the joint space. One important parameter which may be central to any disturbance in movement is the volume of synovial fluid in the joint which is also an index of any pathological change in the joint space. Therefore, there is a need for a method for the estimation of TMJ synovial fluid volume.

During the past decade, saline aspirates of the upper joint space of the TMJ have increasingly been analyzed for the presence of various mediators of pathology (Kopp et al., 1983; Quinn & Bazan, 1990; Holmlund et al., 1991). Quinn & Bazan (1990) have found PGE, in saline aspirates obtained during arthroscopic procedures on painful TMJs. However, the failure of non-steroidal anti-inflammatory analgesics to block chronic facial pain suggests that the prostanoids are not the sole algesic agents in this condition. Arachidonic acid can also be metabolised via the lipoxygenase pathway which is not inhibited by NSAID.

Therefore, it is possible that the chronic facial pain could be due to local generation of products of lipoxygenase activity.

It has been suggested that psychological stress can lead to the peripheral release of neuropeptides such as substance P (Matis et al., 1990). Substance P plays an important role in neurogenic inflammation (Levine et al., 1987) and has also been implicated in the mechanism of migraine (Hardebo, 1984). The temporomandibular joint capsule and disc
are innervated by many substance P containing fibers (Johansson et al., 1986). Holmlund et al. (1991) have demonstrated the presence of high levels of substance P in the temporomandibular joint saline aspirates. Substance P is capable of activating leucocytes and stimulating free radical generation which can contribute to tissue damage (Hartung & Toyka, 1983). It is of interest that hyperalgesia induced by the eicosanoid 15-HPETE in experimental animal models can be blocked by a substance P antagonist (Garland, personal communication).

Therefore, a credible hypothesis for these pains is that, in a metabolically vulnerable person, emotional or even physical stress promotes the release of neuropeptides such as substance P in the joint capsule, muscle or such sites as the periodontal membrane and dental pulp. These induce localized hyperalgesia, vasodilatation, and free radical release. Localized free radical damage will then produce eicosanoid algesic agents such as PGE₂ and 15-HPETE.
10. AIMS OF THE STUDY

The investigations described in the following chapters address four groups of questions related to the different aspects of the pathophysiology of chronic idiopathic orofacial pain.

10.1. Psychological characteristics

Faced with the inconsistencies in the literature regarding the role of psychological factors in the pathophysiology of chronic facial pain, it was decided to use a structured clinical interview according to rigid diagnostic criteria to identify the prevalence of psychopathology in our patients. Using this instrument, it would also be possible to examine the temporal relation between pain and depression. A self-report measure of trait anxiety was also used in order to test the reproducibility of previous reports in relation to vulnerability of TMJ patients to stress.

10.2. Biological markers of depression in patients with facial pain

The aim was to test the hypothesis that chronic facial pain and depression share a common biological pathogenesis. It was hoped that the study in addition to providing insight into the pathophysiology of the condition, may help in the identification of at risk groups and be of assistance in patient management. Two biological markers were chosen:

a. Tyramine conjugation test:

Unlike other biological markers studied in pain and depression, which are state markers, tyramine conjugation test has been shown to be a trait marker in depression independent of severity of illness and age.
b. Platelet monoamine oxidase activity:
This marker has been suggested to reflect the functional capacity of the central serotonin system which is not only involved in the pathogenesis of depression but also in pain modulation. The enzyme activity is stable and under genetic control.

10.3. Oxygen free radical metabolism

The aim was to test the following hypothesis:

1. Stress leads to increased generation of free radicals in human.
2. Oxygen free radicals and in particular hydroxyl radical contribute to the pathogenesis of orofacial pain.
3. Free radical metabolism differs in patients compared to controls in response to stress.

10.4. Temporomandibular joint synovial fluid analysis

The aims were:

1. Determination of the volume of the synovial fluid.
2. Analysis of the synovial fluid for indices of free radical induced tissue damage.
3. Analysis of the synovial fluid for the hyperalgesic products of lipoxygenase activity.
CHAPTER II. PSYCHOLOGICAL CHARACTERISTICS OF

PAIN PATIENTS
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1. INTRODUCTION

Despite the increasing evidence implicating psychological factors in patients with chronic facial pain, this aspect of management is frequently neglected by Oral Surgeons (Speculand & Goss, 1985). There is a risk of either completely ignoring psychological factors and proceeding with an escalating series of physical treatments or alternatively labelling all patients who fail to respond to simple treatment as 'psychiatric cases'.

The dichotomy of pain on the basis of aetiology as being physical(somatic) or psychological lies at the root of some of the misconceptions related to pain management. These misconceptions are derived from a sensory-physiological model of pain that has been dogma in medical training during the past several hundred years. The major premise of the sensory physiologic model of pain is that there is a direct relationship between physical pathology and sensation of pain. The greater the tissue pathology, the greater the pain that should be experienced. Consequently removing the source of pain will eliminate the report of pain. However a compelling literature documents that there is much that is physical in mental disorders and much that is mental in physical disorders.

The aetiology of TMJ syndrome remains obscure and there is often no detectable physical lesion. Considerable effort has been made to test the hypothesis that psychological factors particularly anxiety and depression are involved in the aetiology, pathogenesis or maintenance of the syndrome. Although considerable evidence exists to support the role of anxiety (Fine, 1971; Gale, 1978), depression (Moulton, 1966; Marbach & Dworkin, 1975) and personality types (Southwell et al., 1990) in the condition, more recent studies (Schnurr et al., 1990) suggest that TMJPD patients do not appear to be
significantly different from other pain patients or healthy controls in personality type, response to illness, attitudes towards health care or ways of coping with stress. It has also been suggested that emotional disturbance in pain patients is more likely to be a consequence than a cause of chronic pain (Gamsa, 1990). Owing to methodological problems, interpretation and generalizability of the results of most of these studies have been limited. It remains unclear, whether the extent to which emotional states may cause or promote pain, are a consequence of pain, or are simply correlates of pain.

Some of the methodological problems include the use of self reported questionnaires rather than standardized psychiatric assessments, lack of rigorous psychiatric diagnostic criteria, and failure to determine rates of psychopathology preceding pain.

1.1. Methodological problems

1.1.1. Self reported questionnaires

Haythornwaite et al. (1991) after reviewing the use of self-report instruments that assess depression such as Beck Depression Inventory (BDI) (Beck, 1961) expressed concern over the reliance upon self-report measures when studying depression. These authors cautioned that careful diagnostic procedures are necessary in order to identify depression. Doubts have also been expressed about the extent to which high scores on BDI indicates clinical depression and not depressed mood of a transient nature or some other psychopathological state.
Another widely used assessment questionnaires is the Minnesota Multiphasic Personality Inventory (MMPI). McCall et al. (1961) tested 140 TMJ patients and 70 controls on MMPI. They found 48 items which discriminated between the TMJ patients and the controls at p < 0.05. 23 of the items were related to bodily symptoms and the rest were related to feeling of anxiety and depression. Sternbach (1974) has also reported elevation on the hypochondriasis scale and the hysteria scale in a study on chronic pain patients. However, it should be noted there are a number of limitations in using standardized psychological testing with chronically ill population. For example these instruments are not developed for nor standardized on medical populations and there is some suggestion that the MMPI items on hypochondriasis and hysteria scales are associated with physical symptoms that may be characteristic of any individual with a physical condition (Pincus et al., 1986).

Thompson (1989) summarizes the disadvantages associated with the use of self-rating scales as follows:

1. The reliability is often unknown and virtually inaccessible to study. Reliability is the extent to which the score on a scale reflects the hypothetical true score. Inter-rater reliability is by definition inapplicable and this is the most important statistic available for observer ratings.

2. The validity of self-rated scales is also more open to doubt than observer ratings. Patients themselves may not use words descriptive of emotion in the same way as clinicians. Hence, only commonly used words with obvious meaning to the layman can be used in self-rating scales. Even so there is no guarantee that patients are using these words in the way intended. In particular, items requiring judgements of intensity of
emotion may well be over-rated by the patients.

3. Severely ill patients are usually unable to complete the form either through retardation or agitation or because of indecision or lack of concentration.

4. Unlike the situation in a clinical interview it is very easy for the patient who is inclined to conceal illness during completion of a rating form.

5. Social desirability set: the tendency to answer in a way which is thought by the patient to be socially acceptable.

6. Self rating scales are unable to collect information about important aspects of the syndrome such as psychomotor disturbances or insight.

Nevertheless, self rating scales have an economic advantage when compared with observer rating scales. They save experimenter's time so more patients can be assessed or studies can be carried out with fewer resources of manpower.

1.1.2. Lack of rigorous psychiatric diagnostic criteria

All science depends on the ability to measure natural phenomena. If psychiatric knowledge is to develop, then accurate definition and measurement are essential. In psychiatry this is difficult. The pragmatic researcher accepts the inadequacies of knowledge in his subject and measures as accurately as possible under the circumstances, the proof of the pudding is then in the eating. If the measures were inappropriate or inaccurate then results will be insignificant or unreplicable. Diligence in defining terms (operationalizing them) is the most important element of this pragmatic approach, for a common understanding is crucial to communicability and the general applicability of results.
Speculand & Goss (1985) reviewed 10 studies which attempted to identify personality characteristics common among TMJPD patients. They noted that the terms used were not well defined, giving considerable overlap in characteristics. The publication of DSM-* III (American Psychiatric Association, 1980) and DSM-III-R (American Psychiatric Association, 1987) revolutionized psychiatry with its inclusion of specified diagnostic criteria for virtually all of the mental disorders. The explicit diagnostic criteria improved the poor reliability achieved by previous systems and facilitated clinical communication and research. The manual has been widely used by clinicians, educators, trainees and researchers. Conceptually DSM-III-R is a radical departure from International Classification of Disease, Ninth Edition (ICD9). Its internal structure is rigidly demarcated and individual diagnoses are defined in a relatively precise manner (Feinmann & Bass, 1989). Lack of well demarcated syndromes in ICD-9 has meant that this system has not been as widely used in research compared to DSM-III-R. However, groups working on DSM-IV and ICD-10 are trying to increase the compatibility between systems (Frances et al., 1990).

Spitzer (1989) who served as chairperson of the American Psychiatric Association Task Force to produce DSM-III and DSM-III-R developed the structured clinical interview to ascertain diagnosis according to DSM-III-R. The advent of standardized psychiatric assessment techniques such as SCID represent an important methodological advance in studying psychiatric illness in pain patients. These instruments reduce criterion and information variance, survey for a broad range of major psychiatric conditions and record ages of illness onset. By recording the age of onset of psychopathology the temporal relation between pain and depression becomes apparent. This may help to reach

* DSM : Diagnostic and Statistical Manual of Mental Disorders.
conclusions about the cause and effect relationship of the two conditions. Furthermore, structured interviews allow identification of those whose complaints are clinically significant (e.g. major depression) and not of a transient nature (e.g. depressed mood) and allow comparison of findings across studies.

1.2. Aims of the study

This study was carried out with the following aims:

1. To determine the lifetime prevalence and type of psychiatric illness in patients with chronic idiopathic facial pain.

2. To determine the temporal relation between pain and depression.

3. To determine whether the patients have stress-prone personality predisposition.

2. MATERIALS AND METHODS

34 patients (F:M=30:4, age 40.2±14.5) who had suffered from an orofacial pain condition of at least 6 months duration (mean 5.6±5.5 years, range 0.5-25 years) for which no primary structural pathology was found, underwent the structured clinical interview for DSM-III-R (SCID). The SCID is a semistructured interview which consists of two major sections (SCID-I & SCID-II) for making the major axis I and axis II diagnoses. Ordinarily the Axis I SCID is administered in a single sitting and takes from 60 to 90 minutes, depending on the complexity of psychiatric history and the ability of subject to describe his or her psychopathology succinctly. Usually the SCID II is administered following the Axis I SCID.
The SCID-I consists of probes for the diagnostic criteria for DSM-III-R axis I disorders which contains the following modules:

A. mood syndromes
B/C. psychotic screening
D. mood disorder
E. psychoactive substance use disorder
F. anxiety disorders
G. somatoform disorders
H. Eating disorders
I. Adjustment disorders

At the beginning of the interview, the interviewer obtains an overview of the present illness and past episodes of psychopathology before systematically inquiring about specific symptoms. By the end of the overview, the interviewer should have gathered enough information to formulate a tentative differential diagnosis. Since the DSM-III-R diagnostic criteria are embedded in the SCID and are assessed as the interview progresses, the interviewer is, in effect continually testing diagnostic hypotheses. With a few exceptions, the SCID determines whether an Axis I diagnosis has ever been present (lifetime prevalence) and whether or not there is a current episode (defined as meeting diagnostic criteria within the past month). At the end of each Axis I diagnostic section there is a series of questions about the chronology of the illness that includes: age at onset, presence or absence of symptoms during the past month, and duration of symptoms during the past 5 years.
Raters begin the questioning on a specific symptom with the written probe that is given in the interview schedule. Once subjects respond positively or doubtfully to any items, the interviewer explores the symptom to determine how the symptom should be rated by the SCID scoring scheme, which consists of 3 levels.

1. Present and of clinically significant severity
2. Present but of threshold severity (not counted towards the diagnosis)
3. Absent

The SCID-II is designed to evaluate twelve DSM-III-R axis II personality disorders which include:

1. Avoidant personality disorder
2. Dependent personality disorder
3. Obsessive compulsive personality disorder
4. Passive aggressive personality disorder
5. Self-defeating personality disorder
6. Paranoid personality disorder
7. Schizotypal personality disorder
8. Schizoid personality disorder
9. Histrionic personality disorder
10. Borderline personality disorder
11. Antisocial Personality disorder
12. Narcissistic personality disorder

In order to shorten the time that it takes to evaluate the large number of diagnostic criteria, each subject fills in a self-report personality questionnaire. Each item in the
personality questionnaire corresponds to a question in the SCID II. The personality questionnaire acts as a screening device with intentionally higher rate of false positives (acknowledging a personality problem on the questionnaire that on further questioning turns out not to meet the criterion).

When administering the SCID-II, the interviewer asks only about criteria that correspond to the questionnaire items circled 'yes'. Items circled 'no' on the questionnaire are usually not probed since further probing is not expected to reveal psychopathology. However, there are two circumstances in which items that are circled 'no' on the questionnaire are explored in the SCID-II interviews:

1. When there is clinical basis to suspect the item is true.
2. When the number of SCID-II items coded as present are within one item of diagnostic threshold for a particular disorder.

Training for administration of SCID

My training for administration of SCID involved the following sequence:

1. Studying the basic features, different versions of the interview and the instruction manual.
2. Studying SCID videotapes which include a 6 hour didactic introduction and also videotapes of SCID interviews.
3. Administration of the initial interviews under the supervision of Dr. Feinmann, consultant psychiatrist.
Self-report questionnaires

The patients also filled in two self report questionnaires:

1. Spielberger trait anxiety inventory STAI (Spielberger et al., 1983)
2. Hospital anxiety and depression scale HAD (Zigmond & Snaith, 1983)

A trait is a relatively enduring personality characteristic while a state is a psychological condition occurring at that moment in time. STAI is designed to measure the anxiety trait by asking subjects to rate how they generally feel. About half of the scale is made up of 'anxiety present' items and half of 'anxiety absent' items. Subjects rate the questions on a four point scale.

Anxiety is an integral component of depression and the overlap between the two mood states is at its greatest in the milder disturbances, when they may be inextricably mixed. HAD is designed to measure the state anxiety and depression. It consists of two sets of seven questions, one representing an anxiety subscale, the other representing a depression subscale (Zigmond and Snaith, 1983). Each item is rated using a four point frequency scale which is scored from 0-3. The items are based only on psychic symptoms of depression/anxiety and is independent of physical illness.
3. RESULTS:

Figure 5 shows different orofacial pain conditions diagnosed in 34 patients. Some patients suffered from more than one clinical or psychiatric condition.

Figure 5. Orofacial pain conditions in 34 patients
3.1. SCID

Table 3 shows the prevalence of current and past psychiatric diagnoses for individual patients. Table 4 presents the relative frequency of different psychiatric diagnosis. A lifetime history of major depression was the commonest (50%). However, only 5(14%) were suffering from major depression or dysthymia at the time of examination.

To examine the relationship between depression and pain, the time of onset of pain was compared to that of depression. We examined whether an episode of depression had occurred within one year of pain onset, arbitrarily choosing this as a reasonable time frame for an immediate cause-effect chain of events.

In 13(76%) of those with a positive lifetime history of depression, the depressive episode preceded the pain, but in 5(29%) the depressive episode occurred within 12 month period before the pain onset, 2 of whom were still suffering from depression at the time of examination. In the majority of cases the past depressive episode seemed unrelated to pain and there was a minimum of one year and often longer period free of history of depressive symptoms before the pain onset.

In 4(23%) of those with a positive history of depression, the depressive symptoms developed after pain onset. In one case this was secondary to pain, in two cases the mood disorder was secondary to social and familial problems and in one case it was part of the reaction to a severe stressful life event which led to a post-traumatic stress disorder (PTSD).
Table 3. Psychiatric diagnoses for individual patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Clinical diagnosis</th>
<th>Psychiatric diagnosis current</th>
<th>Psychiatric diagnosis past</th>
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<tbody>
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<td>Bulimia neurosis</td>
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<td>Avoidant PD</td>
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<td>Major depression</td>
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<td>Anorexia neurosis</td>
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<td>AFP,AO</td>
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<td>4.</td>
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<td>M</td>
<td>FAM</td>
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<td>5.</td>
<td>48</td>
<td>F</td>
<td>FAM</td>
<td>PTSD</td>
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<td>Paranoid PD</td>
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Table 3. Psychiatric diagnoses for individual patients

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<td>26</td>
<td>F</td>
<td>FAM</td>
<td>-</td>
<td>Major depression</td>
</tr>
<tr>
<td>28.</td>
<td>22</td>
<td>F</td>
<td>FAM, whiplash</td>
<td>-</td>
<td>PTSD</td>
</tr>
<tr>
<td>29.</td>
<td>32</td>
<td>F</td>
<td>FAM</td>
<td>-</td>
<td>Major depression</td>
</tr>
<tr>
<td>30.</td>
<td>21</td>
<td>F</td>
<td>AFP</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>31.</td>
<td>30</td>
<td>F</td>
<td>FAM</td>
<td>-</td>
<td>Major depression, bipolar (type II)</td>
</tr>
<tr>
<td>32.</td>
<td>23</td>
<td>F</td>
<td>FAM(traumatic)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>33.</td>
<td>52</td>
<td>F</td>
<td>AFP, FAM, whiplash</td>
<td>Dysthymia, Dependent PD, Major depression,</td>
<td>PTSD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Dependent PD</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PTSD</td>
<td></td>
</tr>
<tr>
<td>34.</td>
<td>38</td>
<td>F</td>
<td>AFP</td>
<td>Major depression</td>
<td>PTSD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Paranoid PD</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Relative frequency of psychiatric diagnoses

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Current</th>
<th>Past</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major depression</td>
<td>3</td>
<td>15</td>
<td>17(50%)</td>
</tr>
<tr>
<td>Dysthymia</td>
<td>2</td>
<td>-</td>
<td>2(6%)</td>
</tr>
<tr>
<td>Bipolar disorder</td>
<td>1</td>
<td>-</td>
<td>1(3%)</td>
</tr>
<tr>
<td>Bulimia neurosis</td>
<td>1</td>
<td>-</td>
<td>1(3%)</td>
</tr>
<tr>
<td>Anorexia neurosis</td>
<td>1</td>
<td>-</td>
<td>1(3%)</td>
</tr>
<tr>
<td>PTSD</td>
<td>2</td>
<td>3</td>
<td>5(15%)</td>
</tr>
<tr>
<td>Alcohol dependence</td>
<td>1</td>
<td>-</td>
<td>1(3%)</td>
</tr>
<tr>
<td>Panic disorder + agoraphobia</td>
<td>1</td>
<td>-</td>
<td>1(3%)</td>
</tr>
<tr>
<td>Panic disorder - agoraphobia</td>
<td>-</td>
<td>1</td>
<td>1(3%)</td>
</tr>
<tr>
<td>Generalized anxiety disorder</td>
<td>1</td>
<td>-</td>
<td>1(3%)</td>
</tr>
<tr>
<td>Psychotic disorder NOS</td>
<td>1</td>
<td>-</td>
<td>1(3%)</td>
</tr>
<tr>
<td>Personality disorders:*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avoidant personality disorder</td>
<td></td>
<td></td>
<td>2(6%)</td>
</tr>
<tr>
<td>Dependent personality disorder</td>
<td></td>
<td></td>
<td>2(6%)</td>
</tr>
<tr>
<td>Paranoid personality disorder</td>
<td></td>
<td></td>
<td>2(6%)</td>
</tr>
<tr>
<td>Passive aggressive personality disorder</td>
<td></td>
<td></td>
<td>1(3%)</td>
</tr>
<tr>
<td>Borderline personality disorder</td>
<td></td>
<td></td>
<td>1(3%)</td>
</tr>
</tbody>
</table>

* According to DSM-III-R, personality disorders are inflexible, maladaptive traits of perceiving and relating to and thinking about the environment and oneself. They are often recognizable by adolescence and continue through most or all of adult life. Therefore, a classification into current and past is non-applicable.

Stressful life events within a 12 month period before the pain onset were frequently acknowledged by the patients (64%). However, in 5 cases the severity of the stressful event had led to a post-traumatic stress disorder which was the second commonest diagnosis (15%) in these patients. Two (6%) were experiencing the symptoms of PTSD currently and 3 (9%) had suffered from PTSD in the past. Other psychiatric diagnosis were infrequent. 15 (44%) had a clear lifetime history of psychiatric illness, and 25 (74%) had no current psychiatric illness.
3.2. Self-report questionnaires:

3.2.1. STAI

The patient's mean score of STAI was 42.5±12.3. This is significantly higher (p<0.01) than the average values obtained from large normal population samples (36 ± 10) as well as our own control subjects (37.1 ± 9.2, p<0.01). However, general medical and surgical patients even without psychiatric complications are shown to have similar values (42.7±13.8).

3.2.2 HAD

In relation to current mood disorders, HAD identified depression in all but one case which was secondary to pain. However, two other patients with high depression scores on HAD, when interviewed by SCID, did not receive a diagnosis of major depression or dysthymia.

4. DISCUSSION:

4.1. Depression and pain

Since pain has been shown repeatedly to be associated with depression (Gupta, 1986; Magni, 1987) some researchers have concluded that depression is a precursor of pain, or have suggested that chronic pain is alternate expression of a depressive state (Blumer & Heilbronn, 1982; Lesse, 1974).

Whether pain arises from depression or depression is reactive to pain has been argued extensively in the literature. Some early work suggested that in the majority of cases depression either preceded or developed simultaneously with the onset of chronic pain (Bradley, 1963; Lindsay & Wyckoff, 1981). Others argued that generally depression was 'reactive' or secondary to pain (Sternbach, 1974).
Our results show that chronic pain patients have a higher lifetime prevalence of depression (50%) than the general population which is estimated to be 12% (Schuyler & Katz, 1973). The view that depression is secondary to pain cannot be supported as only 4 (11%) developed depression after the pain onset, and among these 4, only one case was reactive to pain problem. On the other hand, we could not find support for the opposite view that depression is a precursor of pain. Although in the majority of cases with a positive history of depression, the depressive episode preceded the pain onset, only in 5 (29%) the mood disorder occurred in close temporal relationship to the pain disorder. This finding is in agreement with Gamsa (1990) that there is inadequate evidence to substantiate the view that depression precedes and generates pain in the majority of chronic pain sufferers (Gupta, 1986; Romano & Turner, 1985).

It has also been a widely held assumption that because chronic pain is relieved by tricyclic antidepressants, the pain represents an underlying depressive illness (Blumer & Heilbronn, 1982; Katon, 1984). Patients without evidence of classical depression are often described as suffering from 'masked depression' or a 'depressive equivalent' (Lopez Ibor, 1972). Such diagnostic categories have doubtful validity and there are dangers inherent in equating outcome and response to treatment as adequate criteria for diagnosis (Feinmann & Bass, 1989). We have shown the presence of tyramine conjugation deficit which is an established trait marker of endogenous depression in pain patients (see III.2). Such biochemical vulnerability explains the high lifetime prevalence of depression in chronic pain patients without any implication on the cause and effect relationship between pain and depression.
4.2. Stressful life events and PTSD

There has been a substantial body of work to show that chronic orofacial pain is stress related (Lefer, 1966, Fine, 1971, Feinmann & Harris, 1984). Therefore recognition of stressful stimuli responsible for the psychosomatic component operative in a pain condition is essential for establishing both diagnosis and appropriate therapy. Feinmann et al. (1984) in a psychiatric study of patients with chronic facial pain showed that 75% of patients without a psychiatric history and 87% of pain patients with a psychiatric history reported life events within six months before the onset of pain. In our study a considerable proportion of patients (64%) also reported a stressful life event within 12 months before the onset of pain.

In 5 cases the severity of the stressful event had led to PTSD. Although the PTSD has been recently defined, the concept has been recognized for a long time. The essential feature of PTSD is the development of characteristic symptoms following a psychologically distressing event that is outside the range of usual human experience. The stressor producing this syndrome would be markedly distressing to almost anyone, and is usually experienced with intense fear, terror and helplessness. Examples would be assault, a road traffic accident or some form of mass accident. The characteristic symptoms involve re-experiencing the traumatic event, the avoidance of stimuli associated with the event or numbing of general responsiveness, and increased arousal (table 5).

Other psychiatric conditions however, often coexist with PTSD and may themselves be brought on by life events. It has also been shown that patients demonstrating this psychological symptoms carry a concurrent increased risk of physical disorders, such as peptic ulceration, asthma and hypertension, all of which have traditionally been thought of as, in part, stress-related disorders (Davidson et al., 1991).
Table 5. Diagnostic criteria for post-traumatic stress disorder according to DSM-III-R

A. The person has experienced an event that is outside the range of usual human experience and that would be markedly distressing to almost anyone

B. The traumatic event is persistently reexperienced in at least one of the following ways:
   1. Recurrent and intrusive distressing recollections of the event
   2. Recurrent distressing dreams of the event
   3. Sudden acting or feeling as if the traumatic event was recurring
   4. Intense psychological distress at exposure to events that symbolize or resemble an aspect of the traumatic event

C. Persistent avoidance of stimuli associated with the trauma or numbing of general responsiveness, as indicated by at least three of the following:
   1. Efforts to avoid thoughts or feelings associated with the trauma
   2. Efforts to avoid activities or situations that arouse recollections of the trauma
   3. Inability to recall an important aspect of the trauma
   4. markedly diminished interest in significant activities
   5. Feeling of detachment or estrangement from others
   6. Restricted range of affect
   7. Sense of foreshortened future

D. Persistent symptoms of increased arousal as indicated by at least two of the following:
   1. Difficulty falling or staying asleep
   2. Irritability or outbursts of anger
   3. Difficulty concentrating
   4. Hypervigilance
   5. Exaggerated startle response
   6. Physiologic reactivity upon exposure to events that symbolize or resemble an aspect of the traumatic event
Due to traumatic aetiology of many pain conditions, a substantial proportion of patients seen in any pain clinic may suffer from post-traumatic stress syndromes. However, this has not been recognized until recently due to the absence of well defined diagnostic criteria and a lack of objective methods of assessment of psychiatric morbidity. PTSD is still a diagnosis which is frequently missed even by psychiatric professionals (Davidson & Smith, 1990). Davidson (1989) has outlined four main reasons why a diagnosis of PTSD might be overlooked:

a. Not asking the patient about experiences of trauma.

b. Patients' reluctance to disclose painful material.

c. Physicians' discomfort in discussing events which might be gruesome, horrifying or unimaginable.

d. The fact the chronic PTSD presents with non-specific symptoms such as headache, insomnia, irritability, depression, tension, substance abuse, interpersonal or professional dysfunction.

The prevalence of PTSD in our study is 15%, this compares to 9.5% reported by Muse (1985) in a study of 64 chronic pain patients. The point prevalence of PTSD in the general population is about 1% (Jackson, 1991). Thus the prevalence of this syndrome in the present study is substantial when a comparison is made of its relative ranking among other psychological diagnoses associated with chronic pain. Shepherd (1990) in a study of psychiatric morbidity following personal violence, reported behavioral changes in two thirds of victims six months after assault, particularly the avoidance of locations of violence which is also frequently observed in PTSD sufferers.
This study raises a number of questions about stress related post-traumatic chronic pain.

1. What is the real frequency of this syndrome in the chronic pain population?

The present study provides some idea on the prevalence of the condition. However, it is possible that patients with chronic facial pain may differ qualitatively from other groups of pain patients. For instance, Atkinson et al. (1991) in a study of patients with chronic low back pain (CLBP) found that 64.9% had a history of alcohol abuse and suggested that alcohol use disorders rather than depression may increase the risk of developing CLBP. Whereas in our study there was only 1(3%) case of alcohol abuse. Furthermore, departments of Maxillofacial Surgery may care for a significant percentage of latent cases amongst the patients who have sustained an assault or a road traffic accident, the commonest cause of facial injury.

2. However, if one considers the high frequency of traumatic incidents, why is the prevalence of PTSD relatively low?

One explanation is that the complex responses to trauma are influenced by culture, beliefs and social support (Lima et al., 1990). Several studies indicate that preexisting psychopathological conditions predispose to the development of the PTSD. Davidson et al., (1991) in an epidemiological study showed that patients with PTSD had significantly higher family history of psychiatric illness, parental poverty, child abuse and separation or divorce of parents. In our study the majority (80%) of the PTSD sufferers also had personality disorders. However PTSD can develop in people without any such preexisting conditions, particularly if the stress is extreme. Nevertheless, it has been suggested that the psychiatric morbidity following an accident is largely dependent on a subjective response to the event and the amount of distress it engenders, as opposed to the nature

3. What is the relation between PTSD, pain onset and its chronicity?

The association of PTSD and pain, suggests a causal role for PTSD in pain onset. The symptoms of PTSD usually begin immediately or soon after the trauma, although the re-experiencing symptoms may develop after a latency of months or years. It is possible that pain is the result of reexperiencing the pain associated with the traumatic event. Davidson et al., (1991) reported that PTSD sufferers are 90 times more likely to suffer from a somatization disorder than the general population which suggests a connection between PTSD and process of conversion. Other than the stress disorder, other factors such as 'compensation neurosis' or learned pain behaviour may also contribute to the chronicity in certain cases (Muse, 1985).

An important diagnostic problem of medico-legal significance is the neuropathic nature of some post-traumatic psychogenic facial pain which resemble causalgia. That is a persistent burning pain associated with flushing and oedema. The underlying pathophysiological mechanism of such causalgic pains is believed to be abnormal sensitization of damaged sensory nerves to sympathetic stimulation and to a variety of mechanical, thermal and chemical stimuli (Koltzenburg & McMahon, 1991). Furthermore, if the patient has sustained a head injury, careful attention should be given to the possibility that some, or all, of the observed PTSD symptoms might stem from an organic lesion (Roth, 1988).

4. What is the significance of the 'stress related post-traumatic chronic pain syndrome' in patient management?

If PTSD contributes to a painful condition then therapy should be orientated towards the
resolution of the stress disorder. Effective treatment entails use of psychotropic medication (Davidson, 1992). Tricyclic and monoamine oxidase inhibitors have been the most widely studied drugs, and their effect increases with duration of treatment. Pharmacotherapy is probably most effective when administered as part of a broadly based treatment plan. Behavioral treatment directed at exposure to avoided situations or thoughts is often a critical step in treatment (Richards & Rose, 1991). Muse (1986) demonstrated the efficacy of both pharmacological and non-pharmacological desensitization in alleviating anxiety and depression associated with the syndrome. However, these therapeutic approaches do not relieve the associated pain in all cases. This suggests the contribution of other factors, such as compensation neurosis and a learned pain behaviour. However, elimination of anxiety and depression should be beneficial in promoting an early return to increased levels of psychosocial and vocational functioning and the ability to cope with chronic pain.

5. Is every area of the body capable of becoming involved in a stress related post-traumatic chronic pain syndrome or is the face particularly at risk?

The face is one of the most complex areas of the body in terms of pain presentation. The intricate innervation and the concentration of all the special senses give the area a unique significance. In addition the face has a special significance which arises from its emotional and social importance. A major traumatic incident involving the face frequently results in the affected area becoming the focus of the attention of the patient and if there is a susceptible psychiatric make-up, the affected site may become the focus of unresolved conflicts.

In our study various orofacial structures were involved. The clinical diagnoses included atypical odontalgia, atypical facial pain and also facial arthromyalgia including that
associated with whiplash injury. The last condition has frequently been attributed to a physical internal derangement of the meniscus brought about during the traumatic deceleration of the head (Weinberg & Lapointe, 1987). Two cases of PTSD had been diagnosed as this whiplash TMJ internal derangement as a result of whiplash injury. There may be a substantial proportion of these patients, in whom a hidden stress element plays a greater role in the chronicity of the condition than the simple mechanical disturbance. In these cases, therapy should always be directed towards the resolution of the stress disorder.

A means of assessing the subjective distress following a traumatic accident is the use of the Impact of the Event Scale (IES) as suggested by Horowitz et al. (1979). IES is a self-reported questionnaire which can be easily completed by the patients. This must be used at the beginning of an event and its administration at regular intervals helps to assess the patients psychological response and will indicate the course of PTSD symptoms if they develop. If the IES score remains unchanged then referral to a psychiatrist for management of the stress problem is indicated.

4.3. Trait Anxiety and pain

Trait anxiety refers to relatively stable individual differences in anxiety responses, that is to differences between people in the tendency to perceive stressful situations as dangerous or threatening.

It has been suggested that high anxiety is a significant characteristic of TMJ syndrome (Fine, 1971; Gale, 1978; Speculand & Goss 1985). However, Moss & Adams (1984) failed to show any difference in STAI between the TMJ patients and controls. Southwell et al. (1990) on the other hand, in a larger study found a significant difference
in STAI score between TMJ patients and controls who were selected from a denture clinic. These authors, suggested that TMJ patients have personalities that are vulnerable to life stresses, but that they are not more anxious than controls at any given time. Our patient group had similar trait anxiety scores to that of patients studied by Southwell et al., which is significantly different from the normal population value. However, the patients' trait anxiety score is similar to that of general medical and surgical patients. One possible explanation for this similarity could be that stress prone personalities are more vulnerable not only to pain but also other psychosomatic illnesses.

4.4. Prevalence of psychopathology in pain patients

In this study, with the exception of depression and PTSD, other psychiatric disturbances did not have a higher prevalence in the patient group. Almost half of the patients had a clear lifetime history of psychiatric illness and 75% had no current psychiatric problems. Schnurr et al. (1990) also found that TMJPD patients do not appear to be significantly different from other pain patients or healthy controls in personality type, response to illness, attitudes towards health care or ways of coping with stress. Gamsa (1990) has also questioned the view expressed by many authors that emotional disturbance predating pain onset is the prime contributor to chronic pain and criticizes tendency for some physicians to attribute psychological causation to conditions they can neither understand nor treat effectively. This tendency may, in part, be a function of the way in which health care professionals have interacted with less manageable patients. As in any large sample of people, there are likely to be individuals with psychological problems additional to their presenting problems. Slater et al. (1983) estimated that between 20% and 30% of patients consult their physicians for primarily psychological reasons. It is also estimated that only
5% of those with TMJPD signs and symptoms actually seek treatment (Marbach & Lipton, 1978; Rugh & Solberg, 1985). If these patients are the ones who draw most of the attention, then an association between psychopathology and the physical condition is likely to be made, especially if a structural pathology is not readily observable. Generalizations about the role of psychological factors in TMJPD may, in part be based on dentists' and physicians' interactions with these problematic patients.

4.5. Reliability and validity of psychometric assessments

Traditionally, assessment instruments are presented with data indicating their 'reliability' and 'validity'.

Reliability is the extent to which the score on a scale reflects the hypothetical true score and is usually evaluated by comparing the agreement between independent evaluation by two or more interviewers across a group of subjects. Concurrent validity tests the scale against already established instruments.

I. SCID

Because the SCID is new, little reliability data is available. However, recent studies (Skre et al., 1991) has shown that SCID yields highly reliable axis I and axis II DSM-III-R diagnoses. These authors have shown very high inter-rater agreement for most areas of axis-I, with an exception for somatoform disorders and obsessive compulsive disorders.

In our study, it was preferred to use different clinical diagnosis of chronic idiopathic facial pain instead of the diagnosis of somatoform pain disorder in the somatoform disorder module. The DSM-III-R definition of somatoform pain disorder is 'preoccupation with pain in the absence of adequate physical findings to account for the pain intensity'. This definition assumes that the true cause of all pain syndromes is
known. However, this assumption seems unwarranted, because the physical basis for many pain syndromes is unclear (Turk, 1990).

II. STAI

Although the concurrent validity of the trait scale against other personality scales of anxiety varies from 0.53 to 0.85, this does not guarantee that it really measures an unvarying trait. Indeed there is a consensus that trait anxiety tends to change as state anxiety changes. This may be because the instruction to rate 'how you are usually' is too difficult if high state anxiety has been present for some time. The scale is contaminated by somatic items, so its use in a general hospital population may be difficult to interpret. Nevertheless the T score has been shown to correlate satisfactorily with other personality measures of trait anxiety (Thompson, 1989).

III. HAD

Many patients especially in general hospital practice have physical as well as psychiatric conditions. Some of these, could give misleadingly high scores on most of the depression or anxiety rating scales, which include ratings of somatic symptoms on the assumption that they are 'psychogenic'. A scale without this contamination would be valuable in self-assessment of mood disorders in a general hospital. HAD score is independent of physical illness. It has been used as a case finding instrument in general hospital population. The concurrent validity for depression scale using the MADRS (Montgomery & Asberg Depression Rating Scale) as external criteria is 0.77. It has been estimated that 20-25% of patients would be unclassifiable or borderline if the HAD was used as a screening instrument (Thompson, 1989).

In our study HAD was able to identify 80% (4 out of 5) of patients with current dysthymia.
or major depression. However, there were two false positives ie patients had high scores on HAD, but on further questioning using the SCID did not meet the criteria for dysthymia or major depressive episode. Overestimation is a common limitation of self-report questionnaires. The single case that HAD failed to identify was a case of depression secondary to pain with somatic symptoms which HAD is not equipped to identify.

It has been shown that in mild psychiatric disorders self-rating scales are generally adequate. Furthermore TMJ patients are shown to suffer from fewer vegetative (somatic) symptoms associated with depression as compared with other chronic pain patients (McKinney et al., 1990). Therefore, HAD seems appropriate as a case-finding instrument in relation to current mood state. However, if further information is needed to determine the temporal relation between depression and pain, structured clinical interviews are needed.

4.6. Limitations of the Study

Several methodological issues must be acknowledged:

1. Inevitable loss of recall

Remembering psychiatric symptoms appear to follow the same time course and be of similar fidelity to recall of other life events((Uhlenhuth et al., 1977) so that considerable loss of information can occur six to nine months after even major life events.

2. Absence of control group

Although a control group was not employed in the study, the increasingly popular use of
DSM-III-R in psychiatric research on large population samples will allow collection of data in the future for comparison. With regard to STAI, population norms are well established and were used for comparison.

3. Highly selected pain clinic sample

Chronic facial pain is a very common problem. However, it is estimated that only 5% of those with TMJPD signs and symptoms actually seek treatment (Marbach & Lipton, 1978; Rugh & Solberg, 1985). Eastman Dental Hospital acts as a central referral center for difficult facial pain problems and receives patients who have had unsuccessful therapy by other specialisms. These patients are likely to be psychologically different from the chronic facial pain patients in the general population. Furthermore, departments of Maxillofacial Surgery may care for a significant percentage of latent PTSD cases, who have sustained an assault or a road traffic accident, the commonest causes of facial injury.
5. CONCLUSIONS

1. Whereas chronic facial pain does not imply psychopathology a sizeable proportion of patients may experience diagnosable and important psychiatric conditions. The life time prevalence of depression and PTSD is higher than the general population. By being alert to these syndromes, we may be able to provide effective treatment and improve the quality of life, even in chronically burdened patients. The high prevalence of depression does not seem to be related to the causality of either pain or depression in relation to the other. A common metabolic vulnerability may predispose to both idiopathic pain and depression even if both do not occur in the same individual. The pain would seem to be an alternative disturbance rather than masked depression. In relation to post-traumatic TMJ and orofacial pain, the assumption that the pain is only of mechanical or neuropathic origin is unhelpful. Furthermore surgical management appears to intensify the pain and render it intractable. Clinicians should bear in mind that depression and/or PTSD can be chronic or recurrent in a high proportion of those who develop it. Therefore, management needs to include long term follow up and support of these patients.

2. Although, training for recognition and management of the wide range of psychological problems is beyond the scope of dental and oral surgical training, familiarity with the features of dysthymia, major depression and post-traumatic stress disorder and the ability to collect diagnostically relevant historical data from the patients is required by those who
are involved in management of pain patients. Studies addressing the ability of even medical practitioners to identify depression in their patients indicate that the severity or the presence of depression is frequently unrecognized (Haythornwaite et al., 1991).

3. The widespread use of DSM-III-R by researchers and clinicians warrants a quick and cost effective method for screening the patients according to these criteria. Recently, a computerized screening version of the structured clinical interview for DSM-III-R (SCID) has become available. HAD seems appropriate as a case finding instrument in relation to current depression in patients with TMJPD syndrome.

4. Chronic facial pain patients may have personalities that are more vulnerable to stress as a result of heredity and upbringing.
CHAPTER III. BIOLOGICAL MARKERS OF

PAIN AND DEPRESSION
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1. INTRODUCTION

1.1 General considerations

Psychiatry has always been all too aware of the subjective nature of much of its diagnostic framework and hence the temptation to borrow more 'objective' measures from other branches of medicine has always been present.

Research on biological markers in depression was inspired by the clinical phenomenology of the classical 'endogenous' depression characterized by changes in physiological mechanisms and by the discovery of drugs that increased the concentration of amines in the synaptic clefts having an antidepressant effect (Gjerris, 1989).

1.2. What is a biological marker?

A 'marker' at its most basic is something which can be measured independently of the features used to define a category, such as a diagnosis and thus provides some sort of external validation of that category.

Buchsbaum & Haier (1983) define a biological marker as 'a measurable indicator of a disease which may or may not be causal'. They subdivide markers into state and trait. A state marker is one that is related to the severity of an illness, does not reflect the point severity of an illness and is the product of the progression of the illness episode and of immediate environmental influences. Whereas a trait marker is present before the onset of illness, does not reflect the point severity but reflects the vulnerability to an illness.
The search for biological markers of depression or pain has attracted a considerable interest. In addition to providing insight into the pathophysiology, it may also help in the identification of groups at risk and offer practical benefits of greater diagnostic reliability and choice of the most appropriate treatment to be made.

However, there are a number of problems in identifying potential markers (Hale, 1992):

a. Pain and depression are multifactorial. Hence, heterogeneity within the disease entities must be expected.

b. Despite the great progress that has been made in the last 10-20 years, the biological basis of both depression and chronic pain still remains somewhat elusive. Much of our neurochemical insight into both disorders comes from experiments in animal models and its value is therefore limited.

c. Persistent diagnostic and classification unreliability, despite enormous advances since the introduction of operational criteria.

d. Low statistical power in most of the research designs.

1.3. A common biological pathogenesis of pain and depression

The association of different pains in an individual at different stages of life suggests the existence of a pain vulnerable person, ie a biological trait. Up to 80% of patients with chronic idiopathic orofacial pain also complain of other chronic pain symptoms. In childhood, such patients suffer from abdominal or dysmenorrhea and later abdominal pain (usually irritable bowel syndrome), neck pain, back pain and pruritus (Berry, 1969; Feinmann & Harris, 1984). It has also been suggested that this vulnerability may be
genetically transmitted as children of TMJ pain patients seem to suffer from a higher incidence of illness and injury (Raphael et al., 1990).

It has been estimated that 30-60% of patients with 'non-organic' chronic pain suffer from depression. This incidence seems to be higher, not only than in the general population but also than in those with chronic nociceptive pain (Magni, 1987). It has also been reported that a high proportion of subjects with such pains also suffer from emotional disorders. This has led to the hypothesis that chronic pain and depression may share a common biological pathogenesis. This is supported by the finding that both tricyclic antidepressants and monoamine oxidase inhibitor antidepressants have been found to be effective in the treatment of chronic pain (Feinmann, 1985).

The search for common biological markers in depression and chronic pain has attracted considerable interest (Gjerris, 1989; von Knorring, 1989). Patients with idiopathic pain have been shown to have hypercortisolaemia, abnormal dexamethasone suppression test responses, low platelet 3H-imipramine binding, low cerebrospinal fluid 5-hydroxyindolacetic acid and low serum and urine melatonin levels (reviewed by von Knorring, 1989). These studies suggest that there may be a common pathogenesis between depression and idiopathic pain, and point particularly to the possible involvement of 5-hydroxytryptamine. However, most of these markers are state markers. Furthermore, interpretation of the results of many studies of biological markers of depression in pain patients is confounded by the fact that it is not known whether the patients were also suffering from major depression.
1.4. Aims of the study:

We chose two of the biological markers involved in the pathogenesis of depression for study in patients with chronic idiopathic orofacial pain. Both are trait markers, and therefore their value is independent of the severity of illness. The biochemical basis for their abnormalities in the depressive patients is not clear. However, both may be related to central monoaminergic network which is implicated in the pathogenesis of both pain and depression.

To date, there is no study available on either of these biological markers in patients with chronic idiopathic orofacial pain. This study was carried out to:

1. Compare a group of patients with chronic idiopathic orofacial pain with healthy controls.
2. To compare the depressed and non-depressed subgroups of pain patients.
3. To compare drug-free patients and those on tricyclic antidepressants.
4. To study the relation between the two markers.
2. TYRAMINE CONJUGATION DEFICIT IN PATIENTS WITH CHRONIC IDIOPATHIC OROFACIAL PAIN

2.1. BACKGROUND

2.1.1. What is tyramine?
Tyramine (p-hydroxyphenylethylamine) is a dibasic aminoacid which occurs naturally as a product of putrefaction, fermentation and autolysis and is hence present in the gut. Tyramine of dietary origin is rapidly inactivated so that it exerts relatively little pharmacological action. It can also be formed \textit{in vivo} via the enzymatic decarboxylation of tyrosine, by the hydroxylation of dopamine or by the dehydroxylation of phenylethylamine. Tyramine is an indirectly-acting sympathomimetic amine, intravenous administration of which causes the release of catecholamines from nerve terminals resulting in a transient rise in blood pressure. The physiological role of endogenous tyramine is unclear, but it has been suggested that it may regulate noradrenaline turnover (Linnoila et al., 1982) and dopamine synthesis (Juorio, 1982). Its association with brain synaptosomal fractions has been interpreted as evidence for a functional role in neural transmission (Boulton et al., 1975).

2.1.2. Tyramine metabolism
Under normal circumstances, 10-15% of tyramine is metabolised by conjugation via the enzyme phenolsulphotransferase (PST) to form tyramine-O-sulphate. Conjugation of tyramine by PST depends on availability of PAPS which in turn depends on availability of ATP (fig. 6). Most of the remainder of tyramine is metabolised by the enzyme monoamine oxidase (MAO) A and B to p-hydroxyphenylacetic acid (pHPAA) (fig. 7).
Conjugation of Tyramine by Phenolsulphotransferase

\[ \text{ATP} + \text{SO}_4^{2-} \rightarrow \text{APS} + \text{PPi} \]
\[ \text{APS} + \text{ATP} \rightarrow \text{PAPS} + \text{ADP} \]
\[ \text{PAPS} + \text{Tyramine} \xrightarrow{\text{PST}} \text{Tyramine-sulphate} + \text{PAP} \]

ATP: adenosine triphosphate
APS: adenosine 5'-phosphosulphate
PAPS: 3'-phosphoadenosine-5'-phosphosulphate
PST: phenolsulphotransferase
PAP: 3'-phosphoadenosine-5'-phosphate

Figure 6. Conjugation of tyramine by phenolsulphotransferase
PATHWAYS OF TYRAMINE METABOLISM

Under normal circumstances 10-15% of tyramine is metabolised by conjugation via the enzyme phenolsulphotransferase to form tyramine-O-sulphate. Most of the remainder of tyramine is metabolised by the enzyme monoamine oxidase to p-HPAA.

2.2. TYRAMINE-O-SULPHATE AS A BIOLOGICAL MARKER

The interest in tyramine metabolism as a potential biological marker developed out of chance findings, peripheral to the study of the biology of migraine. Youdim et al. (1971) were studying the role of alternative pathways to MAO degradation of monoamines in patients with migraine. They observed a deficit in a metabolic pathway involving tyramine, in a group of patients whom they characterized as suffering from 'dietary' migraine, migraine precipitated by ingestion of certain foodstuffs. They characterized the
abnormality as involving sulfoconjugation, and found a smaller excretion of tyramine-O-sulphate in the urine of patients with migraine.

The link between tyramine, MAO and the use of monoamine oxidase inhibitors (MAOIs) in depression was of interest. Although some patients taking MAOIs suffered from adverse reactions such as headaches which had the clinical characteristics of migraine and hypertensive crisis in response to ingestion of tyramine-containing foods, others could consume such foods with no ill effects. This observation led to speculation that the formation of tyramine-O-sulphate from ingested tyramine might act as a 'safety-valve' to remove excess tyramine under circumstances where the MAO pathway was overloaded or impaired such as in patients taking MAOIs, and that patients experiencing adverse reactions to tyramine containing foods might have some impairment in this pathway which allowed more free tyramine to gain access to the circulation (Sandler et al., 1975). Hence, if it were possible to identify the activity of the tyramine-PST system, a safety test for MAOIs might be developed.

In order to test this hypothesis, tyramine sulfoconjugation was investigated in depressed patients who had experienced an adverse reaction during MAOIs therapy and also in those who had not. Additionally, tyramine conjugation was investigated in depressive patients who had as yet received no therapy, but for whom MAOIs were considered an appropriate treatment and in a group of normal controls (Sandler et al., 1975). Unexpectedly, all three groups of depressives tested showed significant reduction in urinary excretion of tyramine-O-sulphate following an oral load of free tyramine compared with controls.
It appeared that, although the 'safety valve' hypothesis was incorrect, as the sulfoconjugation of tyramine was also impaired in those patients not undergoing adverse reactions to tyramine ingestion while taking MAOIs, a biochemical abnormality had been discovered which appeared to be common to sufferers of depressive illness in general. This finding was later confirmed in a separate study of a group of unipolar depressives which also revealed that the deficit persisted after the recovery (Bonham Carter, 1978). They also showed that tyramine sulphate excretion successfully identified blind those of a group of pregnant women with a past history of depressive illness (Bonham Carter et al., 1980a). Therefore, the conjugation deficit began to emerge as a trait, rather than a state marker for depression, in other words a marker of a vulnerability to depressive illness.

2.3. The biochemical basis of tyramine conjugation deficit

In spite of much effort, the biochemical abnormality underlying the tyramine conjugation deficit remains obscure. A number of theories have been proposed some of which have been rejected, and the rest need to be scientifically tested.

2.3.1. Increase in MAO activity

Originally, it was proposed that an increase in MAO activity might lead to a greater proportion of tyramine being metabolised to pHPAH, thus leaving less available for conjugation with sulphate (Sandler et al., 1975). This hypothesis arose from the fact that MAO inhibitors had been used as an effective treatment in those patients in whom the deficit was first delineated, indirectly pointing to raised activity of MAO in these subjects. Some support for this view came from the findings by several groups of raised platelet MAO in depression (reviewed by Sandler et al., 1981). However, recent studies measuring
both platelet MAO and tyramine sulphate activity in the same patients do not demonstrate any correlation between the two (Jarman, 1992). It should be noted however, that the studies on platelet MAO only measure activity of MAO B, and tyramine is metabolised by both forms of the enzyme. Youdim et al. (1971) measured urinary excretion of pHPAA in an attempt to find an index of in vivo MAO activity but found no difference in excretion levels of the metabolite between low and high conjugators. Additionally the sulfoconjugation deficit has been found in depressives who proved resistant to MAOI therapy (Bonham Carter et al., 1978). Therefore, the evidence is against an increase in MAO activity being the basis for the deficit.

2.3.2. Deficiencies in sulphate conjugation

2.3.2.1. Depleted sulphate reservoir

It was proposed that a depleted sulphate reservoir could form the basis for the deficit. Oral administration of cysteine, containing sulphate in an easily assimilable form, substantially raised the output of tyramine-O-sulphate in both depressives and controls (Bonham Carter et al., 1980b). However, the percentage increase observed was similar in both depressives and controls, indicating that either group was saturated with sulphate and that depressives were no less saturated than controls.

2.3.2.2. Deficiency of enzyme Phenolsulphotransferase (PST)

The enzyme PST catalyses the conjugation of tyramine (fig. 5). The possibility of a generalized deficiency of PST as the source of low excretion of tyramine sulphate was investigated by Bonham Carter et al. (1981). However, no differences in the enzyme
activity were found between patients and controls. Additionally, no correlation between platelet PST and tyramine sulfoconjugation has been found in individuals who have undergone both tests (Jarman, 1992). Nevertheless, the possibility exists that platelet PST activity may not be representative of the enzyme activity in other tissue particularly the gastrointestinal tract.

2.3.2.3. Low availability of ATP and PAPS

A rate limiting step in the production of conjugated tyramine could be the generation of PAPS, which in turn depends on the availability of ATP (fig.5). Whole blood ATP was measured in depressives but its concentration was not significantly different from a control group (Bonham Carter et al., 1981). However, it is still possible that the deficit may have its basis in a deficiency somewhere in the production of PAPS.

2.4. Tyramine conjugation deficit as a trait marker of endogenous depression

The tyramine conjugation deficit in depression was later independently confirmed by Harrison et al. (1984), who demonstrated that the deficit appeared to be associated only with the melancholic subtype of the depressive illness diagnosed according to DSM-III (table 6). The degree of impairment of tyramine sulfoconjugation appeared to be unrelated to severity of illness or age of the patients.
Table 6. DSM-III-R diagnostic criteria for Major Depressive Episode and Melancholia

I. Major Depressive Episode

A. At least five of the following symptoms must be present simultaneously for a two-week period or more. This represents a change from previous levels of functioning, and one symptom must be either depressed mood or anhedonia.

1. depressed mood
2. anhedonia
3. significant weight loss (not due to a voluntary diet), or weight gain, or chronic decrease or increase in appetite
4. insomnia or hypersomnia nearly everyday
5. observable psychomotor agitation or retardation nearly every day
6. loss of energy or fatigue almost every day
7. feeling of worthlessness, or excessive or inappropriate guilt almost every day
8. decrease in ability to concentrate, or indecisiveness almost every day
9. recurrent thoughts of death, or recurrent suicidal ideation

B. The disturbance is not caused or maintained by an organic factor or uncomplicated bereavement. Note: Bereavement can be complicated by a depressive episode. This is suggested when the bereaved reports morbid preoccupation with worthlessness, suicidal ideation, marked functional impairment, psychomotor retardation, or prolonged duration.

C. At no time have delusions or hallucinations been present for two weeks in the absence of prominent mood symptoms.

D. Not superimposed on schizophrenia, schizophreniform Disorder, delusional disorder, or psychotic disorder not otherwise specified.

II. Melancholic Depression

A severe form of Major Depressive Episode that seems to be quite responsive to somatic treatments. The diagnostic criteria include at least five of the following depressive symptoms:

1. anhedonia
2. lack of response to pleasurable stimuli
3. depression is regularly worse in the morning
4. early morning awakening, at least two hours before usual time
5. significant anorexia or weight loss (more than 5% of body weight in a month)
6. observable psychomotor agitation or retardation
7. no significant personality disturbance prior to first Major Depressive Episode
8. one or more previous Major depressive episodes followed by full, or nearly full remissions
9. previous good response to specific and adequate somatic therapy (polycyclic antidepressants, electroconvulsive therapy, lithium)
Hale et al. (1986) extended the previous findings by demonstrating that the deficit could be confined to those depressives with the form of illness categorised as 'endogenous depression' according to the Newcastle Depression Scale (Carney et al., 1965; table 7).

### Table 7. Weights for deriving diagnostic indices of endogenous depression according to the Newcastle Scale (Carney et al., 1965; Carney & Sheffield, 1972)

<table>
<thead>
<tr>
<th>Weight</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Adequate personality</td>
<td>+1</td>
</tr>
<tr>
<td>No adequate psychogenesis</td>
<td>+2</td>
</tr>
<tr>
<td>Distinct quality to mood</td>
<td>+1</td>
</tr>
<tr>
<td>Depressive psychomotor activity</td>
<td>+2</td>
</tr>
<tr>
<td>Nihilistic delusions (feelings)</td>
<td>+2</td>
</tr>
<tr>
<td>Weight loss</td>
<td>+2</td>
</tr>
<tr>
<td>Anxiety</td>
<td>-1</td>
</tr>
<tr>
<td>Blame others</td>
<td>-1</td>
</tr>
</tbody>
</table>

A Newcastle score > 6 is used as the criteria for diagnosis of 'endogenous depression'.
Patients not fulfilling these criteria (neurotic depressives) showed normal tyramine-O-sulphate excretion. The abnormality was also shown in half of the first degree relatives of endogenous depressed patients, which gives additional support to the hypothesis of tyramine-sulphoconjugation as a marker of vulnerability to endogenous depression. However, the test does not seem to be able to identify the endogenous subgroups of patients suffering from bipolar depression, post-natal depression and also elderly depressed patients (Jarman, 1992).

Recently, it has been shown that the tyramine challenge test is a reliable predictor of treatment outcome with tricyclic antidepressants in treatment of depression (Hale et al., 1989). There is also preliminary evidence that the tyramine test can predict response to MAOIs as an antidepressive treatment (Stewart et al., 1988). These findings raises the possibility of a parallel situation in other disorders such as chronic idiopathic pain which are responsive to tricyclic antidepressant drugs.

2.5. Tyramine conjugation deficit in chronic pain

The first demonstration of abnormal tyramine sulfoconjugation was in a group of psychiatrically unclassified patients with so called dietary migraine (Youdim et al., 1971). However, the existence of a dietary subgroup of migraine is questionable (see III.3.1.3.1.). The same authors later had difficulty in replicating this finding. Jarman et al. (1990) aimed to clarify whether the original findings of abnormal sulfoconjugation in migraine was a reflection of a high incidence of endogenous depression in these patients or whether it was a characteristic of migraine itself. They assessed a group of patients with migraine using a structured interview the Schedule for Affective Disorders
and Schizophrenia-lifetime version (Spitzer & Endicott, 1975) and for diagnosis according to the Research Diagnostic Criteria (Spitzer et al., 1977). It was shown that only those patients with a history of endogenous depression had the tyramine conjugation deficiency. They concluded that the original group investigated by Youdim et al. (1971) must have included a significant number of patients with endogenous depression.

To date, no study is available in relation to any other chronic pain population.

2.6. MATERIALS AND METHODS

2.6.1. The tyramine challenge test

From midday the day before the test, patients and controls followed a low-tyramine diet (avoiding foods detailed on a standard monoamine oxidase inhibitor card. At the start of the test subjects emptied their bladder and then swallowed \( \sim 125 \text{mg} \) tyramine hydrochloride capsule containing \( 100 \text{mg} \) tyramine. All urine was collected for exactly 3h and its volume determined. Aliquots were stored frozen at \(-20 \text{ C}\) until the assay.

The tyramine-O-sulphate was measured using a gas chromatographic method with electron capture detection (by Dr. B. Goodwin, Queen Charlotte's Hospital) described by Walker & Sandler (1988):

An internal standard (p-hydroxyphenylpropylamine) was added to the urine sample and the amines isolated using an ion-exchange column and eluted with ammonium hydroxide.
Tyramine-O-sulphate in the column washings was hydrolysed by adjusting to pH 0.9 with 6M HCl and heating in a boiling water bath. After removing ammonia, borrate buffer and NaOH were added to achieve a pH of 10.2 and the solution shaken with ethyl acetate (2x15ml). The solvent was removed by evaporation and the residue transferred to a 'Reacti-vial' using 0.1ml ethanol which was then removed under a stream of nitrogen. For derivatization 0.02ml acetonitrile and 0.1 ml heptafluorobutyryl anhydride were added and the Teflon-capped vial heated for 40 min at 65 C. After cooling, excess reagents were removed under a stream of nitrogen; 0.1 ml heptane and 0.1 ml water were added, vortex mixed and briefly centrifuged. An aliquot of heptane (upper) layer was injected into the gas chromatograph. For gas chromatography-mass spectrometry deuterated p-tyramine(d4) was used as internal standard. For assay of conjugated amines, internal standard was added after the hydrolysis stage, to avoid exchange of the deuterium label. Known amounts of m- and p- tyramine were treated as above and the ratio of tyramine peak height to internal standard was used to construct a calibration curve.

2.6.2. Recruitment of patients and controls

The clinical material consisted of patients attending the pain clinics at the Eastman Dental Hospital who had suffered from an orofacial pain for at least 6 months duration and for which no primary structural pathology was found. The patients were included on a non-selective consecutive basis. Those who declined the somewhat arduous process of clinical interview and dietary restrictions were omitted. The criteria for selection of controls was a clear medical history, freedom from pain and depression as judged by HAD scale, and a clear history of chronic pain of any kind in the past. Age and sex matched controls were selected from patients attending the denture clinic at the department of
prosthodontics, nursing and clerical staff, staff of the research laboratories and students at Eastman Dental and University College Hospitals.

2.6.3. Pilot study

Eight patients and an equal number of controls were included in the pilot study (Aghabeigi et al., 1991). The controls were sex matched. However, we did not attempt to select age matched controls, as previous studies (Hale et al., 1986; Harrison et al., 1984) had not shown any influence of age on tyramine sulfoconjugation. It was found that the pain patients excreted significantly lower ($p < 0.05$) amounts of tyramine sulphate than controls (table 8).

| Table 8. Results of a pilot study comparing tyramine sulfoconjugation in patients with chronic idiopathic orofacial pain and controls *

<table>
<thead>
<tr>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>8</td>
</tr>
<tr>
<td>F:M</td>
<td>3:1</td>
</tr>
<tr>
<td>Age</td>
<td>56/41-76</td>
</tr>
<tr>
<td>Tyramine sulphate output mg/3h</td>
<td>$4.1 \pm 1.7$</td>
</tr>
</tbody>
</table>

In parallel to this study, researchers in Queen Charlotte's Hospital were investigating tyramine sulfoconjugation in elderly patients (age > 75). They showed a significant

* Statistical analysis was carried out using a student's t-test.
negative correlation between age and tyramine sulphate output (Jarman, 1992). Our patient
group had a lower mean age group than the controls. It was possible that the age
discrepancy between our patients and controls could have contributed to the observed
difference. Therefore, it was decided to extend the study and increase the number of
patients for comparison with age and sex matched controls.

2.6.4. Extended study
The available patients and controls included in the pilot study were recalled for a second
test. The study was designed to detect the smallest magnitude of a significant difference
in excretion between the patients and controls reported in the literature (Harrison et
al., 1978). Statistical calculation showed that a minimum of 26 individuals each group
were required to detect a difference of 1.7mg/3h between the groups with a statistical
power of 0.95 at a significance level of 0.05 ($\alpha = \beta = 0.05$)(Bland, 1987).
The study was commenced with a larger number as it was expected that a few patients
might be excluded from the study for various reasons. Two patients, one with a history
of post-natal depression and another one who during the structured interview was found
to have psychotic symptoms were excluded. This is because the tyramine metabolism
seems to be different in post-natal depression(Jarman, 1992) and secondly, a diagnosis of
depression can not be made in the presence of a psychotic disorder.
Finally, the results from twenty nine patients \{F:M=25:4, mean age($\pm SD)= 40.2 \pm 15.2$
years\} and an equal number of age and sex matched controls \{F:M=25:4, mean
age($\pm SD)=40.1 \pm 15.7$ years\} were analysed in this study. The patients had suffered from
an orofacial pain of at least 6 months duration \{mean($\pm SD)=6.6 \pm 6.5$ years, range 0.5-25
years\} for which no primary structural pathology was found.
2.6.4. Psychiatric assessment:

During the 3h period of the test, patients underwent a structured clinical interview for the diagnosis of mental disorders (SCID; Spitzer et al., 1989). In order to assess current and previous incidence of psychiatric disorders according to the criteria set by Diagnostic and Statistical manual of Mental Disorders revised volume III (DSM-III-R) (American Psychiatric Association, 1987). DSM-III-R is currently the most widely accepted system of classification of psychiatric disorders. It includes specified diagnostic criteria for virtually all of the mental disorders. The explicit diagnostic criteria has improved the poor reliability achieved by the previous systems (A detailed description of this may be found in chapter II). When there was a positive history of depression, its melancholic/non-melancholic subtype was determined (table 9) and a further assessment was made using the Newcastle scale (tables 7,10) as an aid in distinguishing endogenous from neurotic depression.

Table 9. Depressive subtypes in 14(n=29) pain patients with a positive history of depression.

<table>
<thead>
<tr>
<th></th>
<th>Current</th>
<th>Past</th>
<th>Total</th>
</tr>
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<tbody>
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<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Non- melancholic</td>
<td>2</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14</td>
</tr>
</tbody>
</table>

2.7. RESULTS

2.7.1. Clinical

The pain diagnosis, history of depression and its subtype and the use of medication for individual patients are shown in table 10.
Table 10.

<table>
<thead>
<tr>
<th>Patient</th>
<th>age</th>
<th>sex</th>
<th>pain condition</th>
<th>medication</th>
<th>history type</th>
<th>Newcastle score</th>
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<td>FAM,AO</td>
<td>None</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>26.</td>
<td>27</td>
<td>M</td>
<td>AO</td>
<td>None</td>
<td>+</td>
<td>NM 3</td>
</tr>
<tr>
<td>27.</td>
<td>36</td>
<td>M</td>
<td>FAM</td>
<td>None</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>28.</td>
<td>59</td>
<td>M</td>
<td>OD</td>
<td>Yes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>29.</td>
<td>76</td>
<td>M</td>
<td>FAM,AFP,OD</td>
<td>None</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Medication: 10-30mg nortriptyline
Depression subtypes:
  *: current depressive episode
  M: melancholic
  NM: non-melancholic
Newcastle score: >6 endogenous depression

FAM: facial arthromyalgia
AFP: atypical facial pain
AO: atypical odontalgia
OD: oral dyseaesthesia
A summary of the incidence of chronic orofacial pain conditions in the patient group is presented in figure 8. Some patients suffered from more than one condition, a common finding.

Figure 8. Orofacial pain conditions in 29 patients
15 patients were medication free at the time of the test and 14 were on low doses of a tricyclic antidepressant for their pain problem. A full account of the psychiatric findings is given in chapter V. In relation to a history of depression, 14 patients (48%) had a positive history of depression. Of these only 3 were suffering from a major depressive episode at the time of examination and 11 had experienced at least one major depressive episode sometime in their life (table 9). With the exception of 1 patient all those with a history of melancholic depression (n=5) had a Newcastle score > 6 (tables 10).

2.7.2. Biochemical

The mean urinary 3h tyramine-O-sulphate output was significantly lower in the patients as compared with controls (p < 0.0004, table 11, fig 9).

Table 11. Tyramine-O-sulphate excretion values group data

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No.</th>
<th>Tyramine-O-sulphate excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>I. Controls</td>
<td>29</td>
<td>5.38</td>
</tr>
<tr>
<td>II. Pain patients</td>
<td>29</td>
<td>3.74</td>
</tr>
<tr>
<td>a. never depressed</td>
<td>15</td>
<td>3.25</td>
</tr>
<tr>
<td>b. history of depression</td>
<td>15</td>
<td>4.33</td>
</tr>
<tr>
<td>melancholic</td>
<td>5</td>
<td>4.26</td>
</tr>
<tr>
<td>non-melancholic</td>
<td>9</td>
<td>4.38</td>
</tr>
<tr>
<td>c. drug free</td>
<td>14</td>
<td>3.59</td>
</tr>
<tr>
<td>d. on medication</td>
<td>15</td>
<td>3.94</td>
</tr>
</tbody>
</table>
Figure 9. Comparison of tyramine-O-sulphate between patients and controls
When the patients were divided into those with a history of depression and those without, the latter showed a lower value compared to the former with a difference which was close to the level of statistical significance (p=0.06, table 12). There was no significant difference between the depressive subgroups of patients ie melancholic/non-melancholic. Half of the patients were on tricylic antidepressants for their pain problem. These did not differ significantly from the drug free patients (table 12).

Table 12. Inter-group comparisons of tyramine-O-sulphate level *

<table>
<thead>
<tr>
<th>Patient subgroups</th>
<th>vs</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>never depressed vs controls</td>
<td>p=0.00009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>history of depression vs controls</td>
<td>p=0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>history of depression vs never depressed</td>
<td>p=0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>melancholic vs non-melancholic</td>
<td>p=0.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>drug-free vs on medication</td>
<td>p=0.55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The combined effects of age, sex, presence of pain and depression on tyramine output were studied using multiple regression analysis. The presence of pain was found to be the only statistically significant factor (b=-2.099, t=-4.08, df=57 p<0.0005). The absence of depression had a negative influence on tyramine output (b=-1.035), which was very close to the level of statistical significance (t=-1.65, df=57 p<0.1). Age and sex had no effect on tyramine output when other variables were kept constant.

* Statistical analysis was carried out using a student's t-test.
2.8. DISCUSSION:

2.8.1. Relationship between pain and depression and tyramine metabolism

The search for biological markers of chronic pain syndromes has become an important means of establishing their aetiology. The demonstration of a common biological abnormality in chronic facial pain and depression can explain the significant relief of symptoms achieved with tricyclic antidepressants in both conditions. It will also explain why antidepressants relieve pain in the absence of clinical depression.

There has been a widely held assumption that because chronic pain is relieved by tricyclic antidepressants, the pain represents an underlying depressive illness (Blumer & Heilbronn, 1982; Katon, 1984). These patients without evidence of classical depression are often described as suffering from 'masked depression' or a 'depressive equivalent' (Lopez Ibor, 1972). Such diagnostic categories have doubtful validity and there are dangers inherent in equating outcome and response to treatment as adequate criteria for diagnosis (Feinmann & Bass, 1989). Our findings support the hypothesis that there may indeed be a common underlying vulnerability to both idiopathic pain and depression even if both do not occur in the same individual, but the pain would seem to be an alternative disturbance rather than 'masked depression'.

Many studies of the biological markers in other chronic pain patients do not specify whether the patients had suffered from major depression. The other methodological weaknesses such as a lack of rigorous psychiatric diagnostic criteria and the use of self-
reporting questionnaires rather than the standardized psychiatric assessments makes any 
comparison with these results difficult.

In order to avoid such pitfalls, a structured clinical interview for the diagnosis of mental 
disorders according to the rigid diagnostic criteria set by DSM-III-R was used to assess 
the presence of psychopathology. The results of the pilot study (Aghabeigi et al., 1991) 
were confirmed at a highly significant level (p < 0.0004). However, an unexpected finding 
was that the pain patients with no history of depression had the lowest tyramine excretion 
values. Multiple regression analysis confirmed that the absence of depression had a 
negative influence on tyramine values (b = -1.035, p < 0.1) which was close to the level 
of statistical significance. Previous studies have largely identified this metabolic 
apnor-mality in melancholic or unipolar endogenous groups of depressed patients. 
However, a recent study (Jarman et al., 1990) noted this biochemical deficit in a group 
of migraine patients but the low values were found in the endogenous depressive 
subgroup diagnosed according to the criteria set by Research Diagnostic Criteria (RDC). 
By contrast, our study indicates a highly significant (p = 0.00009) difference between the 
non-depressed patients and controls which was not demonstrated in the study of migraine 
patients. Furthermore, the psychiatric profile of migraine patients studied by Jarman et 
al. (1990) seems very different from our group, and it is likely that the two patient groups 
are dissimilar in other ways. In our study only 35% of those with a positive history of 
depression were of the melancholic type, where as in the migraine study this figure was 
93%. Part of the difference in the psychiatric profile between our group and the migraine 
patients studied by Jarman et al. (1990) may relate to different diagnostic criteria 
employed. Harrison et al. (1984) have shown that although all the patients with
melancholia meet the RDC criteria for endogenous depression, a considerable proportion of non-melancholic depressives meet these criteria as well. However, in our study there was a close correlation between the diagnosis of melancholic depression as defined by DSM-III-R and the endogenous subtype defined by the Newcastle score. 80% of those with a history of melancholic depression had Newcastle score > 6. However, the correlation between RDC diagnostic criteria and Newcastle criteria seems to be poor (Zimmerman et al., 1986).

Maier & Philipp (1986) undertook a complex comparison of the DSM-III and RDC endogenous/melancholia scale structure from the point of view of construct validity, seen as an essential pre-requisite to the use of such scales for subsequent application in biological research. This requires that the operational definition of the construct which the scale is measuring is coherent, and generally must mean that the scale measures or taps some single underlying category or dimension, ie a latent trait. Maier and Philipp (1986) show that DSM-III criteria of melancholia are superior to the RDC criteria for endogenous depression, both in terms of internal validity, homogeneity and transferability between study populations. Melancholia is seen as a coherent concept, a one dimensional continuum, adequately tapped by DSM-III. However, RDC endogenicity lacks such homogeneity. Harrison et al. (1984) found that the melancholic/non-melancholic subtypes of depression can be identified by the differences in the tyramine metabolism, whereas the test is not sensitive enough to detect the endogenous subtypes diagnosed by RDC criteria. They also attempted to determine which constellation of clinical symptoms of depression were particularly associated with the tyramine conjugation deficit. Patients with reduced tyramine-O-sulphate levels had significantly higher ratings for insomnia,
weight loss, agitation, loss of libido and paranoia on the Hamilton Depression Scale (HDS) compared with other depressives and controls. Nevertheless, the possibility exists that tyramine, like RDC endogenicity is tapping a different latent trait (Hale, 1992). This is obviously not amenable to casual observation, as these differences are certainly not apparent from perusal of items utilised in the scales (Hale & Stokes, 1991).

The underlying mechanism for the deficit in conjugated tyramine excretion is not clear. Furthermore, the actual relevance of the deficit to the underlying vulnerability to depression and pain is an even greater mystery and is the subject of speculation.

Harrison et al. (1984) have hypothesized that the decreased tyramine sulphate excretion output in response to a tyramine load may reflect an attempt to retain tyramine because of a central deficiency of this trace amine. There is some support for this view, as urinary excretion of free tyramine has been shown to be reduced in depressives compared with control subjects (Sandler, 1976). It has been suggested that tyramine may play a role as 'synaptic activator' or 'neuromodulator' (Boulton, 1978). Linnoila et al. (1982) have reported a high positive correlation between tyramine excretion rate and 24h 'whole body' noradrenaline turnover in depressed patients. Based on this finding they have suggested that tyramine may have a significant role in regulating noradrenaline turnover. The monoaminergic network has been implicated not only in the pathogenesis of affective disorders but also in the pain modulating system. It is possible that abnormalities in monoamine metabolism act as the underlying mechanism predisposing to both pain and depression.
A further possibility is that a reduction in ability to transfer sulphate to tyramine may be of relevance in itself in some way to the pathogenesis of depression and pain, or alternatively, it may be reflecting a deficit in sulphation at another relevant site, possibly within the CNS. Alternatively, reduced tyramine sulphaconjugation may not be related to the pathogenesis of depression and pain in itself. Instead, the locus of the gene responsible for the conjugation deficit could be closely linked to a gene or genes which are involved in the pathogenesis of pain and depression, so that the two tend to be inherited and controlled together, with the gene responsible for the conjugation deficit acting as a marker for the presence of the other gene(s). However, at present this is pure speculation (Jarman, 1992).

2.8.2. Tyramine conjugation test as a diagnostic marker

In order to understand the following discussion, it is helpful to appreciate what is meant by such statistical terms as 'sensitivity', 'specificity', 'predictive value of a positive test' and 'predictive value of a negative test' (appendix).

Although pain patients had a significantly lower conjugation value, we did not find a natural cut-off point which could differentiate between patients and controls with a high degree of sensitivity and specificity. The cut off point of 4.1mg/3h as suggested by Hale et al. (1986) for patients with endogenous depression if applied to our data, gives a sensitivity of 65% (ie 35% false negative) and a specificity of 73% (ie 27% false positive). Harrison et al. (1984) have also reported a high degree of false negatives (30%) and false positives (25%) in depression. However, if we choose a tyramine output of 5mg/3h as
suggested by Harrison et al. (1984) as the cut off point, a high percentage (14 out of 16 ie 88%) of the non-depressed pain patients fall at or below this value. Of the two non-depressed patients who had a higher level than this arbitrary cut-off value, one was diagnosed as suffering from post-traumatic stress disorder, following an assault sustained 11 years ago which coincided with the onset of the facial pain problem. The second patient suffered from rheumatoid arthritis and although radiographic examination did not reveal any abnormality, the possibility of latent rheumatoid arthritis of TMJ contributing to the pain can not be excluded. The choice of a tyramine output of 5mg/3h as the cut-off point increases the sensitivity of the test at the expense of a reduction in specificity. In other words, there will be a higher proportion of false positive tests. The probability with which a subject who screens positive and possesses any disease depends not only on the sensitivity and specificity but also on its prevalence. Taking the prevalence value of 14% in the general population, the proportion of false positives in the population (individuals who screen positive but do not have the condition) will be 0.33. The predictive value of a positive test (<5mg/3h) ie the proportion of true positives amongst apparent positives will be equal to 0.26. Therefore, the procedure can not be recommended as a positive diagnostic screening test at this stage. However, the proportion of false negatives will be 0.02, and the predictive value of a negative tyramine test (>5mg/3h) which is the proportion of true negatives amongst apparent negatives is 0.96. This value is higher for the non-depressed group (0.97).

Based on these findings we are suggesting that a patient with a history of chronic idiopathic orofacial pain, particularly in the absence of a history of depression or other psychopathology, with a tyramine sulphate output value greater than 5mg/3h is unlikely
to fall into the chronic idiopathic pain group and careful follow up, supplemented by appropriate investigations is necessary to establish some alternative latent pathology. Therefore, the tyramine conjugation test may serve as an additional means to help the clinician in the differential diagnosis of difficult pain conditions.
3. Platelet Monoamine Oxidase Activity

3.1. Background

3.1.1. Serotonergic system, depression and pain

3.1.2. Platelet MAO as a peripheral marker for central serotonin system

3.1.3. MAO and Pain

3.1.3.1. Migraine

3.1.3.2. Chronic idiopathic pain

3.2. Materials and Methods

3.2.1. Platelet MAO assay

3.2.2. Recruitment of patients and controls

3.3. Results

3.3.1. Pilot study

3.3.2. Extended study

3.4. Discussion
3.1. BACKGROUND

3.1.1. Serotonergic system, depression and pain

The neurotransmitter serotonin (5-hydroxytryptamine) is involved in the pathogenic mechanism of depression and at the same time in the process of pain perception (Magni, 1987). Part of the evidence has come from the fact that depressive syndromes can be relieved by treatment with the serotonin precursor tryptophan, and that therapeutic benefit is also found with selective serotonin reuptake inhibitors (Aberg-Wistedt, 1982). It has also been demonstrated that patients with depressive disorders tend to have low concentrations of the serotonin metabolite 5-hydroxy indole acetic acid (5-HIAA) in their cerebrospinal fluid (Asberg et al., 1984).

In patients with chronic pain syndromes a large number of studies indicate that the serotonergic systems are involved in the nociceptive responses (Messing & Lytle, 1977; Basbaum & Fields, 1978). An increase in serotonergic activity is generally associated with a decrease in pain sensitivity. Activity of descending serotonergic axons in the spinal cord stimulates endorphin neurones which leads to impaired activity in synapses involved in pain transmission (Roberts, 1984). Hyperpathia and spontaneous pain can be provoked by treatment with a serotonin-depleting agent (Sicuteri et al., 1973), clinical pain can be relieved by treatment with the serotonin precursor tryptophan (Millinger, 1986) or treatment with specific serotonin reuptake inhibitors (Johansson & von Knorring, 1979; Eberhard et al., 1988).
In the early seventies, the development of techniques for the measurement of the serotonin metabolite 5-HIAA in the CSF led to increasing interest in the study of the role of the serotonergic system in affective disorders and chronic pain. It has been demonstrated that patients with idiopathic pain have lower levels of 5-HIAA in the CSF compared with controls (Almay et al., 1987a). However, due to ethical problems, a study of CSF serotonin metabolites is not feasible in an out-patient population of patients with chronic facial pain.

3.1.2. Platelet MAO as a peripheral marker for central serotonin system

Recently, evidence has accumulated to show that blood platelets may constitute an easily accessible peripheral marker for central serotonergic activity (Oreland & Hallman, 1989). Blood platelets have receptors and mechanisms for uptake and storage of serotonin in common with central serotonin nerve terminals. Of particular interest are:

1. Platelet monoamine oxidase activity (MAO)
2. \(^1^H\)-imipramine platelet membrane binding
3. Platelet serotonin (5-HT) uptake rate

All 3 markers have been extensively studied in patients with psychiatric disorders. It seems that only platelet MAO activity is stable and does not fluctuate with the disease severity, whereas the other two are state markers which alter with the disease activity. MAO is the major degradative enzyme for several biologically active monoamines, most of which are either neurotransmitters or neurotransmitter candidates. Thus, even though it is present in almost all tissues in the body, it is not surprising that its possible role in
neuropsychiatric disorders has figured most prominently in research publications.

On a sub-cellular level the enzyme is mainly localized to the mitochondrial outer membrane and to the nuclear membrane. A widely accepted working classification of the enzyme into A & B forms exists, based on the differential inhibitory ability of the drug 'clorgyline'. Type A is the form sensitive to clorgyline and this oxidatively deaminates noradrenaline and serotonin, but not phenylethylamine. Type B is inhibited by 'deprenyl' and selectively deaminates phenylethylamine but not noradrenaline and serotonin. Tyramine and dopamine are substrates for both forms.

The human platelet is a pure source of MAO B (Donnelly & Murphy, 1977). Although human brain has a relatively high MAO B content, substantial MAO A activity is also present (Fowler et al., 1980). The enzyme activity is generally stable and under genetic control. There is some evidence that individuals with low activity have higher psychiatric morbidity (with the exception of unipolar depression) than those with high activity. A reduced level of platelet MAO activity has been reported in patients with bipolar disorders (Murphy & Weiss, 1972) and in first degree relatives of bipolar patients (Leckman et al., 1977) as well as patients presenting with psychopathy, suicidal behaviour and alcoholism (von Knorring et al., 1986).

3.1.3. MAO and pain

3.1.3.1. Migraine

Hanington (1978) proposed that there might be a genetic deficiency of MAO in some migrainous patients and that this might account for the reputed ability of tyramine-
containing food such as cheese to initiate attacks (Hannington et al., 1970). Review of the literature shows patients suffering from migraine and cluster headache, though presenting a wide range of platelet MAO values and a considerable overlap with non-headache controls, seem to have a mean decrease in MAO activity (Littlewood et al., 1984). Nevertheless, it is unlikely that any genetic enzymatic deficit can account for most reports of dietary migraine. Ziegler & Stewart (1977) and Moffett et al. (1972) in double blind experiments in which tyramine & placebo were ingested by migraine patients, showed that the same number of dietary migraine patients had headache after tyramine as after placebo. It has often been reported that migraine is strongly influenced by emotional factors, including what the subject has read or been told to expect after eating certain food (Dalton, 1975; Egger et al., 1983), so that the existence of a dietary subgroup may be questionable.

3.1.3.2. Chronic idiopathic pain

Almay et al. (1987b) have demonstrated low platelet MAO activity in patients with chronic idiopathic pain. Same authors have demonstrated a significant positive correlation between the concentrations of 5-HIAA in the CSF and the activity platelet MAO in these patients which provides support for the involvement of serotonergic system in pain susceptibility.

To date, there is no study available on platelet MAO in patients with chronic idiopathic facial pain. Results from other studies on heterogenous groups of pain patients may not be applicable to chronic facial pain patients.
3.2. MATERIALS AND METHODS:

3.2.1. Platelet MAO assay

10ml of venous blood was added to 0.5ml of 5%(w/v) Na2 EDTA in a Universal containers. The sample was centrifuged at 320g for 5 minutes and the platelet rich plasma withdrawn and recentrifuged at 2,500g for 15 min. The platelet pellet was resuspended and washed in 1ml of 0.3M sucrose solution and recentrifuged at 2,500g for a further 15 min. The pellet was resuspended in 1ml of 0.3M sucrose and stored at -20 C until the assay. MAO was assayed blind (by Ms. Hannah, QCH) and in duplicate with ¹⁴C-tyramine as substrate at a final concentration of 150μM. Protein was assayed by the method of Lowry et al.(1951). The results were expressed as nmoles of tyramine oxidised per mg protein in 30 min. All assay data where the amount of platelet protein recovered was less than 1.5mg/ml was excluded as suggested by Glover et al.(1981).

3.3.2. Recruitment of patients and controls

The clinical material consisted of patients attending the pain clinics at Eastman Dental Hospital who had suffered from an orofacial pain of at least 6 months duration for which no primary structural pathology was found. The patients were included on a non-selective consecutive basis. Those who declined the somewhat arduous process of clinical interview and dietary restrictions were omitted. The criteria for selection of controls was a clear medical history, freedom from pain and depression as judged by HAD scale, and a clear history of chronic pain of any kind in the past. Age and sex matched controls were selected from patients attending the denture clinic at the department of prosthodontics, nursing and clerical staff, staff of the research laboratories and students at Eastman Dental and University College Hospitals.
3.4. RESULTS

3.4.1. Pilot study

The initial study was carried out in parallel to the study of tyramine conjugation in pain patients. 18 of the subjects who were included in the tyramine study (mean age±SD = 48.6±13.8, F:M = 14:4) and 16 healthy controls (mean age±SD = 27.2±6.2, F:M = 9:7) were studied for platelet MAO activity.

Figure 10 compares the MAO values between controls, patients and patients subgroups divided according to the presence or absence of a history of depression.

![Figure 10](image_url)

**Figure 10. Comparison of platelet MAO activity between patients and controls**

- pt.: patient
- p.dp.: depressed patients
- p.ndp.: non-depressed patients
- cont.: controls
Table 13 shows the MAO value, tyramine sulphate output, age, sex and history of depression for individual patients. The mean values of platelet MAO activity was not significantly different between patients and controls (patients 17.94±7.15 vs controls 18.05±6.55). The group with a positive history of depression had a higher value but this was not statistically significant (depressed 19.37±8.48 vs non-depressed 16.15±6.55).

Using Spearman's correlation test there was no correlation between the platelet MAO activity and tyramine sulphate output in the urine.

Table 13. Age, sex, history of depression, MAO and tyramine sulphate output in 18 patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>age</th>
<th>sex</th>
<th>history of depression</th>
<th>MAO nmol/mg pr./ 30 min</th>
<th>Tyramine sulphate output mg/3hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>41</td>
<td>F</td>
<td>+</td>
<td>6.64</td>
<td>4.6</td>
</tr>
<tr>
<td>2.</td>
<td>47</td>
<td>F</td>
<td>+</td>
<td>8.50</td>
<td>2.3</td>
</tr>
<tr>
<td>3.</td>
<td>46</td>
<td>F</td>
<td>+</td>
<td>26.73</td>
<td>1.9</td>
</tr>
<tr>
<td>4.</td>
<td>36</td>
<td>M</td>
<td>-</td>
<td>20.62</td>
<td>2.7</td>
</tr>
<tr>
<td>5.</td>
<td>76</td>
<td>M</td>
<td>-</td>
<td>9.22</td>
<td>1.7</td>
</tr>
<tr>
<td>6.</td>
<td>60</td>
<td>F</td>
<td>-</td>
<td>22.88</td>
<td>5.2</td>
</tr>
<tr>
<td>7.</td>
<td>39</td>
<td>F</td>
<td>+</td>
<td>25.84</td>
<td>6.65</td>
</tr>
<tr>
<td>8.</td>
<td>27</td>
<td>M</td>
<td>+</td>
<td>16.23</td>
<td>7.9</td>
</tr>
<tr>
<td>9.</td>
<td>33</td>
<td>F</td>
<td>-</td>
<td>15.66</td>
<td>2.5</td>
</tr>
<tr>
<td>10.</td>
<td>68</td>
<td>F</td>
<td>+</td>
<td>33.08</td>
<td>2.8</td>
</tr>
<tr>
<td>11.</td>
<td>48</td>
<td>F</td>
<td>+</td>
<td>24.98</td>
<td>8.6</td>
</tr>
<tr>
<td>12.</td>
<td>41</td>
<td>F</td>
<td>-</td>
<td>11.15</td>
<td>3.6</td>
</tr>
<tr>
<td>13.</td>
<td>59</td>
<td>F</td>
<td>+</td>
<td>13.82</td>
<td>4.75</td>
</tr>
<tr>
<td>14.</td>
<td>36</td>
<td>F</td>
<td>+</td>
<td>16.63</td>
<td>2.8</td>
</tr>
<tr>
<td>15.</td>
<td>38</td>
<td>F</td>
<td>+</td>
<td>21.25</td>
<td>3.25</td>
</tr>
<tr>
<td>16.</td>
<td>59</td>
<td>M</td>
<td>-</td>
<td>12.83</td>
<td>2.3</td>
</tr>
<tr>
<td>17.</td>
<td>68</td>
<td>F</td>
<td>-</td>
<td>21.42</td>
<td>4.95</td>
</tr>
<tr>
<td>18.</td>
<td>53</td>
<td>F</td>
<td>-</td>
<td>15.43</td>
<td>1.9</td>
</tr>
</tbody>
</table>
3.3.2. Extended study

In order to increase the statistical power of our findings and exclude the possibility of a type II error, blood samples were collected and processed for the assay from another 20 patients and an equal number of controls. However, due to faulty storage, some of the stored samples had to be discarded and only 13 of the patients samples and 8 of the controls were assayed. The numbers are still satisfactory to detect a difference of 7 units (equal to 1 unit standard deviation of previously published studies) with a statistical power of 0.95 at a significance level of 0.05. This second group did not undergo the tyramine test and the psychiatric interview, hence the respective data is not available.

Table 14 compares the patients and controls with regard to age, sex and MAO values.

Table 14. Extended study: platelet MAO results in patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>31</td>
<td>24</td>
</tr>
<tr>
<td>F:M</td>
<td>26:5</td>
<td>13:11</td>
</tr>
<tr>
<td>Age</td>
<td>$44.3 \pm 16.2$</td>
<td>$32.7 \pm 14.7$</td>
</tr>
<tr>
<td>MAO nmol/mg pr./30min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>$18.32 \pm 6.96$</td>
<td>$18.48 \pm 5.44$</td>
</tr>
<tr>
<td>female</td>
<td>$19.03 \pm 7.21$</td>
<td>$18.1 \pm 4.66$</td>
</tr>
<tr>
<td>male</td>
<td>$14.65 \pm 4.21$</td>
<td>$18.9 \pm 6.46$</td>
</tr>
</tbody>
</table>

There was no difference between the patients and controls (fig.11). When subdivided according to gender, there was no significant difference between the groups. However male patients had a slightly lower value compared to controls, but as the number of male
patients was small, the difference was not statistically significant. A multiple regression analysis failed to show any significant influence of age, sex, presence or absence of depression and/or pain on platelet MAO activity.

Figure 11. Comparison of platelet MAO activity between patients and controls

Extended study.
3.4. DISCUSSION:

The role of the serotonergic system in pain and psychiatric research has been the subject of much research, controversy and uncertainty. A plethora of indirect evidence suggests that the serotonergic system is perturbed in pain and major mood disorders (Hallman & Oreland, 1989). The data, however, fail to fit a simple model of either hyperfunctioning or hypofunctioning of the serotonergic system and it is likely that disruption of this system is either a secondary phenomenon, a partial factor or both (Ferrier, 1991). Furthermore, the use of platelet MAO activity as a marker for the turn-over rate of the central serotonin system is controversial.

A correlation between levels of 5-HIAA, the serotonin metabolite in CSF and platelet MAO activity in healthy controls and chronic pain patients has been demonstrated which suggests that platelet MAO activity may be associated with central serotonin turn-over (Oreland et al., 1981). On the other hand, a simple correlation between platelet and brain MAO does not exist (Young et al., 1986) and in a few instances, where it has been possible to make a direct comparison, it has been difficult to detect any correlation between brain and platelet enzyme status (Winbald et al., 1979). Unlike MAO activity which seems to be generally stable, the level of 5-HIAA in CSF fluctuates during a depressive phase (Asberg et al., 1984). Studies on animal models of depression also show a reduction of 5-HIAA in the CSF after prolonged isolation (Valzelli & Bernasconi, 1979) whereas MAO activity is generally stable. Considering that some authors have reported in patients with chronic pain a significantly higher content of 5-HIAA in CSF than in pain-free controls (Ghia et al., 1981), the exact significance of platelet MAO activity in the
possible relationship between chronic pain and depression remains unclear.

Although the blood platelet is the most readily available source of the MAO for routine studies, being easy to obtain in pure preparation, it may not be ideal to provide an insight into the status of central nervous system MAO. Brain contains both types of enzyme and has a huge apparent functional reserve capacity, as evidenced by the need for greater than 85% inhibition of MAO in animals before the onset of any behavioural changes (Green et al., 1977). Additionally, there is such a degree of overlap between patients and controls that platelet activity measurements alone are unlikely to be of practical usefulness. Faced with this problem von Knorring et al. (1986) in a study of biological markers including MAO in pain patients, put forward an interesting solution. They observed that although each of the measures of biological markers gave a low contribution to the discrimination of patients with chronic idiopathic pain from patients with neurogenic pain syndromes and controls, the combination of several measures such as platelet MAO, 5-HIAA in CSF, serum cortisol before and after Dexamethasone Suppression Test and urinary melatonin gave a complete discrimination between groups. This approach was initially undertaken in our study. We attempted to study 3H-imipramine and 5-HT uptake in platelets which are both thought to be related to central serotonergic system. However, due to the fact that these tests need to be carried out under standard conditions e.g. samples should be taken at the same time of the day and the assay should be completed shortly after collection of blood samples, we were unable to perform these tests in a statistically significant number of pain patients and control subjects.
In the present study, we found no difference in mean platelet MAO activity between patients and controls. Almay et al. (1987b) in a study of a heterogenous group of patients with chronic pain showed low MAO values compared with controls. However, review of the literature did not reveal similar reports in relation to platelet MAO activity in chronic pain patients. This may partly be due to a greater tendency of medical journals to publish positive than negative findings.

Other possibilities related to the inconsistency between our findings and that of Almay et al. (1987b) include technical differences as well as patient or control group heterogeneity.

Technical differences:

Among many technical pitfalls, there is no doubt that the method of platelet preparation employed is one of the important ones (Sandler et al., 1981). White et al. (1976) have found specific MAO activity to vary fivefold with changes in the platelet harvesting procedure. Most groups prepare a platelet-rich plasma by slow centrifugation of the whole blood, the separated supernatant is then spun at a faster speed to obtain a platelet button, which is washed and stored frozen. However, Almay et al. (1987b) in their study of chronic pain patients assayed platelet activity in platelet-rich plasma without preparing a platelet button. Although this simplifies the procedure, it introduces a new variable. Yu & Boulton (1979) have shown that plasma can activate platelet MAO substantially. If this activation phenomenon were to vary in different clinical states, then the direct assay in plasma could compound two different variables.
Group heterogeneity:

Pain is multifactorial. Hence heterogeneity within the patients must be the rule. A statistical analysis which finds no significant difference between the groups does not prove that they are the same. There may be a subgroup of unknown size among either the patients or the controls with abnormal activity. If this were the case, then the results of any study would depend on the proportion of abnormal subgroup patients included (Sandler et al., 1981). It is interesting that lower activity is recorded in hospital staff (Berger et al., 1978) or military personnel (Major & Murphy, 1978) compared with random controls. Additionally, 55% of our patients in the first study had a history of depression. Several studies suggest that depressed patients tend to have elevated levels of MAO activity (Sandler et al., 1981)

Oreland & Hallman (1989) postulate that the inconsistency in the literature may be due to the fact that the personality traits of patients and controls perhaps are more important in MAO results than the disease itself. A connection between reduced platelet MAO activity and personality traits such as sensation seeking, impulsiveness and extraversion has now been firmly established (Schalling et al., 1987). Since the enzyme activity is under strong genetic influence (Reveley et al., 1983; Oxenstierna et al., 1986) and since a direct influence of platelet MAO activity on personality traits seems impossible the most likely explanation would be a common genetic mechanism of regulation. According to the vulnerability hypothesis (Buchsbaum et al., 1976) low MAO activity is associated with a predisposition to psychiatric vulnerability. The increased vulnerability may in turn be related to the personality traits associated with low MAO activity. This hypothesis is in line with the personality profiles found in disorders connected with reduced MAO activity i.e. alcoholism, suicidal behaviour and psychopathy.
Furthermore, it is possible that chronic facial pain patients are not identical at least with regard to the biological markers compared with other chronic pain patients. McKinney et al. (1990) compared chronic TMJ pain patients to non-TMJ pain patients and concluded that TMJ patients have lower 'usual' pain intensity and suffering levels, fewer vegetative symptoms associated with depression, less impairment of activity but higher reported stress level than non-TMJ chronic pain patients.

Almost half of our patients were on tricyclic antidepressants at the time of investigation. These patients did not differ from drug free individuals in relation to their MAO activity. Although in-vitro studies clearly show that this group of drugs produce reversible inhibition of platelet enzyme activity (Roth & Gillis, 1974 ; Edwards & Burns, 1978), it appears that circulating levels are too low during treatment to produce this affect to any clinically significant degree in-vivo. This in agreement with findings of Reveley et al. (1979) and also that of Giller et al. (1980). Additionally, the dose of tricyclics used in treatment of chronic facial pain is much lower than the average dose of this drugs in management of depressive illness.

In 18 subjects and 16 controls data were available for both Platelet MAO activity and tyramine sulphate excretion. Using Spearman's correlation test there was no significant correlation between MAO activity and tyramine output. This is in agreement with recent studies measuring both platelet MAO activity and tyramine sulphate output in the same patients (Jarman, 1992). It should be noted however, that the studies on platelet MAO only measure activity of MAO B, and tyramine is metabolised by both forms of the enzyme. Youdim et al. (1971) measured urinary excretion of p-HPAA (p-hydroxyphenyl-
acetic acid) which is the major metabolite of tyramine oxidised by MAO in an attempt to find an index of in vivo MAO activity but found no differences in excretion levels of the metabolite between low and high tyramine conjugators. Additionally the sulphonyl conjugation deficit has been found in depressives who proved resistant to MAOI therapy (Bonham Carter et al., 1978a). Therefore, the evidence is against any relationship between platelet MAO activity and tyramine sulphonyl conjugation.

A recurring problem with studies of platelet MAO activity is apparent lack of reproducibility (Glover et al., 1981). Some of the reasons for the inconsistencies in the literature were discussed above. Sandler et al. (1981) point out if many independent studies using various assay procedure on material from different patient populations show a similar trend, then the findings become more convincing.

In summary,

Our negative findings does not exclude abnormalities of the central serotonin system in patients with chronic idiopathic orofacial pain. Glover et al. (1981) has pointed out that knowledge of chemical pathology of MAO is still in its infancy and emphasizes the need for more basic research into the causes of any variations rather than merely measuring platelet MAO activity in different disease states. Recently the structure of the human gene for MAO A and MAO B has been identified on the human X chromosome (Chen et al., 1991). A knowledge of gene structure may help in evaluating the role of genetic variations of MAO in human disease, as it has been shown that the MAO gene itself is a major determinant of enzyme activity level, through noncoding regulatory elements (Hotamisligil & Breakfield, 1991).
More refined techniques are needed for measuring MAO activity in sites in the body other than platelet. The advent of positron emission tomography is one of the latest non-invasive techniques for the study of cerebral MAO activity (Lammertsma et al., 1991; Ohmomo et al., 1991), central nervous system serotonin uptake (Agren et al., 1991) and pain mechanisms (Jones et al., 1991a, b) which may help to clarify the role of MAO and serotonergic system in patients with chronic facial pain.
CHAPTER IV. OXYGEN FREE RADICAL METABOLISM
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    4.4.2.1. Materials and methods

    4.4.2.2. Results

    4.4.2.3. Discussion
1. BACKGROUND

1.1. Oxygen toxicity

Except for those organisms that are especially adapted to live under anaerobic conditions, all animals and plants require oxygen for efficient production of energy. However, oxygen supplied at concentrations greater than those in normal air has long been known to be toxic to plants, animals and to aerobic bacteria.

The toxicity of oxygen to animals, including man, has been of interest in relation to diving, under water swimming and more recently in the use of oxygen in treatment of cancer, gas gangrene, etc. Of historical scientific interest is the discovery of the adverse influence of hyperoxia in the aetiology of 'retrolental fibroplasia' (formation of fibrous tissue behind the lens). This form of blindness arose abruptly in the early 1940s among infants born prematurely and quickly became wide spread. Not until 1954 was it realized that this disease is associated with the use of high oxygen concentrations in incubators for premature babies, and more careful control of oxygen use has greatly decreased its incidence. Elevated oxygen appears to inhibit growth of retinal blood vessels. On return to a normal atmosphere there is an excessive regrowth of vessels, which sometimes occurs to an extent that causes detachment of the retina and subsequent blindness.

Of interest to researchers in the field of psychosomatic medicine and the role of stress in human diseases, is that high oxygen concentration causes a general "stress-reaction" in animals which stimulates the action of some endocrine glands. Removal of for example thyroid gland decreases the toxic effect of oxygen in some animals whereas administration of thyroxine, cortisone or adrenaline often makes them worse.
1.2. Causes of oxygen toxicity

Perhaps the earliest suggestion made to explain oxygen toxicity was that oxygen inhibits cellular enzymes. However, the rates of enzyme inactivation by oxygen in aerobic cells are too slow and too limited in extent to account for the rate at which toxic effects develop and many enzymes are totally unaffected by oxygen at all.

This led Gershman and Daniel in 1954 (Halliwell & Gutteridge, 1989) to propose that most of the damaging effects of oxygen could be attributed to formation of oxygen free radicals.

1.3. What is a free radical?

Electrons in atoms occupy regions of space known as orbitals. Each orbital can hold a maximum of two electrons spinning in opposite directions. A free radical can be defined as any species (molecule or atom) capable of independent existence that contains one or more unpaired electrons. An unpaired electron is one that occupies an orbital by itself. Most biologic molecules are non-radicals, containing only paired electrons.

1.4. Properties of free radicals

Free radicals broadly have the following properties:

1. Highly reactive with a consequent extremely short life span. This is because electrons are more stable when paired together in orbitals.

2. Self-perpetuating (autocatalytic) and diverse chemical reactivity.

Radicals can react with other molecules in a number of ways. Thus if two radicals meet, they can combine their unpaired electrons (symbolised by '.') and join to form a covalent
bond (a shared pair of electrons). A radical might donate its unpaired electron to another molecule (a reducing radical) or it might take an electron from another molecule in order to form a pair (an oxidising radical). Whichever of these types of reaction occurs, the non-radical species becomes a radical and the process tends to proceed as chain reactions, where one radical gives rise to another.

3. Generated both in vitro and in-vivo.

1.5. Oxygen and its derivatives

Reduction of oxygen produces a series of reactive chemical species.

\[
\begin{align*}
\text{O}_2 \quad \text{one electron} \rightarrow & \quad \text{O}_2^- \quad \text{superoxide} \\
\text{O}_2 \quad \text{two electron} \rightarrow & \quad \text{H}_2\text{O}_2 \quad \text{hydroxide} \text{ (protonated form of O}_2^2^- \text{ peroxide ion)} \\
\text{O}_2 \quad \text{four electron} \rightarrow & \quad 2\text{H}_2\text{O} \quad \text{(protonated form of O}^\cdot \text{)} \\
\text{H}_2\text{O}_2 \quad \text{energy} \rightarrow & \quad 2 \cdot \text{OH} \quad \text{hydroxyl radical}
\end{align*}
\]

Two of these intermediates, the superoxide \( \text{O}_2^- \) and the hydroxyl radical \( \cdot\text{OH} \) species are known as oxygen derived free radicals (OFRs). \( \text{H}_2\text{O}_2 \) has no unpaired electron and does not qualify as a radical. Hence the term reactive oxygen species has been introduced to describe collectively not only superoxide and hydroxyl radicals but also \( \text{H}_2\text{O}_2 \) (non-radical).

Hypochlorus acid (HOCl) is also a powerful antibacterial agent produced by action of the neutrophil enzyme myeloperoxidase on \( \text{H}_2\text{O}_2 \).

\[
\text{H}_2\text{O}_2 + \text{Cl}^- \rightarrow \text{HOCl} + \text{OH}^-
\]

HOCL which is also a non-radical, \( \text{H}_2\text{O}_2 \), superoxide and hydroxyl radical are sometimes collectively called oxidants which are all oxidising agents. However, superoxide has both
oxidising and reducing properties.

1.6. Potential biological sources of oxygen free radicals.

Table 15 shows two main sources of free radicals, which are broadly classified to cellular and environmental sources.

Table 15. Sources of free radicals in biological systems

<table>
<thead>
<tr>
<th>Cellular metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial electron transport</td>
</tr>
<tr>
<td>Auto-oxidation e.g. adrenaline</td>
</tr>
<tr>
<td>Activated phagocytic cells</td>
</tr>
<tr>
<td>Environmental</td>
</tr>
<tr>
<td>Radiation e.g. x-ray</td>
</tr>
<tr>
<td>Drugs e.g. halothane, paracetamol</td>
</tr>
<tr>
<td>Tobacco smoke</td>
</tr>
<tr>
<td>Pesticides e.g. paraquat</td>
</tr>
</tbody>
</table>

Of interest to us are:

1. Mitochondrial electron transport

When mitochondria are functioning, some of the electrons passing through the respiratory chain leak from the electron carriers and pass directly onto oxygen reducing it to superoxide. Indeed the discovery of superoxide dismutase which removes superoxide radical, led to the realization that superoxide radical is formed in living organisms (Halliwell, 1991).
2. Autoxidation

Many molecules oxidize on contact with oxygen, e.g. adrenalin. The first stage in this oxidation is transfer of an electron from adrenaline to $O_2$ forming superoxide.

It is possible that elevated levels of plasma adrenalin under stressful conditions contribute to increased generation of free radicals.

3. Activated phagocytic cells

Activated phagocytic cells generate superoxide radical. At the start of phagocytosis there is a dramatic increase in oxygen consumption unrelated to mitochondrial electron transport. At the same time there is an increased consumption of glucose. An enzyme complex reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase catalyses the reaction:

\[
2O_2 + NADPH \rightarrow 2O_2^- + NADP^+ + H^+
\]

producing superoxide radical in high concentrations. In stressful conditions, the functions of immune cells are generally considered to be modulated by various humoral factors such as hormones, lymphokines and neuropeptides (Nakamura et al., 1989). The enhancement of microbicidal and cytotoxic activities of macrophage and neutrophils which depend to a large extent on the generation of oxygen radicals may be implicated in the cause of stress induced diseases.

Superoxide radical is not particularly damaging in aqueous systems, but can react by a dismutase reaction (catalysed by superoxide dismutase) to form $H_2O_2$ which has some bactericidal properties:

\[
O_2^- + O_2^- + 2H \rightarrow H_2O_2 + O_2
\]
H₂O₂ oxidizes chloride ions to HOCl a powerful antibacterial agent.

\[
\text{H}_2\text{O}_2 + \text{Cl}^- \rightarrow \text{HOCl} + \text{OH}^-
\]

The respiratory burst is attenuated or absent in patients with chronic granulomatous disease a series of inborn conditions in which reduced NADPH oxidase system fails to work. This inherited condition presents in childhood with multiple infections.

Certain metal ions such as Fe²⁺ (ferrous) can react with hydrogen peroxide via a Fenton reaction to form the more toxic hydroxyl radical.

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \text{OH}^-
\]

Fenton reaction

1.7. Hydroxyl radical

Chemists and biologists have examined in detail the role of free radical reactions in the damage done to living cells by high energy radiation. When tissues are exposed to for example Gamma radiation, most of the energy taken up is absorbed by the cell water, largely because there is more water than any other molecule. The radiation causes one of the oxygen-hydrogen covalent bonds in water to split, leaving a single electron on hydrogen and one on oxygen, so creating two radicals:

\[
\text{H}_2\text{O} \rightarrow \text{intermediate stages} \rightarrow \text{H}^- + \cdot\text{OH}
\]

H⁻ is a hydrogen radical (or hydrogen atom) and \cdot\text{OH} is a hydroxyl radical. The hydroxyl radical is the most reactive radical species known to chemistry. It can attack and damage almost every molecule found in living cells. Since \cdot\text{OH} is so reactive, \cdot\text{OH} generated in vivo does not persist for even a microsecond and rapidly combines with molecules in its immediate vicinity.
The best characterized biologic damage caused by OH is its ability to stimulate the free radical chain reaction known as lipid peroxidation (fig. 12). This occurs when the ·OH is generated close to membranes. The ·OH preferentially attacks polyunsaturated fatty acid side chains such as arachidonic acid which is a precursor of prostaglandins and leukotrienes.

Figure 12. Lipid peroxidation
The \( \cdot \text{OH} \) abstracts an atom of hydrogen from one of the carbon atoms in the side chain and combines if to form water.

\[
\text{\(-CH-} + \cdot \text{OH} \longrightarrow \cdot \text{C-} + \text{H}_2\text{O}
\]

This reaction removes the \( \cdot \text{OH} \), but leaves behind a carbon-centred radical (-C-) in the membrane. Carbon-centred radicals formed from polyunsaturated fatty acid side chains usually undergo molecular rearrangement to give conjugated diene structures. Under physiologic conditions, the most likely fate of carbon-centred radicals is to combine with oxygen creating yet another radical:

\[
\cdot \text{C-} + \text{O}_2 \longrightarrow \text{peroxyl radical}
\]

Peroxyl radicals are reactive enough to attack adjacent fatty acid side chains abstracting hydrogen:

\[
\text{peroxyl radical} + \text{fatty acid} \longrightarrow \text{lipid hydroperoxide} + \cdot \text{C-}
\]

Another carbon centred radical is generated and so the chain reaction (equations a,b) continues. One \( \cdot \text{OH} \) can result in the conversion of many hundred fatty acid side chains into lipid hydroperoxides. Accumulation of lipid hydroperoxides in a membrane disrupts its function and causes it to collapse. Lipid hydroperoxides can also decompose and yield a range of highly toxic products among the most unpleasant of which are aldehydes.

A great deal of attention in the literature has been focused on malondialdehyde, measurement of which is used as an index of free radical activity (see IV.4.).
1.8. Methods of measurement of free radicals

Although oxidative stress is thought to be involved in the pathophysiology of several diseases, it is not routinely measured in clinical diagnosis. This is at least partly because accepted and standardized methods for measuring oxidative stress in humans are not yet established. Hydroxyl radical is so highly reactive that measuring its direct formation in vivo is very difficult. Halliwell & Grootveld(1987) in a review of current methods available for measurement of free radicals, state that at present no fully satisfactory method exists. Whatever method is chosen, one should think clearly what is measured and how it relates to the overall lipid peroxidation process. They suggest, whenever possible two or more different assay methods should be used.

1.9. Free radicals and human disease

Oxidant generation is part of normal human metabolism. When produced in excess, oxidants can cause tissue injury. However, tissue injury can itself cause more oxidant generation by such processes as metal-ion release, phagocyte activation, lipoxygenase activation and disruption of mitochondrial electron transport chains, so that more electrons escape to oxygen to form superoxide radical. This may (or may not, depending on the situation) contribute to a worsening of the injury. It follows that almost any disease is likely to be accompanied by increased formation of reactive oxygen species. At the cellular level free radicals have a great propensity for causing cellular oxidative damage through their innate high reactivity, this may be why they have been called "agents and products of doom"(Dormandy, 1989). However, the beneficial actions of free radicals are now being recognized; for instance a free radical intermediate is produced during prostaglandin synthesis which is thought to self-deactivate cyclo-oxygenase oxidatively
forming a negative feedback loop and therefore controlling the prostaglandin cascade
(Ogino et al, 1978).

There is a massive literature concerning the role of free radical reactions in human
diseases. Table 16 lists some of the conditions in which free radical involvement has been
proposed.

Table 16. Some clinical conditions in which the involvement of oxygen radicals has been
suggested.

<table>
<thead>
<tr>
<th>Inflammatory-immune injury</th>
<th>Heart and cardiovascular system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis</td>
<td>Alcohol cardiomyopathy</td>
</tr>
<tr>
<td>Autoimmune disease</td>
<td>Keshan disease</td>
</tr>
<tr>
<td>Ischemia-reperfusion states</td>
<td>Kidney</td>
</tr>
<tr>
<td>Stroke/myocardial infarction</td>
<td>Autoimmune nephrotic syndrome</td>
</tr>
<tr>
<td>Organ transplantation</td>
<td>Heavy metal nephrotoxicity</td>
</tr>
<tr>
<td>Drug and toxin-induced reaction</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>Iron overload</td>
<td>Peptic ulcer</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>Pancreatitis</td>
</tr>
<tr>
<td>Radiation injury</td>
<td>Halothane induced jaundice</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>Brain</td>
</tr>
<tr>
<td>Malaria</td>
<td>Vitamin E deficiency</td>
</tr>
<tr>
<td>Sickle cell anaemia</td>
<td>Potentiation of traumatic injury</td>
</tr>
<tr>
<td>Fanconi's anaemia</td>
<td>Parkinson's disease</td>
</tr>
<tr>
<td>Lung</td>
<td>Eye</td>
</tr>
<tr>
<td>Cigarette-smoke effect</td>
<td>Cataractogenesis</td>
</tr>
<tr>
<td>Hyperoxia</td>
<td>retinopathy of prematurity</td>
</tr>
<tr>
<td>Emphysema</td>
<td>Ocular haemorrhage</td>
</tr>
</tbody>
</table>
2. AIMS OF THE STUDY

In a review of the literature our attention was drawn to studies claiming to demonstrate that emotional stress and pain in animals were associated with an increased generation of free radicals (Aleksandrovskii et al., 1988; Golikov et al., 1987; Vitrichenko, 1985) and that stress induced damage to the gastric mucosa was related to free radical production (Itoh & Guth, 1985; Salim, 1989). Duthie et al (1989) demonstrated in stress-susceptible pigs an elevated level of TBA-RS in the plasma. These pigs have an inherited tendency to develop rapidly malignant hyperthermia on exposure to stress. Furthermore, it is said that free radical related disease is significantly more common in women. Chronic facial pain is four times more common in females than males and there has been a substantial body of work to show the condition is stress related (Lefer, 1966; Fine, 1971; Feinmann & Harris, 1984). Nevertheless, underlying biochemical mechanisms linking stress, pain and free radicals are not clear. However, activation of phagocytic cells under stress and autoxidation of raised levels of adrenaline may play a part.

To date, there has been no study of free radical metabolism in relation to chronic facial pain. Therefore it was decided to test the hypothesis that oxygen free radicals and in particular hydroxyl radical contribute to the pathogenesis of chronic facial pain.

Two methods were chosen for investigation of free radical metabolism in our patients.

The first, was a recently developed method by Grootveld & Halliwell (1986) which attempts to measure \( \cdot OH \text{ in vivo} \). The method is based on measurement of the products...
formed by the attack of hydroxyl radical on aromatic compounds particularly 2,3-dihydroxybenzoic acid by HPLC separation combined with highly sensitive electrochemical detection.

The second method was the thiobarbituric acid test which is one of the oldest and most frequently used tests for measuring the lipid peroxidation endproducts. Unlike the first method, TBA test is very simple and can be applied to crude biological systems.
3. MEASUREMENT OF 2,3,- DIHYDROXY BENZOIC ACID :

3.1. BACKGROUND :

Under physiological conditions, attack of 'OH on molecules containing benzene ring (aromatic compounds) results in formation of hydroxylated products. Indeed, aromatic hydroxylation has been used as a method for measuring 'OH production in vitro (Halliwell, 1978 ; Richmond & Halliwell, 1982). Grootveld & Halliwell (1986) suggested that if an aromatic compound reacts with 'OH to form a specific set of hydroxylated products that can be accurately measured in body fluids, and one or more of these products is not identical to enzyme produced hydroxylated products, then formation of the "unnatural" products could conceivably be used to assess 'OH formation in vivo. Aspirin is such a compound which can be safely administered to humans in doses that produce concentrations in body fluids which are sufficient to scavenge 'OH. Measurement of products of the reaction between 'OH and salicylate molecule is therefore used in as a means of assessing of hydroxyl radical formation in vivo (Grootveld & Halliwell, 1986).

Aspirin (O-acetylsalicylate) is rapidly hydrolysed to salicylate in the body. Some salicylic acid is excreted as such, some is conjugated with glycine to produce salicyluric acid, some is hydroxylated to gentisic acid (2,5-dihydroxybenzoic acid) and some is conjugated with glucoronic acid (fig. 13). Attack of hydroxyl radical upon salicylate produces two products that have not been reported as normal products of enzymic salicylate metabolism, namely 2,3,-dihydroxybenzoic acid and to a smaller extent catechol (fig. 14).
Figure 13. Aspirin metabolism
Figure 14. Products of attack of hydroxyl radical on salicylate
As the concentration of these compounds in plasma would be extremely low, a HPLC separation method combined with highly sensitive electrochemical detection was developed by Grootveld & Halliwell for the measurement of these compounds. The same authors in a later publication demonstrated the presence of these compounds in diethyl ether extracts of human urine (Grootveld & Halliwell, 1988). Their methodology was adopted for measurement of 2,3,-DHB in our study of patients with chronic facial pain.

3.2. Materials and Methods:

Reagents. HPLC-grade solvents were obtained from BDH Chemicals Ltd. and aromatic compounds from Sigma. Standard solutions were made up and stored as described by Grootveld & Halliwell (1986).

HPLC. HPLC was carried out using an HPLC Spectraphysics pump, a 5μm ODS reverse-phase column 25cm x 4.6mm (Brownlee). The mobile phase and electrochemical detection were identical to that described by Grootveld and Halliwell (1986) ie sodium citrate (30mM)-sodium acetate (27.7mM) buffer pH 4.75 at a flow rate of 1.0 ml/min. The mobile phase was sparged continuously with HE gas during elution. Detection was by an EDT LCA15 electrochemical detector equipped with a glassy carbon working electrode and an Ag/AgCl reference electrode. The injection loop (10μl volume) was cleaned with at least 3ml each of methanol, water and buffer after each injection, until injection of buffer did not produce any electroactivity as a result of residues in the loop from previous injection.

Plasma preparation and analysis. 10ml of venous blood was collected into heparinized bottles, from subjects before and 2hrs after ingestion of 1.2g of aspirin. The samples were centrifuged at 3000rpm/5min and the plasma layer was aliquoted and stored at -20°C.
until analysis.

**Extraction procedure.** 200μl of plasma was treated with 25μl of 1M HCl and 25μl of 7μM 3,4-DHB (internal standard) and extracted twice with 10ml of diethyl ether for chromatography. The combined extracts were evaporated to dryness in a water-bath at 40°C and then reconstituted with 250μl of 50mM HCl.

### 3.3. PILOT STUDY

10 patients and six age and sex matched control subjects were included in this study. Venous blood samples were collected 4 hours after ingestion of 600mg of aspirin and centrifuged at 3000rpm/5min. Plasma was assayed according to the standard protocol. The mean level of 2,3-DHB in patients was $354.1 \pm 93.5\mu M$ compared to $106 \pm 73.3\mu M$ in controls (Table 17). The difference was statistically significant ($p < 0.001$).

**Table 17.** Concentration of 2,3-DHB in patients with chronic facial pain and controls

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pilot study</td>
<td>$354.1 \pm 93.5\mu M$</td>
<td>$106 \pm 73.3\mu M$</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td>Extended study</td>
<td>$38.4 \pm 30.2\mu M$</td>
<td>$21.8 \pm 18.3\mu M$</td>
<td>$p &lt; 0.05$</td>
</tr>
</tbody>
</table>

### 3.4. EXTENDED STUDY

As the results of the pilot study were very encouraging, a larger number of patients and controls were investigated. 20 patients with chronic facial pain and an equal number of healthy age and sex matched controls were included. Although patients had a significantly higher value ($p < 0.05$), the magnitude of difference from the control group was not as high as the pilot study. (Table 17)
3.5. DISCOVERY OF A METHODOLOGICAL PROBLEM

It was realized that the measured levels of 2,3,-DHB in our laboratory were much higher than others (Grootveld & Halliwell, 1986; Ward, personal communication). This prompted us to investigate the validity of our method. The following experiments were carried out:

I. Assay of a blank sample:

It was considered that the putative peak might not be 2,3,DHB. Therefore, a blank serum sample from a patient before ingestion of aspirin was run parallel to the sample collected after ingestion of aspirin. Surprisingly, there was a peak with exactly the same retention time as 2,5-DHB(fig.15). Figure 16 shows the same sample spiked with 2,5-DHB. Initially, it was considered that the subject may have consumed other non steroid anti-inflammatory drugs which could have metabolites with retention times identical to that of 2,5-DHB. However, plasma samples from nine medication free controls also showed the same peak.

In order to identify the source of this peak the following were considered as possible sources:

1. The anticoagulant content of blood container

Plasma samples collected into heparinized bottles, EDTA bottles as well as serum samples were compared. All contained the unknown peak.

2. Contamination of glassware

All glassware were meticulously cleaned several times with double distilled water. Nevertheless, the unknown peak remained unchanged.

3. Water impurity

In the extraction procedure, the test samples were substituted with water. Both the
double distilled water as well as HPLC grade water (BDH chemicals) used in preparation of buffers, contained the unknown peak.

Figure 15. Chromatogram of a blank plasma sample before ingestion of aspirin

Figure 16. Chromatogram of the same sample spiked with 2,5-DHB
Eventually, diethyl ether itself was found to contain a compound with exactly the same retention time as 2,5-DHB. Although diethyl ether was especially made for chromatographic use, it was found that it contains a stabilizer with an aromatic ring. The stabilizer pyrogallol (1,2,3-trihydrobenzene) was found to have exactly the same retention time as 2,5-DHB. It not only made the identification of 2,5-DHB impossible, but also because of the large size of its corresponding peak it overlapped the peaks related to 2,3- and 3,4-DHB, and therefore it was not possible to measure 2,3- and/or 2,5-DHB in diethyl ether extracts of biological fluids.

3.6. Modification of the extraction procedure and method development

Ethyl acetate is allegedly specific for the extraction of phenolic compounds (Krstulovic et al., 1982). Therefore it was decided to use ethyl acetate for the future extraction procedures and to validate the method fully before proceeding to investigate any number of patients or controls.

3.6.1. Separation of standards

Figure 17 shows the separation of a standard mixture of 2,3-DHB, 2,5-DHB and also 3,4-DHB which was used as an internal standard. The relative retention time of 2,3-DHB in relation to 3,4-DHB was 0.78. 2,5-DHB ran close to the internal standard but was clearly separable from it (relative retention time 0.86, resolution from 3,4-DHB 2.2).
3.6.2. Calibration curve

There was a linear relation between the detector response and concentration of standards within the range of 50nm to 1μM (fig. 18).
3.6.3. Measurement of concentration of salicylate in plasma:

Grootveld and Halliwell (1986) point out that for generation of measurable amounts of 2,3-DHB, it is important that administered aromatic compound to achieve adequate concentrations in the blood. Therefore, serial measurements of salicylate concentration of plasma after ingestion of 1.2g aspirin was performed as follows:

3.6.3.1. Sample collection

A size 18'G intravenous canula was inserted in the antecubital fossa of a control subject and a blood sample was collected. After each collection, the patency of the canula was maintained by irrigation with 1ml of sterile water. 1.2g of aspirin was ingested, and serial venous sample collection was carried out at various time intervals up to 5.5 hours post-aspirin. At each sample collection the first 2ml of blood was discarded.

3.6.3.2. Salicylate measurement

Salicylate measurement was carried out using a colorimetric assay (Sigma). The results showed that salicylate concentration reached its peak after 150min(fig.19). However, plasma levels reached close to the peak concentration 1-2 hours after ingestion of aspirin. The time interval of 2 hours between the ingestion of aspirin and blood collection was arbitrarily chosen as the standard for future experiments.
3.6.4. Comparison of plasma samples collected before and after ingestion of aspirin and measurement of the extraction efficiency

Plasma samples collected before and 2 hours after ingestion of aspirin were run parallel. Aliquots from each were also spiked with different concentration of individual standards namely 2,3,DHB, 2,5,DHB and 3,4, DHB. This was to confirm the identity of observed peaks, as well as measuring the extraction efficiency.

The blank samples extracted with ethyl acetate only contained the internal standard which was added during the extraction procedure. Post-aspirin samples, contained all 3 peaks, which were increased in height when spiked with standards.

The extraction efficiency of 2,3,-DHB was similar to previous reports (Grootveld & Halliwell,1986). However, the extraction efficiency of 2,5-DHB was very low (20%).
which is in contrast to previous reports of 76% (Grootveld & Halliwell, 1986). The explanation for this observation was not clear.

The possibility that 2,5- DHB was binding to plasma proteins such as albumin or immunoglobulin was tested. It was possible to extract 2,5- DHB efficiently from buffer, albumin (5g/dl) or human immunoglobulin (3g/dl). However, as 2,3, DHB is the metabolite of interest, this methodological deficiency was accepted.

3.6.5. Improvement of resolution:

Resolution ($R_s$) describes the degree of separation of one component from another and is defined as the difference in retention volumes of the two solutes divided by their average peak width (fig. 20).

$$RS = \frac{(V_{R2} - V_{R1})}{0.5 \times (W_1 + W_2)}$$

![Figure 20. Measurement of resolution](image)

A minimum resolution of 1.5 is generally recommended. This resolution corresponds to two peaks which are just resolved to the baseline. Figure 21 and figure 22 show
chromatographs of plasma extracts from two control subjects who had consumed 1.2g of aspirin 2hrs prior to blood collection. Due to the large size of catecholamine peak which is efficiently extracted by ethyl acetate, in one of the subjects it is not possible to detect the presence of 2,3, DHB.

*Figure 21. Plasma sample from a healthy volunteer after ingestion of 1.2g of aspirin*

*Figure 22. Plasma sample from another healthy volunteer after ingestion of 1.2g of aspirin*
To improve the resolution a C8 and a C18 column were placed in series. This increased the column efficiency and provided the required resolution of 2,3, DHB and adjacent peaks (Table 18). However, this was achieved at the expense of doubling the analysis time. This is still below 20 minutes which is considered acceptable.

Table 18. Comparison of the resolution of 2,3,- 2,5,- and 3,4,-DHB as obtained by a single C18 column and the combination of C8 and C18.

<table>
<thead>
<tr>
<th></th>
<th>Resolution with a C18 column</th>
<th>Resolution with a C18 and C8 in series</th>
</tr>
</thead>
<tbody>
<tr>
<td>R, between 2,3, &amp; 2,5,-DHB</td>
<td>1.6</td>
<td>3.6</td>
</tr>
<tr>
<td>R, between 2,5, &amp; 3,4,-DHB</td>
<td>2.2</td>
<td>3.6</td>
</tr>
</tbody>
</table>

3.6.6. Measurement of 2,3,DHB in urine

A pharmacodynamic study of 2,3,DHB, would involve not only measurement of plasma levels of 2,3,DHB, but also measurement of its excretion in the urine. Therefore, urine samples collected 3hrs after ingestion of 1.2g of aspirin were assayed and found to contain a much higher concentration of 2,3, DHB than plasma( 5μM vs 50nM).
3.7. DISCUSSION:

Our findings once again brings to light a common problem in chromatographic assays which is frequently overlooked. The appearance of a well resolved band at the retention time of the standard does not guarantee that the band represents a pure compound. It is always possible for a second compound to have the same retention time as the standard. Therefore during method development it is important to check for any interfering peaks that might overlap analyte bands. Commonly used procedures include (Dolan & Snyder, 1989):

1. Chromatographic cross-checks
2. Wavelength-ratioing and diode-array detectors
3. Other spectroscopic tests as by liquid chromatography-mass spectrometry.

Grootveld & Halliwell (1986) point out that in view of the large number of ether-soluble molecules present in biological fluids, the identity of retention time with that of a standard is inadequate evidence to attribute the peak to 2,3,DHB. These authors identified the presence of 2,3,-DHB in plasma and urine samples collected after ingestion of aspirin by electrochemical analysis and by mass spectrometry (Grootveld & Halliwell, 1988).

The reason for the lack of a similar observation by the originators of the method is not clear. One possibility is that the HPLC column used by them had a low efficiency, and therefore, 2,5,DHB was not separable from and overlapped with the internal standard 3,4,DHB. This could lead to inaccuracies in the quantification of 2,5,DHB and also 2,3,DHB if an internal standard method is used.
In our study, blank plasma samples were an additional means of confirming the identity of aspirin metabolites. By comparing the chromatograms of samples from subjects before and after ingestion of aspirin, a clear picture is provided of peaks which are attributable to aspirin metabolite. An incidental finding was that measurement of salicylate by the above method (oxidation potential 0.96) was not reliable as blank plasma showed a peak with an identical retention time to that of salicylate (See V.3.2.3.).

The question also arises, that if we were measuring an artefact during the initial phases of the study, why was there a significant difference between the patients and controls.

Over the past 15 years, high performance liquid chromatography has made the transition from an instrument used only by experts in research labs to a tool used for routine applications by relatively unskilled workers. Traditionally, identification and correction of HPLC problems have been a result of years of work at the bench. The advancement of technology, allows the liquid chromatography system to locate many of its own problems. However, a thorough understanding of the fundamentals of HPLC, particularly those of separation and quantification problems is essential for accurate measurements.

Calculated concentrations for various sample components can be in error as a result of system malfunction of various kinds such as leaks, changes in pressure and abnormal chromatograms.

The precision and accuracy of a liquid chromatographic method should be determined at the time method development and during method validation. Simulated samples (in the same sample matrix as the samples for assay) must be prepared, containing known concentrations of the standard over the range to be reported by the method.
Unfortunately, during the initial phases of this study, we did not attempt to validate the method, as it was introduced and validated by one of the leading biochemical laboratories in the field of free radical research. Had we checked the reproducibility of our extraction procedure, many unnecessary experiments could have been avoided. In summary, our initial observation of a significant difference between patients and controls could be due to the following factors:

1. Inaccuracies and lack of reproducibility in the extraction procedure.
2. Unfamiliarity with the quantification errors which could arise in HPLC measurements. These include errors in sample processing, sample injection, separation, detection and calibration (Dolan & Snyder, 1989).

Having standardized our methodology for measurement of 2,3-DHB, work is now in progress in our research laboratory to study this aspect of free radical metabolism in patients with chronic idiopathic orofacial pain.
4. THIOBARBITURIC ACID TEST:

4.1. Background:

The thiobarbituric acid (TBA) reaction was introduced by Kohn and Liversedge in 1944. MDA is an endproduct of lipid peroxidation of polyunsaturated fatty acids by free radicals. The TBA test is based on the reaction of this molecule with two molecules of TBA with to give a pink chromophore. In acid solution the product absorbs light at 532nm and is readily extractable into organic solvents such as butan-1-ol. Widespread use of the TBA reaction by the food and dairy industries established the TBA test as an important test of lipid rancidity. However, conflicting results have been reported on the plasma levels of MDA with a range of between 0.6\(\mu\)M (Wong et al., 1987) and 47\(\mu\)M (Aznar et al., 1983). Recently Frei et al. (1988) used a method based on HPLC and microperoxidase/isoluminol dependent chemiluminescence for qualitative and quantitative measurement of hydroperoxides in human plasma and found concentrations no higher than 0.03\(\mu\)M in the Plasma. Therefore, it is generally assumed that most of the MDA is derived from the breakdown of peroxides during the acid heating stage of the test.

4.2. Specificity and sensitivity of TBA test:

Since many different peroxidic intermediates of polyunsaturated fatty acids release MDA, the test is not specific for any stage of lipid peroxidation. Furthermore many non-lipid molecules directly react with TBA to form chromogens (Gutteridge, 1986). Bile pigments which often occur in plasma are TBA reactive and their reaction product absorbs light at 532nm. Aminoacids, carbohydrates and nucleic acid are also TBA reactive after damage by free radicals. Therefore, a considerable amount of TBA-RS in plasma is non-lipid. It
has been shown that the source of TBA-RS of blood are only 43% lipoprotein. The rest are 11-15% proteins and the remainder in non-lipoprotein and protein part of blood (Bonnefont et al., 1989).

As far as the sensitivity is concerned, when TBA test is applied to biological samples the sensitivity is high because the test amplifies oxidative changes that have begun in the sample, and is therefore used as a measure of the potential of the material to undergo the chain reactions of lipid peroxidation. Halliwell (personal communication) points out that although false positives are frequent, false negatives are rare.

4.3. Method standardization

The method described by Rowley et al. (1984) in a study of lipid peroxidation in serum and synovial fluid of patients with rheumatoid arthritis was chosen and with slight modification used for this study. It was as follows:

10ml of venous blood was collected into heparinized bottles and centrifuged at 3000rpm/5min. The plasma layer was aliquoted and stored at -20°C until the assay. The assay consisted of adding 125µl of plasma to 250µl of 1%(w/v) TBA dissolved in NaOH(50mmol/l), 250µl of HCl(25%v/v) and 200µl of water. In the blank the test material was substituted with an equal volume of water. The tubes were tightly capped and heated at 100°C for various times (see IV.4.3.2.1.). When cool, 3 ml butan-1-ol was added and the TBA reactive material was extracted into the butan-1-ol by vigorous vortexing for 2 minutes. After centrifugation at 3000rpm for 15 minutes the upper organic layer was measured against the blank in a spectrophotometer at 532nm.
4.3.1. MDA-TBA calibration curve and expression of the results

TBA test is often calibrated with malonaldehyde, and the results expressed in terms of the amount of MDA produced in a given time. As MDA is unstable, its precursor tetramethoxypropane was used in our assay to construct a calibration curve (fig.23).

![Calibration Curve](image)

**Figure 23. Calibration curve, TBA assay**

However, expression of the results as MDA concentration was considered unsatisfactory. The amount of free MDA has been shown to be extremely low and most of the MDA that reacts in the TBA test is not present in the sample assayed but is formed by decomposition of lipid peroxides during the acid heating stage of the TBA assay. Therefore, the results are expressed as Δ absorbance at 532nm. However, the molar extinction coefficient of MDA-TBA adduct (1.54×10^4 at 532nm) can be used to calculate the amount of MDA by reference to the calibration curve.
4.3.2. Study of factors affecting TBA test:

The simplicity of the test has led to many different versions of the test being adopted. A comparison of results between laboratories is therefore extremely difficult. Our method standardization included experiments to assess the following:

1. Heating time
2. Assay reproducibility
   2.1 Intra-assay variation
   2.2 Inter-assay variation
3. Storage temperature and duration
4. Time interval between blood collection and incubation temperature before blood centrifugation
5. Diurnal variation in sample collection
6. Test controls
   6.1. Promoters of lipid peroxidation
   6.2. Inhibitors of lipid peroxidation
      6.2.1. antioxidants
      6.2.2. ascorbic acid
      6.2.3. iron chelators
6. Salicylate and 2,3,DHB

4.3.2.1. Heating time

The rate of chromogen formation in TBA test is dependent on the duration of the heating time. Aliquots of plasma sample from a normal subject were treated according to the standard protocol and heated at 100C for various durations ranging from 15 to 90 minutes. It was found that TBA reactivity gradually rises with the increase in heating time.
and reaches its maximum after 60 minutes. Further heating did not lead to any more rise in TBA reactivity (fig.24). Therefore, 60 minutes was chosen as the optimal heating time.

Figure 24. Study of the effect of heating time on TBA reactivity of plasma

4.3.2.2. Assay reproducibility

I. Intraassay variation

Plasma collected from a normal subject was divided into 40 aliquots, which were assayed in four groups of 10, in four consecutive days. The coefficient of variation in TBA reactivity of samples varied from 3 to 13% with a mean of 8% (fig.25).
II. Inter-assay variation

Inter-group comparison showed a gradual increase in the mean value from the day 1 to the day 4 (fig. 25). Although the consecutive groups were not significantly different from each other, however, the results of the day 4 were significantly different from the day 1. Although this could be due to inaccuracies in the test, we considered the possibility that the storage of samples could lead to an increase in TBA reactivity. The test of this assumption was the following step.

![Graph showing reproducibility of TBA test](image)

Figure 25. Reproducibility of TBA test
4.3.2.3. Storage temperature and duration

Aliquots of plasma sample from a normal subject were stored at 4C, -20C and -70C. These were assayed on days 1, 4, 8, 15, 22 and 29 (figs. 26, 27).

Figure 26. Reproducibility of TBA test, effect of storage time and temperature

Figure 27. Reproducibility of TBA test, effect of storage temperature
There was no significant difference between the mean TBA reactivity of plasma samples stored at 3 different temperatures mentioned above. Although the intraassay variation was acceptable the interassay variation was not. There was no uniform trend in the variability of TBA reactivity of the samples from day 1 to day 29. Therefore, it was decided to perform comparative studies of TBA reactivity in a single assay.

4.3.2.4. Effect of time interval between blood collection and incubation temperature before blood centrifugation

The time interval between blood collection and its centrifugation was variable and its effect on TBA reactivity needed to be studied. Aliquots of blood samples were incubated at 4C, 22C (room temperature) and 37C. These were centrifuged at hourly intervals ranging from 2 to 6 hours after blood collection. Plasma layer was stored at -20C until the assay.

Plasma showed higher reactivity when incubated at 37 and left longer than 4 hours before centrifugation. However, our standard protocol for sample preparation was to store the samples either at 4C or at room temperature and to centrifuge the samples within 2 hours after collection.

4.3.2.5. Diurnal variation in sample collection

As 43% of TBA-RS of blood are lipoproteins, it was considered that, time of blood collection in relation to a meal consumption with the subsequent rise in plasma lipid content may be important in TBA reactivity. Blood samples were collected from a normal
subject at fasting and 3 hours after eating a meal rich in lipids, to ensure a rise in plasma lipid. Plasma was prepared and processed according to the standard TBA protocol. However, there was no difference between fasting and post-meal samples.

4.3.2.6. Controls

Gutteridge (1986) points out that when using TBA assay one should take into account the effect of added iron salts and metal chelators on lipid peroxides already present in the lipid samples. When applied to complex biological material, the TBA test will undoubtedly be influenced by such factors.

4.3.2.6.1. Promoters of lipid peroxidation

Iron has the ability to decompose lipid peroxides with the release of peroxy radicals which are precursors of MDA. This stimulatory effect of iron on TBA reactivity is often used as a positive control in TBA tests. Aruoma et al. (1989) in the study of the effect of metal ions on TBA reactivity of liver microsomes found that:

a. Addition of Fe$^{3+}$ has no effect on lipid peroxidation.

b. Addition of Fe$^{2+}$ slowly starts the peroxidation of microsomes.

c. Combination of Fe$^{2+}$ and Fe$^{3+}$ shows considerable increase in TBA reactivity.

However, we found that addition of either Fe$^{3+}$ or Fe$^{2+}$ (final concentration in the assay 100μM) increased plasma TBA reactivity by 62% and 71% respectively. Combination of Fe$^{2+}$ and Fe$^{3+}$ caused an increase in TBA reactivity similar to that of Fe$^{2+}$ alone.
4.3.2.6.2. Inhibitors of lipid peroxidation

I. Antioxidants

Peroxyl radicals formed during the TBA reaction can abstract H\(^+\) atoms from polyunsaturated fatty acids to start a chain reaction giving rise to more TBA reactive material. To prevent peroxidation during the TBA reaction, therefore, antioxidants have been added to the TBA reagents (Aruoma et al., 1989).

50\(\mu\)l of 2% butylatedhydroxytoluene in ethanol was added as an antioxidant to a test sample and was compared with a similar test sample for TBA reactivity. There was no difference between the two.

II. Ascorbic acid

Ascorbic acid (AA) is a powerful reducing agent (electron donor), an antioxidant and reacts with superoxide, peroxide and hydroxyl radical to form dehydroascorbic acid (DHA). Under certain physiological conditions, however, ascorbic acid can promote lipid peroxidation by the reduction of ferric iron (Fe\(^{3+}\)) to ferrous iron (Fe\(^{2+}\)):

\[
\text{Fe}^{3+} + \text{Ascorbic Acid} \rightarrow \text{Fe}^{2+} + \text{DHA}
\]

The ferrous iron can then take part in a fenton reaction to generate \(\cdot\text{OH}\) radical. In liver microsomes, combination of ascorbate and Fe\(^{3+}\) has been found to be the most potent promoter of TBA reactivity (Aruoma et al., 1989). Although we found that the combination of AA and Fe\(^{3+}\) is a potent stimulator of TBA reactivity, however, ascorbic acid alone was found to be the most potent promoter of TBA reactivity in our assay.
Nevertheless, ascorbic acid at maximum concentrations attainable in the blood (40-80μM) did not cause any increase in TBA reactivity.

In order to test whether increased TBA reactivity induced by AA is due to the reduction of Fe^{3+} or other metal ions by ascorbic acid, various concentrations of EDTA which is a potent iron chelator were added to the test mixture containing ascorbic acid. This failed to show any blocking effect on the apparent pro-oxidant activity of ascorbic acid in our assay system.
III. Iron chelators

Bigwood and Read (1989) have suggested that to eliminate the contribution of pseudo-MDA reactive material such as hydroperoxy-dienoic acids and alpha,beta-unsaturated aldehydes which can react with TBA and generate a chromogen similar to MDA-TBA, a powerful Fe$^{3+}$ chelator such as EDTA or desferrioxamine must be introduced into the assay immediately prior to the introduction of TBA. This was therefore tested with the following results:

a. Addition of EDTA (final concentration in the assay 150μM) led to an unexpected increase of 74% in TBA reactivity compared with blank plasma samples.

b. Addition of desferrioxamine in concentrations ranging from 15 to 600μM had no effect on TBA-RS of plasma.

4.3.2.7. Salicylate and its metabolite 2,3,-DHB

As it was intended to assess in-vivo activity of OH by measurement of 2,3,-DHB as well as TBA reactivity for all samples, it was necessary to study the effect of aspirin on TBA-RS of plasma. This was carried out by both in-vivo and in-vitro experiments:

I. In-vivo

A blood sample was collected from a normal subject before and 2 hours after ingestion of 600mg of aspirin. Plasma samples were prepared and assayed for TBA-RS. There was no difference between the two.

II. In-vitro

Salicylate was added in serial concentrations ranging from 0.05 to 0.8mM to plasma samples from a medication free subject (peak concentration in blood after ingestion of 1.2g of aspirin is 0.8mM). Blank plasma sample was run in parallel. There was no difference between the groups.
III. Effect of 2,3-DHB

It has been suggested that 2,3-DHB might arise as a product of attack of hydroxyl radical generated *in vivo*, on the salicylic acid molecule. This compound is a chelating agent and in view of its ability to interfere with iron dependent free radical reactions, it was decided to test whether it affects TBA reactivity of plasma. 2,3-DHB was added to plasma samples from a medication free subject (final concentration in the assay ranging from 40 to 500 µM). This failed to alter TBA reactivity of the plasma.

4.3.3. Discovery of a methodological problem

Ultraviolet range cuvettes (supplied by Hughes and Hughes) were sometimes used in our research laboratory and proved satisfactory for spectrophotometric studies in both ultraviolet and visible ranges of wavelengths. However, late in the course of method standardization it was noted that uv-range cuvettes but not the visible range were slowly dissolved by butan-1-ol which was used in the extraction of MDA-TBA adduct. This could have contributed to some extent to the inter and intra-assay variations observed during the method standardization process.

4.3.4. Summary of method standardization:

1. Only visible range cuvettes were used in spectrophotometric studies of TBA test.
2. 60 minutes heating time was chosen as the optimal duration for production of TBA-RS.
3. The use of promoters and inhibitors of lipid peroxidation to act as positive and negative test controls was abandoned, as it became clear that there was no similarity between plasma and microsomal systems used in the study of TBA reactivity. This is possibly due to the fact that a large proportion of TBA reactive material in plasma is non-lipid.
4.4. CLINICAL STUDY:

4.4.1. A comparison of plasma TBA reactivity between patients with chronic facial pain, patients with chronic venous ulcers and controls.

14 patients with chronic idiopathic orofacial pain and 9 controls were included in a pilot study. The controls were not age and sex matched. Venous blood samples were collected into heparinized bottles and centrifuged at 3000rpm/5min. Plasma layer was removed, aliquoted and stored at -20C until the assay. In parallel with this study plasma samples from 16 patients with chronic venous ulcers were supplied from Middlesex hospital for the assay. Patients with facial pain had a significantly higher level of TBA reactive material than both controls and patients with venous ulcers. The control group did not differ from the venous ulcer group.

![Figure 29. TBA-RS in patients vs controls and venous ulcer sufferers](image-url)
However, caution is needed in interpretation of these results. The experiments were carried out before the discovery the methodological problem ie solubility of the cuvettes. Although, all samples were assayed simultaneously, using the same batch of cuvettes, it is possible that the methodological problem could have contributed to the observed differences.

4.4.2. A comparison of the effect of experimental stress between patients and controls

A review of the literature revealed that both pain and emotional stress are associated with an increased generation of free radicals as measured by the TBA test. In order to elucidate the relative contribution of either pain or stress to this phenomenon, it was decided to study the indices of free radical generation in patients and controls in relation to stress.

In order to study these, a controlled situation was needed and therefore it was necessary to induce stress experimentally. A stress inducing test should be capable of producing:

1. psychological changes that indicate increased distress, as the individual's perception and interpretation of the situation is of great importance for the observed physiological activation and variation;
2. physiological changes that indicate sympathoadrenal activation as reflected in parameters such as heart rate;
3. muscular exertion as part of the fight-flight defence reaction and
4. hormonal changes as reflected in plasma and urinary catecholamines.
One of the tests which seems to satisfy the above requirements is the Stroop Colour Word Test (SCWT) (Tulen et al., 1989). It produces mental overstimulation as a result of cognitive conflict combined with time-pressure effects. Since its introduction by Golden (1979) it has been found to be a useful model to study the effect of standardized mental stress in human and is now used in a number of different fields of psychosomatic research (Kilminster et al., 1988; Jern et al., 1989; Mazzuero et al., 1989; ).

The study was conducted to answer the following questions:

1. Do patients with stress related facial pain have increased level of oxygen free radical activity? The methodology was standardized and the methodological problems had been overcome.

2. Does experimental stress lead to an increased generation of free radicals in human?

3. Do patients with chronic facial pain differ from normal controls in their response to stress?

4.4.2.1. MATERIALS AND METHODS:

Subjects and Procedure:

10 patients with chronic orofacial pain and an equal number of healthy controls participated in two experimental sessions. The first was the stress session which was followed after a week by resting session. At the beginning of each session the subjects were given 600 mg of aspirin orally. This was the basis of the method for in-vivo measurement of OH radical activity using HPLC measurement of 2,3,-DHB (see IV.3.).
10ml venous blood sample was collected into heparinized bottles 2 hours following the ingestion of aspirin, centrifuged at 3000rpm/5min, aliquoted and stored at -20C until assayed for either lipid peroxidation products or 2,3- dihydroxybenzoic acid. During the stress session subjects underwent a 20 minute version of the Stroop Colour Word Test (CWT). The resting session consisted of 20 minutes of relaxation in a quiet comfortable environment.

**Stroop Test**

The CWT was presented on an automatic slide projector. In short, four words: red, green, blue and yellow were presented at random in one of four colours (red, green, blue and yellow) at random intervals of 0.5-1 seconds, while the duration of the stimuli varied at random also between 0.5-1 seconds. The subjects had to indicate the colour of the words, by writing the initial for each colour on an answer sheet. Beforehand a one minute practice session was administered so that the subjects would be familiar with the test requirements. Prior to the start of each experimental session, the subjects were requested to relax so that the basal heart rate could be recorded for 5 minutes before commencing the test.

**Psychological Measurements:**

The spielberger state anxiety inventory (Spielberger et al., 1983) was administered before and after the sessions to assess the subjective perception of the stress and anxiety (see II.2.).
Physiological measurements:

Heart rate was recorded every 15 seconds using a Novamatrix Model 500 Pulse Oximeter with the sensor attached to the ear lobe starting from 5 minutes before each session and continued during twenty minutes of the stress test or relaxation period.

Biochemical measurements:

Measurement of 2,3-DHB was abandoned following the discovery, that the stabilizer content of diethyl ether used for extraction of samples interferes with the chromatographic separation (see IV.3.5.). Unfortunately, some of the samples were used during the HPLC assays prior to the discovery of the methodological problem. However, samples from seven patients (age range 16-60 mean 45, F:M = 5:2) and eight controls (age range 20-40, mean 25, F:M = 5:3) were available for the thiobarbituric acid test which was carried out according to the standardized protocol (see IV.4.3.).

Having acknowledged the poor specificity of the TBA test, plasma samples from 6 of patients and an equal number of controls were also assayed using high performance liquid chromatography for MDA-TBA adduct (by Dr. S. Chirico, King's College Hospital, London).
4.4.2.2. RESULTS

Psychological:

Pre- and post-session values of Spielberger state anxiety are presented in table 19. The statistical analysis was carried out using an unpaired t-test for inter-group and a paired t-test for intra-group comparisons. The pre-session values of the stress and the resting session were not significantly different between patients and controls. The pre-session values of the resting session were lower in both groups as compared to the stress session, although the difference was not significant. There was a statistically significant increase after the stress session in both patients (p<0.02) and controls (p<0.007). Controls showed a significant decrease in their score after the relaxation session (p<0.02).

Table 19. Spielberger anxiety state score

<table>
<thead>
<tr>
<th></th>
<th>Stress session</th>
<th>Resting session</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pre</td>
<td>post</td>
</tr>
<tr>
<td>Controls</td>
<td>31.5±6.43</td>
<td>41.6±9.1</td>
</tr>
<tr>
<td>Patients</td>
<td>30.4±6.67</td>
<td>38.1±9.8</td>
</tr>
</tbody>
</table>

Physiological:

The CWT caused a significant increase in heart rate in both groups (fig.30). The increase in heart rate was more marked in patients (14 vs 7 BPM). During the control session the heart rate slowed down a few beats in both groups (fig.31). The basal heart rate
difference between groups was not statistically significant.

Figure 30. Changes in heart rate in patients and controls during the stress session

Figure 31. Changes in heart rate in patients and controls during the resting session
Biochemical:

The findings of the biochemical analysis are summarized in table 20. Figure 32 shows the amount of TBA-RS and figure 33 presents the amount of MDA-TBA adduct in plasma of patients and controls. There was no significant difference in TBA-RS between patients and controls either during stress or resting session. Control subjects had a lower value of MDA-TBA adduct during the stress session. However, this was not statistically significant.

Table 20. Biochemical data under stress and resting session

<table>
<thead>
<tr>
<th></th>
<th>Stress session</th>
<th></th>
<th>Resting session</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TBA-RS</td>
<td>MDA-TBA</td>
<td>TBA-RS</td>
<td>MDA-TBA</td>
</tr>
<tr>
<td></td>
<td>ΔAbs. 532nm</td>
<td>μmol eq. MDA</td>
<td>ΔAbs. 532nm</td>
<td>μmol eq. MDA</td>
</tr>
<tr>
<td>Patients</td>
<td>0.119±0.012</td>
<td>3.40±2.91</td>
<td>0.118±0.018</td>
<td>3.31±2.15</td>
</tr>
<tr>
<td>Controls</td>
<td>0.116±0.012</td>
<td>2.28±1.27</td>
<td>0.116±0.008</td>
<td>3.01±2.68</td>
</tr>
</tbody>
</table>
Figure 32. TBA-RS in patients and controls during stress and resting session

Figure 33. µM eq. MDA in patients and controls during stress and resting sessions
4.4.2.3. DISCUSSION

The psychological and physiological measurements indicate that the experimental stress had been administered correctly, as both patients and controls showed a significant increase in subjective perception of stress as shown by Spielberger score as well as the heart rate during the stress session. The lower pre-session Spielberger scores during the resting session as compared to the stress session is probably due to the subjects being more familiar with the environment during the resting session which was carried out a week after the stress session.

Control subjects were members of the research laboratories who were more familiar with the environment, hence they were able to relax more easily and this may explain the significantly lower postrelaxation value in this group.

The increase in heart rate was variable. Patients generally showed a higher rise in pulse rate than controls, but the difference did not reach the level of statistical significance. There was a wide range of difference in the amount of increase in pulse rate between subjects (5-37BPM in patients, 3-13 in controls). Tullen et al. (1989) reported a uniform pattern of increase in heart rate (6-7BPM) in their subjects. This is probably due to the fact that their subjects were from a homogenous group of trained sportsmen, whereas our subjects were from a heterogenous group.

Paradoxically, there was a negative correlation between the increase in Spielberger score and rise in the heart rate during the stress session ($r=0.48$) which was close to the level
of statistical significance ($p<0.07$). In other words cognitive perception of stress is inversely related to physiological changes. This finding is in agreement with those of Salmon and Kaufman (1990) who showed a statistically significant negative correlation between the endocrine response and preoperative anxiety in a group of patients undergoing major surgery.

Our subjects were given 600mg of aspirin orally at the beginning of each session and a blood sample was collected after 2 hours. As it was found that aspirin does not affect TBA reactivity of plasma (see IV.4.3.2.7.), it was considered safe to use the plasma samples for both lipid peroxidation and 2,3,DHB assays.

We found that the measurement of 2,3,DHB was flawed by a number of methodological problems, the main one being the presence of pyrogallol ($1,2,3$,trihydrobenzene) as a stabilizer in diethyl ether which was recommended for extraction of plasma samples in the original method (Grootveld & Halliwell, 1986). Since then, we have been using ethyl acetate for the extraction procedure and the method is standardized. However, as all plasma aliquots were used before the methodological problems were discovered, it has not been possible to obtain an accurate value of 2,3,DHB content of any of the samples. A second Stroop test was not considered appropriate as it has been shown that there is a reduction in the response magnitude if the test is repeated (Tulen et al., 1989).

In this study, there was no demonstrable difference in the levels of TBA-RS or MDA-TBA adduct between patients and controls. This is in contrast to the results of our pilot study. Furthermore, the experimental stress as induced by CWT did not alter the TBA
reactivity of plasma in either group, although control subjects had a reduced level of MDA-TBA adduct during the stress session. The measurement of lipid peroxidation is faced with a number of problems (see IV. 1.8.). Although the thiobarbituric acid test is probably the most widely used single assay for measuring lipid peroxidation, it has been criticised for its lack of specificity when applied to human body fluids (Gutteridge & Halliwell, 1990). HPLC separation of MDA-TBA adduct before measurement solves only part of the problem because some compounds (especially amino acids and sugars) react in the assay to form an authentic MDA-TBA adduct.

We acknowledge that the numbers are small, and the possibility of a type II error exists. However, as we were not satisfied with the available methodology for measurement of free radical activity, the study was not pursued on a larger number of subjects. With increased understanding in free radical chemistry and introduction of more specific assays, some of the problems presented above will be avoided. Further research to clarify the relationship between stress, pain and changes in the body chemistry in a larger number of subjects is warranted.

Halliwell (1991) points out that almost any disease is likely to be accompanied by increased formation of reactive oxygen species, and expanding free radical theories to an ever increasing list of diseases is not the only way forward. In rheumatoid arthritis, autoimmune disease, iron overload and reoxygenation injury there is reasonable evidence that some tissue damage is radical mediated, but for many of the other conditions, it has merely been shown that radicals are produced, without an assessment of their role in the disease process. The careful use of a range of antioxidants combined with new methods
for measuring oxidant generation in humans, is needed to evaluate the exact contribution of oxidant generation to disease pathology. Measurement of antioxidants in local tissues as well as systemic circulation would be of interest.
CHAPTER V.

TEMPOROMANDIBULAR JOINT

SYNOVIAL FLUID ANALYSIS
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1. INTRODUCTION

Although the diagnosis of the common temporomandibular joint disorders are mainly made by clinical examination, research into underlying pathophysiology of this common problem, is essential to increase our understanding of "internal derangement" in order to achieve consistently successful treatment outcome. In some patients marked localized pain and tenderness of TMJ suggests a capsulitis; if persistent this may lead to developments of adhesions in the joint space and subsequent internal derangement and limited opening. These features can be confirmed with arthroscopy. Surgical exposure also reveals a joint capsule that is invariably thickened implying a chronic capsulitis (Harris, personal communication). However, the underlying pathophysiological changes leading to pain and internal derangement of the TMJ are unknown. It has been suggested that joint disease activity at least in the early stage is only detectable by a biochemical approach (Kopp, 1991) and synovial fluid analysis is an important model that can be used for investigation of the pathogenesis of TMJ disorders (Israel et al., 1991).

Synovial fluid occupies a key position in joint physiology. Although aspiration and analysis of synovial fluid is commonly performed in other joints (Cohen, 1967) owing to difficulties associated with the sample collection and problems associated with interpretation of the results, very few methodologically sound studies of TMJ are available.

Toller (1961) was unable to aspirate any free fluid from the TMJ in more than 250 attempts on human subjects, although in patients with rheumatoid arthritis aspiratable
effusions up to 0.5ml in TMJ have been reported (Applegren et al., 1991). However, in most normal joints the aspirateable volume is very small in relation to the internal area. The normal human knee for example usually contains 0.5 ml aspiratable fluid. This volume if spread evenly throughout the joint, would have a thickness of only 24μm (Levick, 1984). Furthermore, the aspiration is never total and has been estimated to constitute only 52% of the total fluid volume (Rekonen et al., 1973). Toller (1961) "guesstimated" the total volume of TMJ synovial fluid to be no more than 50 μl. Therefore, direct aspiration of sufficient amounts for biochemical analysis is rarely feasible.

During the past decade, saline aspirates of the upper joint space of TMJ have increasingly been analysed for the presence of various mediators of pathology (Kopp et al., 1983, Quinn & Bazan, 1990; Holmlund et al., 1991; Israel et al., 1991; Applegren et al., 1991). We also used saline aspirates for various biochemical assays. However, caution is needed when interpreting the results.

Contamination of the aspirate with blood is an important source of error in published results of TMJ synovial fluid analysis. When measuring any compound in the synovial fluid, correction should be made for its presence due to plasma or blood cell contamination, particularly if the sample is not centrifuged before being frozen. The lysis of blood cells at the thawing stage could possibly lead to a misleadingly high value of inflammatory mediators in the aspirate.

Additionally, it is possible that completed mixing of injected saline and synovial fluid may
not be achieved in all cases owing to presence of fibrous adhesions. Hence, it is not known whether the saline aspirate is a representative sample of the synovial fluid. Therefore in interpreting the saline aspirate analysis for various compounds, it is crucial to obtain a dilution factor which will allow for correction of the results.

Another important issue is the lack of a reliable control. For obvious ethical reasons it has not been possible to obtain samples of the synovial fluid from normal TMJs and therefore the normal values of TMJ synovial fluid is not known for comparison to the values obtained from patients with TMJ pain and dysfunction.

The following chapter can be divided into three main sections. The first one is concerned with the measurement of hyperalgesic eicosanoids in the joint. The second part addresses the question of measurement of the TMJ synovial fluid volume. The method developed for this purpose, also allowed the determination of the concentration and absolute amount of putative mediators of pathology in the joint. Finally, using the method established form the second part of the study, the role of oxygen free radicals in the pathogenesis of TMJ disorders was investigated.
2. PART I. MEASUREMENT OF HYPERALGESIC EICOSANOIDS

2.1. Background

Hyperalgesia is a term used to describe a reduction in pain threshold to stimuli that are not normally painful. An important family of pain mediators which may be involved in painful arthritic conditions are eicosanoids. Their biosynthesis involves the controlled and stereospecific free radical peroxidation of arachidonic acid by lipoygenase or cyclooxygenase enzymes (fig. 34).

![Figure 34. Pathways of arachidonic acid metabolism](image)
Polyunsaturated fatty acids in membranes can also be peroxidized in the absence of enzymes by exposure to reactive oxygen species and/or to transition metal ions in a free radical chain reaction known as lipid peroxidation (see IV.1.7., fig. 12). The products formed are complex and non-stereospecific, but there are relationships between enzymic and non-enzymic peroxidation which are also mediated by free radical reactions. Thus, low level of lipid peroxides can activate both cyclo-oxygenase and lipoxygenase enzymes and thereby increase eicosanoid formation (Laughton et al., 1991). Similarly, some inhibitors of lipid peroxidation, such as vitamin E, have been shown to inhibit lipoxygenases (Redanna et al., 1985). Inhibitors of lipid peroxidation often act by scavenging free radicals, and is therefore widely believed that lipoxygenase inhibition by such chain breaking antioxidants is due to scavenging of free radicals that are formed within the active site of the enzymes (Laughton et al. 1991).

Quinn & Bazan (1990) have found PGE₂ in saline aspirates obtained during arthroscopic procedures on painful TMJs. However, the failure of non-steroidal anti-inflammatory analgesics to block chronic facial pain suggests that the prostanoids are not the sole algesic agents in this condition. Furthermore, non-steroidal analgesics do not block the synthesis of lipoxygenase products. Therefore, it was decided to test the hypothesis that the chronic facial pain could be due to the local generation of products of lipoxygenase activity.
2.2. Materials and Methods

2.2.1. Patients

15 patients (age range 15-41, mean ± SD = 28.3±7.4 years, F:M=9:6) with chronically painful TMJs unresponsive to medical therapy with a tricyclic antidepressant undergoing arthroscopy under general anaesthesia were included. Informed consent was obtained for aspiration of the contralateral symptomless joint.

2.2.2. Aspiration technique

An assistant distracted the mandible downwards and forwards. After palpation of the periauricular depression and the outer rim of the glenoid fossa, a 19"G needle was used to puncture the skin. The point of entry was 1cm in front of the tragus on a line drawn from the tragus to the outer canthus of the eye. The needle was angled upwards, forwards and medially towards the posterior slope of the articular eminence. After contact with the bone, the needle was slightly withdrawn and its position in the upper joint space was confirmed by manipulation of the mandible, which caused the needle to move and by the easy flow of the injected saline. If the needle was outside the joint space a slight resistance was felt when the syringe was pressed. On occasions there was an obvious deviation of the mandible to the opposite side upon injection of saline. Individual anatomical variations such as a downwardly prominent rim of the glenoid fossa occasionally required modification of the approach. After injection of 1 ml of saline the mouth was opened and closed to ensure adequate equilibration of the saline within the synovial fluid and then it was aspirated. The aspirates were put into heparinized bottles. The needle was left in place on the symptomatic side to distend the joint space by injecting more saline in order to carry out the arthroscopic cannulation. Routine arthroscopic examination was carried out and pathological changes were assessed and photographed.
2.2.3. Preliminary preparation of samples

50 μl of the aspirate was used for haemoglobin determination using a commercial colorimetric assay (Sigma) and the rest was centrifuged (3000rpm/5min) to sediment any cells. The volume of the supernatant was measured and then aliquoted and stored at -70C until the assay. To increase the sensitivity of the haemoglobin assay to allow measurement of samples with only slight blood contamination (down to 1mg/ml) the manufacturer's instructions were modified by increasing the volume of test fluid in the assay from 20μl to 50μl.

2.2.4. Leukocyte count:

Any cells pelleted by centrifugation were gently resuspended in the same measured volume of the aspirate. The cell content was measured by visual counting using a haemocytometer.

2.2.5. Measurement of eicosanoids:

Prostaglandin E₂ was measured by the Amersham Amerlex MTM (Amersham Code RPA 530) magnetic separation system using an iodinated methyl oximate derivative of PGE₂. Leukotriene B₄ (Amersham code TRK.940) and 15-hydroxyeicosatetraenoic acid (15-HETE) (Amersham code TRK.920) were measured using commercial Amersham assay kits utilizing tritiated analytes. All assays were performed according to the manufacturer's instructions.

2.3. RESULTS

2.3.1. Markers of inflammation

Inflammation in the TMJ was assessed both visually and by assay of various inflammatory markers. The arthroscopic findings are shown in Table 21. Leukocyte numbers were measured in the specimens collected for this study. As in previous studies where we have
counted leukocytes in the TMJs of identical groups of patients most specimens contained very small number of leukocytes < 10^{-1} \text{O} '/ml. None of the specimens studies in this series contained significant numbers of leukocytes as estimated by microscopic examination. This compares with a mean number of 2 \times 10^7 leukocytes/ml in the joint fluids of patients with rheumatoid arthritis.

Table 21. 15-HETE levels in 11 bilateral saline aspirates and the arthroscopic findings of the symptomatic TMJ

<table>
<thead>
<tr>
<th>Patient</th>
<th>symptomatic 15-HETE pg/ml</th>
<th>HB mg/dl</th>
<th>symptomless 15-HETE pg/ml</th>
<th>HB mg/dl</th>
<th>Arthroscopic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>980</td>
<td>2.08</td>
<td>926</td>
<td>29.3</td>
<td>mild hyperaemia</td>
</tr>
<tr>
<td>2.</td>
<td>581</td>
<td>0</td>
<td>646</td>
<td>0</td>
<td>adhesions, irregular joint surface</td>
</tr>
<tr>
<td>3.</td>
<td>491</td>
<td>1.92</td>
<td>671</td>
<td>1.6</td>
<td>synovitis, adhesions, irregular joint surface</td>
</tr>
<tr>
<td>4.</td>
<td>378</td>
<td>0</td>
<td>478</td>
<td>0</td>
<td>no obvious abnormality</td>
</tr>
<tr>
<td>5.</td>
<td>594</td>
<td>0</td>
<td>601</td>
<td>0</td>
<td>synovitis, adhesions</td>
</tr>
<tr>
<td>6.</td>
<td>808</td>
<td>3.04</td>
<td>688</td>
<td>6.72</td>
<td>arthroscopy abandoned due to bleeding</td>
</tr>
<tr>
<td>7.</td>
<td>551</td>
<td>4.64</td>
<td>551</td>
<td>3.04</td>
<td>adhesions</td>
</tr>
<tr>
<td>8.</td>
<td>771</td>
<td>19.8</td>
<td>671</td>
<td>3.84</td>
<td>irregular joint surface</td>
</tr>
<tr>
<td>9.</td>
<td>443</td>
<td>0</td>
<td>834</td>
<td>17.6</td>
<td>adhesions, irregular joint surface</td>
</tr>
<tr>
<td>10.</td>
<td>803</td>
<td>0</td>
<td>904</td>
<td>0</td>
<td>no obvious abnormality</td>
</tr>
<tr>
<td>11.</td>
<td>673</td>
<td>0</td>
<td>514</td>
<td>0</td>
<td>synovitis</td>
</tr>
</tbody>
</table>

mean±SD 643 ± 182 680 ± 150

Limit of assay sensitivity 16pg/ml
2. Eicosanoid levels in joint washes

The level of PGE$_2$ was measured in 15, LTB$_4$ in 13 and 15-HETE in 11 pairs of TMJ aspirates. With both PGE$_2$ and LTB$_4$, the levels in the joint aspirate were below the limit of detection of the assay. By contrast all joint washes contained levels of 15-HETE significantly higher than the limit of sensitivity of this assay (table 21, fig.35). Table 21 compares the levels of 15-HETE from the 11 bilateral aspirate and also shows the findings on arthroscopic examination of the symptomatic joints. Blood contamination did not affect the 15-HETE levels, as there was no correlation between the haemoglobin content and the 15-HETE level of the aspirates. The mean value of 15-HETE in the symptomatic joint was 643±182pg/ml which compares to 680±182pg/ml in the symptomless joint. Using a student’s t-test there was no significant difference between the two sides.

![Graph showing 15-HETE levels in symptomatic and symptomless joints](image)

**Figure 35.** 15-HETE levels in symptomatic and symptomless joints
2.4. DISCUSSION

We have attempted to measure lipid mediators with hyperalgesic activity in the TMJs of patients with facial arthromyalgia. A number of products of both cyclo-oxygenase and lipooxygenase action on cell membrane arachidonic acid are hyperalgesic. The best recognised of these are PGE₂ and prostacyclin (Samuelsson et al., 1978). The lipooxygenase products which have been reported to be hyperalgesic include LTB₄, 15-hydroperox-eicosatetraenoic acid (Follenfant et al., 1990) and 5,15,diHETE (Levine et al., 1986). It was unlikely that prostanoids were the sole hyperalgesic agents in the painful TMJ as non-steroidal analgesics are ineffective in the treatment of this chronic facial pain. However, the possibility existed that products of 5 or 15 lipooxygenase activity could be involved.

Specific assays of three hyperalgesic eicosanoids in the joint washes from painful TMJs has revealed that there are very low levels of both PGE₂ and LTB₄ such that the very sensitive assays used could not detect any of these analytes. This reflects the low level of inflammation in these joints also seen at arthroscopy. However, it proved possible to measure 15-HETE in all the samples analysed. 15-HETE is the reduction product of 15-HPETE and is therefore a marker of the presence of this very potent hyperalgesic lipid. 15-HPETE not only induces acute hyperalgesia in animals but repeated stimulation of the paws of rats with this lipid induces a state of chronic hyperalgesia (Follenfant et al., 1990). It is of interest that the hyperalgesia induced by 15-HPETE in experimental animal models can be blocked by substance P(SP) antagonist (Garland, personal communication). High levels of SP have been demonstrated in TMJ saline aspirates (Holmlund et al., 1991). The implications of these observations are discussed in section V.5.
The findings described here do not support the report of Quinn and Bazan (1990) who claim that the painful TMJ contains large amounts of both PGE\(_2\) and LTB\(_4\). In our study there were no measurable levels of the above mediators. It is unclear what accounts for the difference in results. The method of collection may be important as any trauma to the joint could result in haemorrhage into the joint space with activation of leukocytes and generation of eicosanoids. We took care to manipulate the joints very gently while collecting the joint aspirate. We also centrifuged joint aspirates to sediment any leukocytes. This step is not reported in the paper by Quinn & Bazan (1990) and the autolysis of cells when the stored joint aspirates were thawed could have contributed to the high levels of eicosanoids. Given that the joint aspirates produced by these authors were diluted 20-30 fold, the concentrations of PGE\(_2\) and LTB\(_4\) in the joint aspirates are much higher than have previously been reported in the literature for rheumatoid or gouty joints (Henderson, 1988). Facial arthromyalgia patients would not be expected to demonstrate such high levels of eicosanoids in the absence of overt arthritis.

It was not possible to correlate arthroscopic and biochemical findings, also symptomless joints had comparable levels of 15-HETE to that of the symptomatic joints. However, these aspirates from symptomless joints can not be regarded as a normal control. The demonstration of 15-HETE in the asymptomatic joints probably reflects a latent or potential pathological process. This is discussed further in V.5.

In conclusion, our findings are compatible with an intermittent or chronic hyperalgesic state induced by generation of 15-lipoxygenase products such as 15-HPETE. The failure to find significant quantities of prostaglandins may explain the lack of clinical efficacy of
non-steroidal analgesics in the treatment of chronic facial pain. Work is now in progress to determine if the analgesic dipyrone known to be able to inhibit chronic hyperalgesia in animals (Follenfant et al., 1990) and man (Marquez & Ferreira, 1987) has any efficacy in the treatment of TMJ pain.

3. PART II. MEASUREMENT OF TMJ SYNOVIAL FLUID VOLUME

3.1. Background
The importance of estimation of the synovial fluid volume is that it provides an estimate of the joint space. One important parameter which may be central to any disturbance in joint movement is the volume of synovial fluid in the joint which is also an index of any pathological change in the joint space volume. An increased synovial fluid volume as a result of effusions or haemarthroses is a potential source of morbidity, giving rise to a sense of tension or even pain if formed rapidly (Jayson & Dixon, 1970a; Myers & Palmer, 1972), impairment of synovial blood flow above a critical effusion pressure (Jayson & Dixon 1970b; Lucht et al., 1983) and even direct mechanical limitation of movement (Nade & Newbould, 1984). On the other hand, a reduced volume of the synovial fluid may adversely affect its lubricating property and transport of the nutrients to the articular surfaces (Levick, 1987).

Toller (1961) "guesstimated" the total volume of TMJ synovial fluid to be no more than 50μl. This small volume does not allow the use of established methods applied to larger joints for accurate estimation of the synovial fluid volume. These methods include the use of radioisotope dilution (Rekonen et al., 1973; Ekman et al., 1981), non-isotope dilution
using a contrast medium (Pereira et al., 1990) and wash-out technique (Geborek et al., 1988).

However, if one considers the small amount of TMJ synovial fluid, any dilution of injected media by this fluid will be very high. For example, injection of 1ml of a contrast medium will produce a dilution of up to 20 folds. Synovial fluid content will be approximately 5% of the injected medium. This is within the range of experimental error when using a radioisotope or contrast medium for larger joint volume measurement, and therefore can not be used accurately for TMJ synovial fluid volume determination.

To overcome the problem, a marker was needed with the following properties:
1. It should be safe to administer to the patients.
2. It should attain a measurable level shortly after administration.
3. Low levels should be measurable in highly diluted saline aspirates.
4. It should have equal concentrations in both plasma and synovial fluid.

We chose to administer 1.2g of aspirin to patients prior to aspiration, and the haemoglobin and salicylate levels were estimated both in the plasma and the aspirate. By comparing the salicylate concentration in the aspirate with that of the plasma, it was possible to calculate the volume of synovial fluid. Furthermore, concentration and absolute amounts of the mediators of pathology in the joint could be calculated. The technique neither involves radioactivity nor is dependent on the efficiency of the washout. From a clinical standpoint the administration of salicylate also has a therapeutic benefit by reducing postoperative pain and discomfort.
3.2. MATERIALS AND METHODS

3.2.1. Patients

Nine patients (age-range 15-47, mean $38.6\pm9.8$; F:M=3:6) with painful temporomandibular joints unresponsive to 12 weeks medical therapy with a tricyclic antidepressant, underwent arthroscopy under general anaesthesia. None of these patients had any history of allergy to aspirin, gastrointestinal disorders or a bleeding tendency. 1-2 hours prior to the operation 1.2g of aspirin was administered orally. Informed consent was obtained for all procedures.

3.2.2. Aspiration technique and preliminary preparation of samples

Aspiration technique was as described in part I (see V.2.2.2.). Additionally a venous blood sample was collected simultaneously.

3.2.3. Salicylate measurement

It was not possible to measure accurately salicylate using colorimetric and enzymatic methods which are useful for assaying levels in the normal serum or in cases of overdose. Therefore, HPLC was considered to have the required sensitivity for the expected levels of salicylate in the saline aspirate (25 times less than plasma).

The method described by Grootveld & Halliwell(1986) was used with slight modifications. A uv-vis detector was used instead of an electrochemical detector as it was found that blank plasma contains an electroactive compound with the same retention time as standard salicylate which could confound the results. Although spectrophotometric
detection is not as sensitive as electrochemical detection, it was satisfactory for our purpose and concentrations down to 1 \mu M could be measured. Prior to the assay, the samples were extracted by the following procedure:

200 \mu l of sample (plasma or saline aspirate) were treated with 25 \mu l of 1M-HCl and extracted with 10ml of diethyl ether on a vortex mixer for 2 minutes. Pyrogallol (1,2,3,-trihydrobenzene) which is present in diethyl ether as a stabilizer served as an internal standard for calculation of the relative retention time. After separation, the ether layer was evaporated in a water bath at 40\degree C and the residue was dissolved in 225\mu l of the mobile phase containing 5\%(v/v) 1M-HCl. Samples not analysed immediately were stored at -20\degree C until used. HPLC was carried on a SpectraPhysics chromatography system. The column was a Shandon 5 ODS reverse-phase (25cmx4.6mm). The mobile phase was 30mM sodium citrate/27.7mM sodium acetate buffer(pH 4.75)-methanol (94:6) at a flow rate of 1ml/min. The mobile phase was sparged continuously with He gas during elution. Detection was at 254 nm on a uv/vis detector. The injection loop (10\mu l volume) was cleaned with at least 3ml each of methanol, water and buffer after each injection, until injection of buffer did not produce any change in absorbance as a result of residues in the loop from the previous injection.

3.3. RESULTS:

Out of 9 pairs of saline aspirates, 3 samples from the symptomless sides were excluded from the study because of heavy blood contamination. Table 22 compares the results between the symptomatic and symptomless joints. A paired t-test was used for statistical analysis of the results from 6 patients from whom bilateral samples were analysed. The
results of 9 samples from the symptomatic side against 6 of the symptomless side were compared using an unpaired t-test (table 22).

Table 22. Comparison of the results between symptomatic and symptomless joints

<table>
<thead>
<tr>
<th></th>
<th>symptomless (n=6)</th>
<th>symptomatic (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirate volume (µl)</td>
<td>865±69</td>
<td>811±303</td>
</tr>
<tr>
<td>Blood contamination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of aspirates</td>
<td>33%</td>
<td>33%</td>
</tr>
<tr>
<td>Hb mg/ml</td>
<td>0.58±0.98</td>
<td>1.43±2.99</td>
</tr>
<tr>
<td>Salicylate Conc. (µM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>8.57±6.02</td>
<td>11.95±10.53</td>
</tr>
<tr>
<td>range</td>
<td>(4-19.9)</td>
<td>(3.8-29.95)</td>
</tr>
<tr>
<td>Dilution factor *</td>
<td>48.7±21.4</td>
<td>43.2±28.1</td>
</tr>
<tr>
<td>SF volume (µl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>20.4±5.5</td>
<td>36.1±26.5</td>
</tr>
<tr>
<td>range</td>
<td>(12.6-55.3)</td>
<td>(14.3-80.9)</td>
</tr>
</tbody>
</table>

* The ratio of salicylate concentration in the plasma to that in the saline aspirate

The yield of aspirate was variable ranging from 270 to 1050µl (mean 840µl). There was no significant difference between the symptomatic and symptomless sides. Blood contamination was unpredictable. Almost half of the samples showed blood contamination. The retention time of salicylate (20min) relative to the internal standard(pyrogallol) was 2.5 (fig 36).
Figure 36. Chromatographic separation of salicylate and pyrogallol

The response of the detector was directly proportional to salicylate concentration in the range of 1-800μM (fig.37).

Figure 37. Salicylate calibration curve
The extraction efficiency of salicylate was 75%. This was determined by adding standard salicylate to plasma samples from volunteers who had not taken aspirin and carrying them through the extraction procedure. Figures (38a,b,c,) show chromatographs of plasma and joint aspirates from a patient. The sample from the symptomless joint shows a higher concentration which in this case was due to blood contamination. The salicylate concentration was determined by comparing its peak height to that of the external standard taking into account the extraction efficiency factor.

Figure 38a. Chromatograph of a plasma sample from a patient who had taken 1.2g of prior to blood collection
Figure 38b. Chromatograph of saline aspirate from the symptomatic joint with minimal blood contamination

Figure 38c. Chromatograph of saline aspirate from the symptomless joint with moderate blood contamination
The ratio of concentration of salicylate in the plasma relative to that of the joint wash, ie the dilution factor, was not significantly different between symptomatic and symptomless joints. In cases where there was blood contamination, corrections were made to exclude plasma salicylate from the calculation. The volume of synovial fluid was calculated using a concentration-volume equation (fig.39), the mean value of which was found to be 37μl and not significantly different between the symptomatic and symptomless joints. Using Pearson's correlation test, no significant correlation was demonstrable between the volume of the aspirate and the dilution factor (r=0.44).

\[
V x C_p = (K + V) x C_a \\
V C_p = K C_a + V C_a \\
V C_p - V C_a = K C_a \\
V(C_p - C_a) = K C_a
\]

\[
V = \frac{K C_a}{C_p - C_a}
\]

\(V\) = volume of the synovial fluid in TMJ

\(C_p\) = concentration of salicylate in the plasma/synovial fluid

\(K\) = volume of the injected saline = 1000μl

\(C_a\) = concentration of salicylate in the saline aspirate

**Figure 39.** Concentration-volume equation : calculation of TMJ synovial fluid volume
3.4. DISCUSSION

3.4.1. TMJ synovial fluid volume

Taking into account the volume of the injected saline and the ratio of salicylate concentration in the plasma and the saline aspirate, the volume of the synovial fluid in TMJ was calculated by using a concentration-volume equation (fig.39). The technique neither involves radioactivity nor is dependent on the efficiency of washout. Furthermore, its administration has a therapeutic benefit by reducing postoperative pain and discomfort. However, it should be noted that this method is based on the assumption that the salicylate concentration in the synovial fluid is similar to those in plasma. A number of studies support this assumption (Rosenthal et al., 1964; Cleland et al., 1985; Grootveld & Halliwell, 1986). However, Sitar et al. (1985) found that although metabolites of salicylate equilibrate completely between synovial fluid and plasma, salicylate concentration in synovial fluid was approximately 80% of that of the plasma. It has been suggested that this may be secondary to lower albumin concentration in synovial fluid affecting the protein bound portion of the drug (Wallis & Simkin, 1983).

Studies with 14C-labelled acetyl salicylic acid in rabbits suggests that complete equilibration of salicylate between plasma and joint fluid is reached within 60 minutes (Paul & Routh, 1952). Our study of plasma salicylate level in a healthy volunteer showed that a steady state is reached 1h after ingestion of a single dose (1.2g) of aspirin which persisted for up to 5h. (fig.20). This is in agreement with animal studies of salicylate concentration in plasma (Higgs et al., 1987). Therefore in order to avoid the pharmacokinetic lag period between the primary absorption into plasma and secondary absorption into synovial fluid, we chose to perform the aspirations 1-2h after ingestion.
of aspirin.

It is possible that the pathology itself may influence the salicylate level in the joint. Concentrations of the protein bound salicylate may be higher in the inflamed joint which may have a higher total protein and albumin concentrations due to increased capillary permeability. Although a symptomless joint can not be regarded as normal, nevertheless, salicylate levels were comparable between symptomatic and symptomless joints. There was also no difference in total protein content between symptomatic and symptomless joints in our previous studies (Wasil, personal communication).

The knowledge of the pharmacokinetics of salicylate in synovial fluid is usually derived from serial observations in knee joint effusions of patients with rheumatoid arthritis (Sholkoff et al., 1967; Netter et al., 1989). These data might not be applicable to patients with facial arthromyalgia. The rheumatoid patients studied have usually been long term recipients of salicylate, and because of associated hypoalbuminaemia the drug metabolism may be different from non-rheumatoid subjects. Additionally some workers have provided evidence that albumin is qualitatively different in patients with rheumatoid arthritis (Wallis & Simkin, 1983).

It is important to remember that all synovial joints need not be the same. In fact studies on factors which affect exchange across synovial membranes such as total protein content and hydrostatic and osmotic pressure of synovial fluid have revealed significant inter-articular differences between for instance wrists and knees of normal dogs (Simkin, 1991). These findings imply corresponding differences in microvascular physiology. It would not
be surprising if these physiological differences made individual joints more, or less susceptible to specific arthropathies.

In this study the average volume of synovial fluid was estimated to be $37\mu l (0.98-135\mu l)$. It is possible that completed mixing of injected saline and synovial fluid may not be achieved in all cases owing to presence of fibrous adhesions. This may result in underestimation of synovial fluid volume. This may explain the large standard deviations for salicylate concentrations and the corresponding synovial fluid volume in our study.

Given that $37\mu l$ is the volume of TMJ synovial fluid, as with most normal joints this volume is very small in relation to the internal surface area of the joint. The TMJ internal surface area has not been measured accurately. Nevertheless, as the upper joint space can be distended passively with $1.2ml$ of fluid, this can be estimated to be $5.45-6.75cm^2$ given that $5.45cm^2$ is the surface area of a sphere with a volume of $1.2ml$ and $6.75$ is that of a cube with a similar volume. Therefore, there is approximately $6\mu l$ of synovial fluid per $cm^2$ surface. If the fluid were evenly "sandwiched" between the joint surfaces, it would form a layer only $121\mu m$ thick. For the human knee the mean film thickness is calculated to be $24\mu m$ (Levick, 1984). Unfortunately there is no similar data available relating to other human joints or of an animal TMJ. However, the dog knee has been estimated to have a thicker film of synovial fluid ranging from 67 to $270\mu m$ (Levick, 1987). It is unlikely that the film is of a uniform thickness and the significance of different thicknesses of the synovial fluid in various joints and the relation between the volume of this fluid and joint function is not clear. An increased synovial fluid volume as a result of effusions or haemarthroses is a potential source of morbidity, giving rise to a sense of tension or even pain if formed rapidly (Jayson & Dixon, 1970a; Myers & Palmer, 1972),
impairment of synovial blood flow above a critical effusion pressure (Jayson & Dixon 1970b; Lucht et al., 1983) and even direct mechanical limitation of movement (Nade & Newbould, 1984). However, a clinically detectable effusion in the TMJ dysfunction is not a common finding. In all but one case, we have been unable to directly aspirate any fluid from the TMJ. The effusion occurred in a single symptomatic joint in which the aspirate volume was 50μl more than the volume of injected saline, indicating the presence of a mild effusion into the joint. The patient had developed TMJ symptoms following a visit to his dentist which had persisted for 5 weeks before he underwent an arthroscopy which revealed severe hyperaemia of retrodiscal tissues. On the other hand, a reduced volume of the synovial fluid may adversely affect its lubricating property and transport of the nutrients to the articular surfaces. Nevertheless, it has been shown that synovial fluid is capable of forming a permanent though extremely thin (10-50nm) layer between two static surfaces, even under considerable loads, due to the voluminous trapped hyaluronate particles and/or short-range electrical repulsive forces (Ogston & Stanier, 1953). In vivo somewhat thicker films might possibly be maintained between loaded surfaces by movement (Levick, 1987). For obvious ethical reasons, it has not been possible to obtain samples of the synovial fluid from normal TMJs and therefore the normal level of TMJ synovial fluid volume is not known for comparison relative to the values obtained from patients with TMJ pain.

In paired samples the synovial fluid volume was not significantly different between symptomatic and symptomless joints. If the additional 3 symptomatic samples are taken into analysis, the symptomatic joints had larger volumes of synovial fluid which may be biologically important. However, this was not statistically significant from the
symptomless group using an unpaired t-test. It should be noted that symptomless joint can not be regarded as a reliable control as it is functionally and anatomically related to the contralateral symptomless side. This is discussed further in V.5.

3.4.2. Determination of concentration and absolute amounts of analytes

The yield of aspirate after injection of 1ml of saline was variable, ranging from 270 to 1050µl (mean 840µl). The volume collected is to a large extent dependent on the operator's technique and probably the patency of the joint space. Correct positioning of the needle in the joint space is confirmed by the criteria mentioned in the aspiration technique. The mean volume of the aspirate collected in this study is much higher than that in other reports (Kopp et al., 1983) or our own earlier report (Aghabeigi et al., 1990). However, there are instances that despite the correct position of the needle as evidenced by the arthroscopy because of the presence of adhesions or meniscus perforation, the injected saline is unavailable for aspiration.

It is possible that completed mixing of injected saline and synovial fluid may not be achieved in all cases owing to presence of fibrous adhesions. Hence, it is not known whether the saline aspirate is a representative sample of the synovial fluid. Therefore in interpreting the saline aspirate analysis for various compounds, it is crucial to obtain a dilution factor which will allow for correction of the results. In this study there was no demonstrable correlation between the volume of the aspirate and its synovial fluid content as measured by the salicylate concentration. Some investigators report the results of joint assays per volume. This may not be accurate as it is possible to obtain a large amount of highly diluted aspirate which is trapped in a compartment formed by fibrous adhesions.
Conversely, it is possible for the injected saline to equilibrate fully, but due to blockage of the needle during the aspiration, only a small volume of aspirate to be collected. Therefore, theoretically there is no direct relation between the volume of the aspirate and its synovial fluid content. The expression of results per protein content of the aspirate is also not satisfactory in making comparisons between diseased and normal joints as there may be significant differences between symptomatic and symptomless joints. The simultaneous determination of salicylate concentrations in the plasma and joint washes provides therefore a solution to the problems outlined above as it allows the determination of the concentration and absolute amount of putative mediators of pathology in the joint. This is particularly important if any comparisons between the levels in various joints are to be meaningful.

4. PART III. OXYGEN-FREE RADICAL INDUCED DAMAGE IN THE TMJ

4.1. Background

In an attempt to account for the pain and joint derangement our attention was drawn to studies claiming to demonstrate that emotional stress and pain in animals were associated with an increased generation of free radicals (Aleksandrovskii et al., 1988; Golikov et al., 1987; Vitrichenko, 1985) and that stress induced damage to the gastric mucosa were related to free radical production (Itoh & Guth, 1985; Salim, 1989). Furthermore, it is said that free radical related disease is significantly more common in women. Additionally, there have been reports of free radical activity in knee joint synovial fluid from rheumatoid patients (Rowley et al., 1984) which correlate with the clinical severity of the disease. Free radicals can attack lipids leading to lipid peroxidation, yielding
hyperalgesic eicosanoids such as prostaglandins, leukotrienes and various hydroxy and hydroperoxy acids (Samuelsson et al., 1978; Samuelsson, 1983; Salmon & Higgs, 1989). Free radicals can also attack protein molecules, proteoglycans and glycosaminoglycans. In vitro experiments have shown that free radicals depolymerize hyaluronic acid producing lower synovial fluid viscosity (Greenwald & Moy, 1980). It is possible that a free radical induced altered viscosity of synovial fluid could lead to meniscal hesitation and clicking as originally proposed by Toller (1961). There is also evidence associating free radicals with cartilage damage (Merry et al., 1990) and a recent study has demonstrated that oxygen-derived free radicals stimulate bone resorption (Garret et al., 1990).

Therefore, it was decided to test the hypothesis that oxygen free radicals contribute to the pathogenesis of temporomandibular joint pain and dysfunction.

4.2. Pilot Study (Aghabeigi et al., 1990)

10 patients with chronic TMJ pain not responding to conservative measures were included in the study (2 males, 8 females, age range 20-57 years). Under general anaesthesia which was administered for the purpose of TMJ arthroscopy (n=9) or exploration (n=1), 1 ml of normal saline was bilaterally injected to and aspirated from the upper joint space. Samples were assayed for lipid peroxidation products using the thiobarbituric acid test. Lipid peroxidation products were detected in all samples (mean 0.312±0.303 ΔAbs. 532nm). Comparison of the levels of lipid peroxidation products in symptomatic and symptomless joints revealed a significantly increased level (p<0.05) of TBA-RS in the affected joints (mean 0.356±0.275 vs 0.093±0.040 Δ Abs. 532nm).
Limitations of the pilot study:

Subsequently, it was noted that a methodological problem could have led to false high readings. Ultraviolet range cuvettes (Hughes & Hughes) were sometimes used in our research laboratory and proved satisfactory for spectrophotometric studies in both ultraviolet and visible ranges of wavelengths. However, in the course of method standardization it was noted that uv-range cuvettes but not the visible range were slowly dissolved by butan-1-ol which was used in the extraction of MDA-TBA adduct. Furthermore, the centrifugation step was initially performed at ambient temperature rather than 4°C of the standard method. The higher temperature of test solution would have accelerated the reaction of butan-1-ol with the uv-range cuvettes. The combination of the above had led to misleadingly high readings.

Additionally, the contribution of blood contamination to the TBA reactivity of samples was not measured, which could have confounded the accuracy of the results. Furthermore, as the yield of aspirate was variable, it was not known whether a representative sample of the synovial fluid had been obtained nor whether there was a direct relationship between its volume and synovial fluid content (see V.3.4.2.) Having standardized the methodology for the collection and assay of saline aspirate the paired joint washes and plasma samples were assayed for the presence of lipid peroxidation products by using the TBA test, and aspirate haemoglobin was estimated as a measure of blood contamination.
4.3. Materials and Methods:

4.3.1. Patients:

18 patients (age range 22-49, mean 33.2±8.1, F:M = 13:5) with chronically painful TMJs unresponsive to medical therapy with a tricyclic antidepressant undergoing arthroscopy under general anaesthesia were included in this study. 3 patients complained of bilateral TMJ pain prior to the procedure in which case the predominantly painful side was compared to the contralateral side. None of these patients had any history of allergy to aspirin, gastrointestinal disorders or a bleeding tendency. 1-2 hours prior to the operation 1.2g of aspirin was administered orally. Informed consent was obtained for all procedures.

4.3.2. Aspiration technique and preliminary preparation of samples

As described in V.2.2.2. and V.2.2.3.

4.3.3. Biochemical measurements:

4.3.3.1. Thiobarbituric acid test. The method was as described in IV.4.3. with the exception of using 1.5ml of butan-1-ol instead of 3 ml for extraction of TBA-RS.

4.3.3.2. Haemoglobin assay (see V.2.2.3)

4.3.3.3. Salicylate assay (see V.3.2.3.)

4.3.4. Data analysis:

Corrections were made to adjust for any TBA-RS or salicylate contribution to the aspirate by blood contamination. The adjusted value(AAC) was calculated from the following
equation:

\[ \text{OAC} = \text{AAC} \times (1-H) + \text{PC} \times H \]

where:

\( \text{OAC} \) : Observed Aspirate Concentration of TBA-RS or Salicylate

\( \text{AAC} \) : Adjusted Aspirate Concentration of TBA-RS or Salicylate

\( H \) : \text{Hb of aspirate} / \text{Hb of blood}

\( \text{PC} \) : Plasma concentration of TBA-RS or Salicylate

In order to estimate the synovial TBA-RS concentration from the aspirate in which it was diluted by the injected saline, the ratio of salicylate concentration in the plasma to that in the joint wash was used as a dilution factor. This was calculated in 8 pairs of aspirates where sufficient volume were available for the biochemical assays.

Final corrected value of \[\text{TMJ TBA-RS adjusted TMJ salicylate}\] = Adjusted TBA-RS x plasma salicylate / adjusted TMJ salicylate

Statistical analysis for the comparison of symptomatic and symptomless joints was carried out using a paired t-test.

4.4. RESULTS

Table 23 compares the results from 18 bilateral saline aspirates. The yield of aspirate was variable ranging from 500 to 1050\( \mu \)l (mean 859\( \mu \)l). There was no significant difference between symptomatic and symptomless sides. Almost half of the samples showed blood contamination. TBA-RS were detectable in both symptomatic and symptomless joints. Using a paired t-test there was no significant difference in TBA reactivity between
symptomatic and symptomless sides. Exclusion of TBA reactivity due to blood contamination gave a significantly different value from the initial reading (p<0.03 symptomatic, p<0.06 symptomless). However, the difference between the symptomatic and symptomless sides after correction was not significant.

Table 23. Comparison of TBA-RS in 18 bilateral saline aspirates

<table>
<thead>
<tr>
<th></th>
<th>symptomatic</th>
<th>symptomless</th>
</tr>
</thead>
<tbody>
<tr>
<td>aspirate volume (µl)</td>
<td>873±162</td>
<td>846±210</td>
</tr>
<tr>
<td>Blood contamination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of samples</td>
<td>50%</td>
<td>55%</td>
</tr>
<tr>
<td>Hb mg/ml</td>
<td>6±5</td>
<td>9.5±12.8</td>
</tr>
<tr>
<td>TBA-RS Δ Abs. 532nm*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>initial value</td>
<td>0.013±0.011</td>
<td>0.014±0.013 n.s.</td>
</tr>
<tr>
<td>adjusted value</td>
<td>0.011±0.010</td>
<td>0.010±0.007 n.s.</td>
</tr>
</tbody>
</table>

* TBA-RS are measured spectrophotometrically at the light wavelength of 532 nm.
  n.s. = not significant

Table 24 compares the results from 8 patients from whom bilateral samples were assayed for salicylate. The ratio of salicylate in plasma to that in the joint wash was taken as the dilution factor. After exclusion of TBA-RS due to blood contamination of the joint wash, the adjusted value was further corrected by its multiplication by the dilution factor (see data analysis V.4.3.4. equation 2). A paired t-test did not show a significant difference between either the adjusted values or the corrected multiplied value.
Table 24. Comparison of the results of 8 bilateral samples

<table>
<thead>
<tr>
<th></th>
<th>symptomatic</th>
<th>symptomless</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBA-RS Δ Abs. 532nm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>adjusted value</td>
<td>0.011±0.009</td>
<td>0.008±0.005 n.s.</td>
</tr>
<tr>
<td>corrected value :</td>
<td></td>
<td></td>
</tr>
<tr>
<td>adjusted value x dilution factor</td>
<td>0.198±0.126</td>
<td>0.177±0.122 n.s.</td>
</tr>
</tbody>
</table>

4.5. DISCUSSION

Measurement of TBA-RS is probably the most widely used single assay for measuring lipid peroxidation despite being criticised for its lack of specificity when applied to human body fluids ie, the TBA reactivity is not entirely due to free radical generation as other substances such as bilirubin, aminoacids and nucleic acid also react with TBA. However, the test sensitivity is high because it amplifies oxidative changes that have begun in the sample, and is therefore used as a measure of the potential of the material to undergo the chain reaction of lipid peroxidation(Gutteridge,1986). Halliwell (personal communication) points out that although false positives are frequent, false negatives are rare. Therefore, the TBA test is usually used as the first step in investigating the potential of a material to undergo the process free radical mediated lipid peroxidation, before embarking on more sophisticated methods of measurements of free radical activity. Therefore, our
demonstration of the presence of TBA-RS in saline aspirates collected from TMJs of patients with facial arthromyalgia, implies the involvement of free radicals in the pathogenesis of TMJ disorders. Subsequently, we attempted to measure lipid peroxides using more specific assays such as HPLC measurement of MDA-TBA adduct. This was unsuccessful because of the small volume of the sample and its high dilution which was beyond the sensitivity range of the assay system.

Another supporting piece of evidence for the involvement of free radicals in the pathogenesis of TMJ disorders is our demonstration of high concentrations of a hyperalgesic mediator (15-HETE) whose synthesis involves the free radical mediated process of lipid peroxidation of arachidonic acid in the TMJs of patients with facial arthromyalgia (see VI.2.). It is of interest that the hyperalgesia induced by 15-HPETE in experimental animal models can be blocked by a substance P (SP) antagonist (Garland, personal communication). Holmlund et al. (1991) have demonstrated the presence of high levels of substance P (SP) in the temporomandibular joint. SP is capable of activating leukocytes to promote free radical generation which can then contribute to tissue damage (Hartung & Toyka, 1983).

An interesting finding was that symptomless joints had comparable levels of TBA-RS and 15-HETE to that of symptomatic joints. However, these aspirates from symptomless joints can not be regarded as a normal control. This is discussed further in the next section (V.5).
5. GENERAL DISCUSSION

In this study, most of the problems encountered during the initial phase of method development were overcome. Aspiration technique was standardized and performed by a single operator. With experience, successful aspiration of a good volume of saline was consistently achieved in most instances. Blood contamination of some of the samples was identified as an important source of error. Particularly if the sample is not centrifuged before being frozen. Blood cell lysis at thawing could lead to misleadingly high levels of inflammatory mediators. None of the published studies on TMJ saline aspirates comment on this and it is not surprising that their reported levels of neuropeptides, prostaglandins and leukotrienes measured in highly diluted saline aspirates of TMJ are higher than those in synovial fluid of inflamed knee joints!

It is possible that completed mixing of injected saline and synovial fluid may not be achieved in all cases owing to presence of fibrous adhesions. Hence, it is not known whether the saline aspirate is a representative sample of synovial fluid. Therefore in interpreting the saline aspirate analysis for various compounds, it is crucial to obtain a dilution factor which will allow for correction of the results. We were not able to show any correlation between the volume of the aspirate and its synovial fluid content as measured by the salicylate concentration. However, the assumption of equal concentration of salicylate in synovial fluid and plasma needs more rigorous pharmacokinetic studies. Alternatively, to avoid the problems associated with the interpretation of the results of pharmacokinetic studies of various drugs in plasma and synovial fluid, effort could be directed towards the development of sensitive techniques for measurement of electrolytes
such as calcium or sodium which always have equal concentrations in plasma and synovial fluid. Recently we have standardized the method for measurement of calcium ions in the saline aspirates (Meghji, personal communication).

However, one problem has been the lack of a reliable control for comparison. For obvious ethical reasons it has not been possible to obtain samples of the synovial fluid from a normal TMJ and therefore the normal values of TMJ synovial fluid is not known for comparison to the values obtained from patients with TMJ pain and dysfunction. In the above studies, there was no significant difference between the symptomatic and symptomless joints with respect to 15-HETE and TBA-RS levels or the synovial fluid volume. Although we have obtained and analysed the aspirates from symptomless joints, by no means we imply that the symptomless joint can be regarded as a reliable control as it is functionally and anatomically related to the contralateral symptomatic side.

The reliability of a negative clinical temporomandibular joint examination has been recently questioned based on arthrographic data (Wetesson et al., 1989), arthroscopic findings (Israel et al., 1991) and biochemical indices of joint diseases associated with cartilage breakdown (Israel et al., 1991). However, the biochemical analysis of TMJ synovial fluid may be of value in early assessment of TMJ disease. Kopp (1991) points out that joint disease activity at least in the early stage is only detectable by a biochemical observation.

The TMJs are unique in the body in that both joints are anatomically and functionally inseparable and hence pathology in one joint will undoubtedly affect the other. Patients
frequently complain of bilateral symptoms either simultaneously or sequentially. Also the mirror imaging of inflammatory responses in rheumatoid arthritis and osteoarthritis affecting other pairs of joints in the body which lack the unique anatomical and functional characteristics of TMJ has stimulated a considerable research interest. Kidd et al (1989a) injected latex particles in the rabbit knee joint and found histological evidence of inflammation in the contralateral joint without clinical signs. This was abolished by neural blockade. Therefore, it has been proposed (Kidd et al., 1989b) that the provocation of sensory fibres in one joint, activates preganglionic sympathetic neurons in the autonomic cell column projecting to the contralateral side. In this way unilateral joint damage could result in selective activation of sympathetic neurons on both sides of the spinal cord. Such increased sympathetic tone would lower the threshold of the primary nociceptive fibres, which in turn could then be activated by events that usually non-noxious. Thus a sequence of movement, nociceptor activation and neuropeptide release from sensory nerve fibres could induce the disease process in the contralateral as well as in the damaged joint. The relief of symptoms following a variety of surgical procedures on the joint may well be the result of deafferentation suspending this neural mechanism.

Despite beliefs to the contrary, immunohistochemical techniques have revealed that human synovium is richly innervated (Mapp et al., 1989). Animal studies have also demonstrated the presence of neuropeptide containing fibres in TMJ capsule (Johansson et al., 1986; Ichikawa et al., 1989). Recently the presence of these fibres in human TMJ capsule has also been demonstrated (Harrison, personal communication). These nerves can be stimulated to release neuropeptides by a peripheral mechanism such as antidromic axon reflex after tissue injury or a central stress related mechanism (Matis et al., 1990).
Holmlund et al. (1991) have demonstrated the presence of high levels of SP in the temporomandibular joint. These neuropeptides have a variety of complex influences on inflammation (Foreman, 1987). They produce oxygen free radicals, release interleukins from macrophages and release PGE$_2$ from synoviocytes. Free radicals mediate the synthesis of hyperalgesic eicosanoids such as 15-HPETE. Both SP and 15-HPETE induce a state of prolonged hyperalgesia without continuous tissue destruction, by sensitizing peripheral nociceptors (Ferreira et al., 1990). This may be relevant in explaining chronic TMJ pain without evident structural changes. It is of interest that hyperalgesia induced by 15-HPETE in experimental animal models can be blocked by a SP antagonist but not by non-steroidal anti-inflammatory analgesics. There is thus good evidence implicating neuropeptide generated free radicals in the pathogenesis of temporomandibular joint disorders.

In summary a credible hypothesis for these pains is that emotional with or without physical stress promotes the release of neuropeptides such as substance P in the joint capsule. These induce localized hyperalgesia, vasodilatation, and free radical release in the joint capsule and synovium. Free radical damage will then alter joint lubrication and produce eicosanoid algesic agents such as 15-HPETE leading to a chronic hyperalgesic state. Ultimately both adhesions and fibrocartilage damage may arise (fig. 40). Asymmetrical masticatory activity or bruxism may be important in localizing the presenting site, but the contralateral symptomless joint also shows latent biochemical damage which probably reflects a potential pathological process and could account for
dysfunction without pain. However, the lack of positive objective clinical or arthroscopic findings in some painful joints and the well recognized effect of predisposing life events on a vulnerable personality emphasize the role of central modulating factors. This is being pursued by current work exploring personality factors and the effect of cognitive therapy.

Figure 40

Diagrammatic hypothesis of hyperalgesic temporomandibular joint pain
CONCLUSIONS
CONCLUSIONS:

This study aimed to answer some of the questions related to the pathophysiology of chronic idiopathic orofacial pain. There were some exciting findings which have important implications in the diagnosis and management of the condition. There were also negative findings as well as methodological problems which did not allow any definite conclusion to be reached. The overall picture of these results, once again emphasizes the multifactorial nature of pain.

I. Psychological vulnerability

The majority of the patients (74%) had no current psychiatric illness and almost half had a clear lifetime history of psychiatric illness. However, a sizable proportion had suffered from major depression and post-traumatic stress disorder. By being alert to these syndromes, we may be able to provide effective treatment and improve the quality of life, even in chronically burdened patients. Although, training for recognition and management of the wide range of psychological problems is beyond the scope of dental and oral surgical training, familiarity with features of dysthymia, major depression and post-traumatic stress disorder and the ability to collect diagnostically relevant historical data from the patients is required by those who are involved in management of pain patients.

Patients had a higher lifetime prevalence of major depression than the general population. However, this did not seem to be causally related to the pain in any way. This supports our finding of a common metabolic abnormality predisposing to both idiopathic pain and depression, even if both do not occur in the same individual, but the pain would seem to
be an alternative disturbance rather than 'masked depression'.

Stress was found to be an important contributing factor as 64% of patients reported a stressful life event within 12 months before the pain onset. Furthermore, higher trait anxiety scores in the patients than the general population suggests that they may have personalities that are more vulnerable to stress. In 5 cases the severity of the stressful event appeared to have led to PTSD the onset of which coincided with the pain onset. This has important diagnostic, therapeutic and medicolegal implications. Due to the putative traumatic aetiology of many facial pain conditions, a substantial proportion of patients may in fact suffer from post traumatic stress syndrome. Unfortunately with most post-traumatic TMJ and orofacial pains the assumption that the pain is only of mechanical or neuropathic origin is unhelpful. If PTSD is indeed found to contribute to a painful condition then therapy should be oriented towards the resolution of stress disorder.

The widespread use of DSM-III-R by researchers and clinicians provides a quick and cost effective method for screening patients if any future comparison with different study populations is to be made. Recently, a computerized screening version of the structured clinical interview for DSM-III-R has become available and is being used in our department.

II. Biochemical predisposition to pain and depression

The finding of abnormal tyramine conjugation in both pain and depression is of great interest. Even more so, is the finding that the pain patients with no history of depression had the lowest tyramine-sulphate excretion values. This can explain the significant relief
of symptoms achieved with tricyclic antidepressants in both conditions. It will also explain why antidepressants relieve pain in the absence of clinical depression.

The diagnostic and therapeutic implications of abnormal tyramine conjugation test in pain patients are also interesting. The high predictive value of a negative tyramine test justifies its use as an additional means to help the clinicians in differential diagnosis of difficult facial pain.

Tyramine test has been shown to be a reliable predictor of outcome of treatment to tricyclics in depression. Similar studies are being undertaken to assess its value in treatment of chronic facial pain with tricyclic antidepressants. A reliable predictor of treatment would allow rational selection of particular treatment for particular patients, prevent premature abandonment of treatment, or even spare inappropriate patients from exposure to unnecessary treatment.

A widely held belief is that abnormalities of the central serotonergic system underlies the pathophysiology of both pain and depression. The lack of difference in platelet MAO activity between pain patients and controls in this study does not reject this belief. More refined techniques are needed for measuring MAO activity in sites in the body other than platelets and also techniques for measuring the functional capacity of the serotonergic system. The advent of positron emission tomography is one of the latest non-invasive techniques for the study of MAO activity in the central nervous system which may help to clarify the role of MAO and the serotonergic system in patients with chronic facial pain.
III. Oxygen free radicals

The role of oxygen free radicals in facial pain was investigated using two approaches. The first was the study of OFR in the systemic circulation and the second was the study of OFR contribution to local damage in the TMJ.

Unfortunately, at present no fully satisfactory method exists for measurement of free radicals. The measurement of 2,3,- dihydroxy-benzoic acid which had been suggested to be of value for in-vivo measurement of OH hydroxyl radical, was found to be flawed by a number of methodological problems, the main one being the presence of pyrogallol(1,2,3, trihydrobenzene) as a stabilizer in diethyl ether which was recommended for the extraction of samples in the original method. Since then, we have been using ethyl acetate for the extraction procedure and the method is standardized. The modified method is now being used for a new study of the systemic role of OFR in chronic facial pain.

The discovery of the methodological problem once again brings to light a common problem in chromatographic assays which is frequently overlooked. The appearance of a well resolved band at the retention time of the standard does not guarantee that the band represents a pure compound. Therefore, during method development it is important to check for any interfering peaks that might overlap analyte bands.

The non-specific but sensitive thiobarbituric acid test however, did not show a significant difference between pain patients and controls. However, acute experimental stress did not seem to affect free radical metabolism in either pain or control group. With increased understanding in free radical chemistry and introduction of more specific assays, some of the problems faced in our study will be avoided. Future research to clarify the relationship between stress, pain and changes in the body chemistry is warranted.
In relation to temporomandibular joint synovial fluid, demonstration of TBA-RS implies that OFR may play a role in the local pathogenesis of TMJ pain and dysfunction. However, the unavoidable lack of a reliable control and absence of similar data in relation to normal TMJ synovial fluid makes interpretation of the results difficult. For ethical reasons aspiration of normal human TMJ synovial fluid may not be possible, studies on animal TMJ synovial fluid may be another alternative.

IV. Temporomandibular joint synovial fluid analysis

In this study most of the problems encountered during the initial phase of the investigation were overcome. Methodological problems of previous published reports of TMJ saline aspirate analysis were identified and avoided. Of particular importance was the surprisingly low levels of prostanoids i.e. the products of cyclooxygenase activity. Our finding of considerable levels of the hyperalgesic products of 15-lipoxygenase enzyme such as 15-HETE may explain the failure of non-steroidal anti-inflammatory drugs in the relief of pain associated with the condition. However, the reason for the activation of the cell membrane or cells containing 15-lipoxygenase is unknown. Immunohistochemical studies should be directed towards the localization of the enzyme in tissue sections of TMJ capsule and meniscus from normal joints and from joints from patients with facial arthromyalgia. Furthermore, other hyperalgesic products of lipoxygenase pathway in TMJ should be defined. Also of interest, would be therapeutic intervention using agents which are known to block lipoxygenase activity. At present, the analgesic efficacy of such an agent (dipyrone) in the management of chronic TMJ pain is being investigated.

Development of a method for the measurement of TMJ synovial fluid volume is valuable
not only for understanding TMJ pathophysiology, but also for a comparison of analyte concentrations in different joints. As equilibration of injected saline in the joint is not certain, the correlation between the concentration of various compounds in saline aspirates and their actual amount in the synovial fluid remains unknown. Simultaneous measurement of salicylate in both plasma and synovial fluid should overcome this problem. Although, the basic assumption of equilibration of salicylate in plasma and synovial fluid needs further validation, the principle underlying the method can be used to estimate the synovial fluid volume by measuring other constituents which are known to equilibrate fully between plasma and synovial fluid. Work is now in progress for the measurement of calcium ions in plasma and saline aspirates.

Figure 41 is a flow chart proposing the sequential changes giving rise to TMJ pain and dysfunction. The pain mechanisms are also applicable to non joint orofacial tissues giving rise to atypical facial pain, atypical odontalgia and oral dysaesthesia. The boxed areas are those examined by this research project, unboxed areas indicate other work or hypothesis.

The interaction of varied combinations of emotional and physical stress acting in a vulnerable individual gives rise to neuropeptide release within the joint capsule. This may activate free radical and eicosanoid release from leukocytes and possibly cytokines. The eicosanoids especially 15-HPETE together with the neuropeptides such as substance P and calcitonin gene related peptide (and possibly cytokines IL-1 and IL-6) produce a hyperalgesic capsulitis and hence pain with function. It is postulated that the oxygen free radicals may also depolymerize hyaluronic acid and damage the articular cartilage. The outcome will be altered lubrication and adhesion formation impairing condylar/meniscal
movement. If severe and prolonged, the meniscus may become adherent in an anteriorly displaced position. Fibrocartilage damage may even progress to bone resorption with pathological remodelling and osteophyte formation.

* Peripheral Mechanism

** Central Mechanism

- Depression
- Biochemical/Psychological Vulnerability
- End Organ Changes
- Neuropeptide Release
  - Leukocytes
  - Cytokines
- Eicosanoids
  - Oxygen Free Radicals
  - Cartilage Damage
  - Bone Resorption
  - Adhesions
  - Dysfuction
- Hyaluronic Acid Depolymerization
- Altered Lubrication
- Hyperalgesia
- Pain

**e.g. bruxism or trauma**

**acute or chronic stress**

**Figure 41**

Pathophysiology of chronic idiopathic orofacial pain
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ACKNOWLEDGEMENTS

Throughout this study I had the privilege of receiving supervision and help from distinguished clinicians and scientists of various disciplines, to whom I would like to express my gratitude.

Professor Malcolm Harris, Professor and Head of Department of Maxillofacial Surgery and Oral Medicine, Eastman Dental and University College Hospitals has been a constant source of encouragement, help and supervision. This study would not have been possible without his tremendous support. His kindness is deeply appreciated.

Dr. Brian Henderson, Professor of Oral Biochemistry, Eastman Dental Hospital, introduced me to the meaning of science. His critical evaluation of my work throughout this study has had a valuable influence on the outcome.

Dr. Charlotte Feinmann, Consultant Psychiatrist, Eastman Dental Hospital, helped me with the study of psychological characteristics of my patients. I am very grateful to her. I am also obliged to Dr. Gary Jackson, Department of Psychiatry, Middlesex Hospital for reading the chapter on post-traumatic stress disorder and his constructive comments.

Professor Merton Sandler, Emeritus Professor of Chemical Pathology and Dr. Vivette Glover, Senior Biochemist from Bernhard Baron Memorial Research Laboratory, Queen Charlotte's and Chelsea Hospital, advised me on the study of biological markers of depression. Mrs. Patricia Hannah and Dr. Brian Goodwin from the same institute gave
valuable help with the biochemical assays.

Professor Barry Halliwell, Dr. Sue Chirico and Dr. B. Ward, from Department of Biochemistry, King's College, University of London, advised and assisted in my study of oxygen free radical metabolism.

Dr. Joe Edwards, Department of Rheumatology, Middlesex Hospital, gave valuable advice on TMJ synovial fluid analysis.

Dr. Mohammad Wasil and Dr. Sajeda Meghji, Research Fellows, Oral & Maxillofacial Surgery Research Laboratory, Eastman Dental Hospital, patiently taught me the laboratory research techniques.

Mr. Colin Hopper, Consultant Oral & Maxillofacial Surgeon, Eastman Dental Hospital, allowed me to use his patients for the study of TMJ synovial fluid and offered valuable assistance in TMJ synovial fluid collection and preparation.

Dr. Rosamond Hopps, Department of Oral Biology, Eastman Dental Hospital, kindly supervised the initial stages of my research.

Dr. John Bullman, Department of Dental Public Health, Eastman Dental Hospital, offered help with various statistical analysis. Miss Gemma Harris gave valuable mathematical help with TMJ synovial fluid data analysis.
And last but not least, without the help and cooperation of all the patients and control subjects who voluntarily gave their valuable time and blood (and other body fluids), this study could not have been accomplished.
Diagnostic Screening Tests: (Armitage, 1971)

Sensitivity (x):
The sensitivity of a screening test is the probability (Pr) that a subject with the disease will screen positively and hence be correctly identified. There is an analogy with significance tests. Sensitivity is analogous to the 'statistical power' of a test.

Statistical power = 1-β, where β (type II error) is the probability of not rejecting the null hypothesis when it is false, i.e., false negative.

Specificity (y):
The specificity of a screening test is the probability that a subject without the disease will screen negative and hence also be correctly identified. The analogy with the significance test is that specificity is equal to 1-α (significance level of a test).

α (type I error): probability of rejecting null hypothesis when it is true, i.e., the significance level of a test.

Using D+ or - to refer to the disease presence or absence and T+ or - to refer to the test result, these definitions may be written as:

Sensitivity = Pr (T+ / D+)
Specificity = Pr (T- / D-)

Proportions of false positives and false negatives in a population:
A tree diagram helps to illustrate the general case, where p is the prevalence of disease and x and y refer to sensitivity and specificity.
For any member of the population there are four possible outcomes:

D+T+  D+T-  D-T+  D-T-

Each subject can experience only one of these outcomes and is referred to as a genuine positive, false negative, false positive or genuine negative depending on which one occurs. To obtain the probability of any outcome such as D+T+ the probabilities belonging to the branches of the tree leading to the outcome are multiplied together.

The proportion of false negatives (D+T-) in the population is \( x(1-y) \). The proportion of false positives (D-T+) in the population is \( (1-x)(1-y) \). There are costs for both of these mistakes; the false negatives are lost opportunities for treatment and the false positives may have to undergo further unnecessary tests.
Predictive values of a test:

1. The predictive value of a positive test

This is the probability with which an individual who screens positive has the disease ie

\[ \Pr(D^+ / T^+) = \frac{px}{px + (1-x)(1-y)} \]

2. The predictive value of a negative test

This is the probability with which an individual who screens negative does not have the disease.

\[ \Pr(D^- / T^-) = \frac{(1-p)y}{(1-p)y + p(1-x)} \]
RELATED PUBLICATIONS

1. Chapter I

Aghabeigi B. (1992)
Pain Pathophysiology.

2. Chapter II


3. Chapter III

Tyramine Conjugation deficit in patients with chronic idiopathic temporomandibular joint and orofacial pain.
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4. Chapter IV

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5. Chapter V

Temporomandibular joint synovial fluid analysis.
The pathophysiology of pain

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The two most common types of pain presented to dentists are acute orofacial pain and chronic craniofacial pain. This article presents some newer concepts in the understanding of the pathophysiology of pain. Pain perception, modulation and transmission are briefly described, followed by a discussion of the physiological and biochemical aspects of chronic and recurrent facial pain.

Pain is the most common symptom that compels patients to seek medical and dental therapy and constitutes a serious health and economic problem. It has been estimated that in the industrialised countries 15-20% of the population have acute pain and between 25 and 30% suffer from chronic pain. This costs American society 79 billion dollars annually.

Dentists and dental specialists are concerned with two of the most common pains. The first is acute orofacial pain arising from the teeth and associated structures and the second is chronic craniofacial pain, which is believed to account for 40% of all chronic pain problems. An understanding of the pathophysiology of pain is needed by all those who are involved in pain relief. The aim of this article is to present some newer concepts, particularly those which may have an important effect on patient management.

Definitions

Traditional views of pain are reflected in definitions such as that of the Butterworth's Medical Dictionary: 'the distressing sensation excited by noxious stimuli of sufficient intensity acting on nerve endings'. It is often difficult to define psychogenic pain as 'pain which occurs without any organic cause and is due to a disorder of mind'.

Although much pain results from noxious stimulation, it can also occur from non-noxious stimuli, as well as spontaneously when there is no stimulus at all. Wall and Devor have shown that sensory impulses from the dorsal root ganglia (the site where sensory neurones make their first synapse) are constantly being transmitted into the central nervous system (CNS) even under normal conditions. This means that we have a potential source of neural impulses present at all times which can reach a conscious level of pain as a result of any decrease in central inhibitory control.

Thus, newer concepts regard pain as a subjective psychological state rather than an unpleasant sensory activity that is induced solely by noxious stimulation. The definition of pain adopted by the International Association for the Study of Pain (IASP) defines pain as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage'. This definition avoids linking pain to a stimulus and regards pain always as an affective state, that is, an emotional experience and not merely the perception of a pure sensation. However, the affective state of pain differs from other affective states in that it is always referred or projected to some part of the body with varying degrees of precision. Unlike elation or sorrow, pain is always 'felt' in some part of the body, even when that part is no longer present, as in the case when pain is felt in a 'phantom limb' after its amputation. As pain is a totally subjective experience which cannot be simultaneously shared and reported by another individual, it is clinically important to accept the subject's description of the pain experience. Conversely, it is unhelpful to question or reject it.

Classification

A classification into acute and chronic or recurrent pain is chosen here (Table I) as the management, and to some extent the pathophysiology, is different. Acute pain may be considered to be a protective mechanism for the body which, by stimulating the sympathetic nervous system, is often accompanied by the autonomic signs of stress and anxiety. It is also of considerable diagnostic value to the clinician in determining the nature and site of the disturbance. Also, the control of acute pain in most instances is accomplished by the use of non-steroidal anti-inflammatory analgesics or the opioids. On the other hand, chronic pain does not serve any apparent biological function and is socially and psychologically destructive. It can be recurrent or continuous. The sympathetic and neuroendocrine responses have usually become less apparent and vegetative (somatic) signs emerge similar to those seen in depressive syndromes. Furthermore, pain control is invariably achieved by specific forms of medication such as anticonvulsants for trigeminal neuralgia, ergonamine for migraine and tricyclic antidepressants for chronic idiopathic pain, as well as for the prophylaxis of migraine.

A detailed classification and description of acute orofacial pain is beyond the scope of this article. However, as the subject of chronic orofacial pain is controversial, it deserves consideration and is dealt with in detail below. Such pains may be subgrouped into three types (Table I):

(1) Neuropathic: arising from damage anywhere in the nervous system, e.g. trigeminal neuralgia, post-herpetic neuralgia.
(2) Nociceptive: where an identifiable continuous nociceptive input is present, e.g. osteo- or rheumatoid arthritis or cancer.
(3) Chronic and recurrent idiopathic orofacial pain.

The following sections contain brief descriptions of the mechanisms of pain perception, transmission and modulation.

Chemical basis of nociception

Pain is provoked when a variety of substances are released or injected into the tissues. These pain-producing substances can be released by trauma, infection, allergic reaction,
Table I Classification of common orofacial pain

<table>
<thead>
<tr>
<th>Acute</th>
<th>Chronic</th>
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<tbody>
<tr>
<td>Oral: Dental: pulpitis, cracked tooth</td>
<td>Neuropathic: trigeminal neuralgia, post-herpetic neuralgia, causalgia</td>
</tr>
<tr>
<td>Dental: gingivitis, periodontitis, periodontia, periodontia</td>
<td>Nociceptive: cancer, osteoarthritis, rheumatoid arthritis</td>
</tr>
<tr>
<td>Dental: various causes of ulceration</td>
<td>Chronic and recurrent idiopathic profacial pain:</td>
</tr>
<tr>
<td>Temporomandibular joint:</td>
<td>Facial anaesthesia (TMJ dysfunction syndrome)</td>
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<tr>
<td>Traumatic acute dysfunction</td>
<td>Atypical facial pain (idiopathic facial pain)</td>
</tr>
<tr>
<td>Maxillary sinus: sinusitis and carcinoma</td>
<td>Atypical odontalgia</td>
</tr>
<tr>
<td>Safety: quinsy, carcinoma</td>
<td>Oral dysaesthesia</td>
</tr>
<tr>
<td>Tonsils: quinsy, carcinoma</td>
<td>Tension headache</td>
</tr>
<tr>
<td>Refrained: cardiac arrhythmia, cervical</td>
<td>Migraine</td>
</tr>
<tr>
<td>spondylitis</td>
<td>Facial migrainous neuralgia</td>
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<tr>
<th>Corticosomes</th>
<th>Phospholipase A2</th>
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<tr>
<td>Cyclooxygenase</td>
<td>Cell Membrane Phospholipid</td>
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<tr>
<td>Arachidonic Acid</td>
<td>Prostaglandins</td>
</tr>
<tr>
<td>(Eicosatetraenoic Acid)</td>
<td>PG E2, PGI2, Thromboxanases</td>
</tr>
<tr>
<td>Phospholipase</td>
<td>Leukotrienes</td>
</tr>
<tr>
<td>A2</td>
<td>LT B, 15-HPETE, etc.</td>
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Fig. 1 Pathways of arachidonic acid metabolism.

neurogenic reflexes and central emotional changes from cell membranes, mast cells and nerve endings. This leads to the excitation of free nerve endings which act as nociceptors or peripheral sensor organs that respond to the noxious stimuli. This group of substances includes histamine, bradykinin, potassium, acetylcholine, prostaglandins, leukotrienes, and the neuropeptides.

Among these noxious substances prostaglandins have attracted much attention in the recent past. They are produced by the metabolism of cell membrane arachidonic acid through the cyclooxygenase pathway (fig. 1). Prostaglandins E2 (PG E2) sensitise the nerve endings and lowers their threshold to all kinds of stimulation. The analgesic action of aspirin and the other non-steroidal anti-inflammatory analgesics is believed to be due to inhibition of cyclooxygenase.

Arachidonic acid can also be metabolised via the lipooxygenase pathway. This is not inhibited by non-steroidal anti-inflammatory analgesics (fig. 1). Leukotriene B4 (LT B4) is a hyperalgesic substance which is derived from the 5-lipoxygenation of arachidonic acid. This is the result of the action of the lipooxygenase enzyme on the fifth carbon atom bond of arachidonic acid. LT B4 has been identified in the saline aspirates of the painful temporomandibular joint, as has been 15-HPETE (hydroxyeicosatetraenoic acid)—which is the breakdown product of 15-HPETE (dihydroxyeicosatetraenoic acid), another known hyperalgesic agent produced via the lipooxygenase pathway. This may explain why the non-steroidal anti-inflammatory analgesics are not fully successful in relief of chronic temporomandibular joint pain.

Although acute and some types of chronic hyperalgesia are due to a continuous generation of pain mediators by damaged tissue, there is evidence that certain mediators such as the neuropeptide substance P and PGE2 as well as 15-HPETE can induce a state of prolonged hyperalgesia which is not due to continuous tissue destruction, but sensitising the peripheral nociceptors. Furthermore, there is evidence that activation of peripheral nociceptors as a consequence of tissue damage can lead to hyperexcitability of central neurones at the spinal dorsal horn resulting in unexpected prolonged hyperalgesia. This may be avoided in clinical practice by the infiltration of a local analgesic prior to surgery, even when carried out under a general anaesthetic. These observations may also be relevant in explaining other chronic painful conditions without evident structural changes.

It has also been suggested that stress can lead to the peripheral release of neuropeptides such as substance P. Substance P plays an important role in neurogenic inflammation and has also been implicated in the mechanism of migraine. The temporomandibular joint capsule and disc are innervated by many substance P containing fibres and the possibility of the release of neuropeptides in stress-related facial arthralgia is currently under investigation.

Pain transmission

Free nerve endings which act as nociceptors are present in all orofacial tissues including the skin, oral mucosa, the temporomandibular joint, periodontium, tooth pulp, periodontium and muscles. Bone itself has substance P and calcitonin gene-related peptide (CGRP) containing nerve endings. These become excited by noxious stimuli and the impulses are transmitted via three major classes of nociceptive afferents:

(1) C-polymodal fibres. These have a diameter of 0.3–3 μm and are unmyelinated with a conduction velocity (cv) of 0–5 m/s. They respond to somato-cutaneous, thermal and chemical stimuli.

(2) A-delta fibres. These are 2–5 μm in diameter and thinly myelinated with a cv of 5–30 m/s.

(3) A-beta fibres. These are 6–22 μm in diameter and heavily myelinated with a cv of 35–75 m/s. In one study almost half the myelinated dental axons in the trigeminal nerve conducted at A-beta velocities.

Studies of electrically stimulated teeth have found that the most sensitive fibres are activated by low-stimulus intensities and evoke 'prepain' sensations, whereas higher intensities cause sharp pain, and the highest cause an unpleasant ache. As single fibre studies in animals show that A-beta fibres are more sensitive than A-delta fibres and that C-fibres are least sensitive, it is probable that prepain, sharp pain and dull ache correspond to A-beta, A-delta and C-fibre activation.

Abnormal conducting patterns may occur in nerves after experimentally-induced demyelination and this has been suggested as a pathogenesis of trigeminal neuralgia. A paroxysmal pain which is characteristically provoked by the stimulation of myelinated fibres by light touch. Plaques of demyelination are frequently found in the descending
trigeminal tract and in the lemniscal systems, but it is not possible to argue that such plaques are either necessary or sufficient to cause tic douloureux. Nevertheless, trigeminal neuralgia is associated with plaques of demyelination in multiple sclerosis and sites of nerve compression by cerebral arteries.

Although teeth are extensively innervated by A-beta, A-delta and C-fibre axons, it is not possible to prove that all these fibres are all nociceptive afferents. It is likely that some are autonomic efferents. Although nerve fibres enter the dentinal tubules, the actual mechanism of dentinal sensitivity is unclear. Three major theories are:

1) Activation of the intradental extensions of pulpal nerves.
2) A transduction mechanism involving the odontoblast or its dentinal process.
3) A hydrodynamic mechanism within the dentinal tubules and pulp.

The first theory has largely been abandoned as there is no satisfactory evidence to show that there are nerves in the outer dentine, which is the most sensitive. Furthermore, agents that cause pain when applied to the skin do not do so when applied to dentine. Substance P has been measured in dentine and dental pulp and is interesting to note that approximately four times as much is transported peripherally as centrally. Substance P is the neurotransmitter at the primary sensory synapse. This neuropeptide is synthesised by the nerve cell body and can be released antidromically. In fact it could have retained an ability to transduce and propagate an impulse. However, dentine remains sensitive despite the destruction of not only the intradental nerve fibres but also the odontoblast layer. This gives support to the concept that the existence of nerve fibres in dentine is not a necessary prerequisite for its sensitivity.

The second theory considers the odontoblast to be a receptor coupled to the nerves in the pulp. It used to be argued that because the odontoblast was of neural crest origin it could have retained an ability to transduce and propagate an impulse. However, dentine remains sensitive despite the destruction of not only the intradental nerve fibres but also the odontoblast layer. This gives support to the concept that the existence of nerve fibres in dentine is not a necessary prerequisite for its sensitivity.

The hydrodynamic theory of dentine sensitivity fits much of the experimental and morphologic data. It proposes that the fluid movement through the tubules distorts the adjacent pulp and is sensed by its fine nerve endings. This theory explains why topical anaesthetics fail to block dentine sensitivity and why pain is produced by dehydration, hypertonic solutions, thermal change, and mechanical probing. The increased sensitivity at the dentino-enamel junction is explained by the profuse branching of the tubules in this region.

Once generated the impulse in the orofacial nerves travels mainly via the trigeminal nerve but also by the sensory roots of the facial, glossopharyngeal, vagus and upper cervical nerves. Substance P is the neurotransmitter at the primary sensory synapse. This neuropeptide is synthesised by the nerve cell body and can be released antidromically. In fact it is interesting to note that approximately four times as much Substance P is transported peripherally as centrally.

Substance P has been measured in dentine and dental pulp and is believed to play a role in the reception and transmission of pain in dentine. The central processes of these neurons enter the brainstem and synapse on second order neurones at various levels of the trigeminal brainstem sensory nuclear complex. The central processes of these neurones enter the brainstem and synapse on second order neurones at various levels of the trigeminal brainstem sensory nuclear complex (Fig. 2) which consists of:

1) The main sensory trigeminal nucleus, which is rostrally located and receives periodontal and some pulpal afferents.
2) The spinal tract of the trigeminal nucleus, which is more caudally located. The spinal tract is divided into the subnucleus oralis, subnucleus interpolaris and the subnucleus caudalis.

The subnucleus caudalis extends into the cervical spinal cord and merges with the spinal dorsal horn. It serves as the principal relay site of orofacial nociceptive information and is similar in structure and projection to the spinal dorsal horn, which is the principal component of the spinal nociceptive mechanism. Evidence suggests that the subnucleus caudalis is homologous to the substantia gelatinosa of the spinal dorsal horn and acts as a 'gating' mechanism capable of modulating sensory information. From the brainstem, sensory information may then be relayed directly to third order neurones in the thalamus and from there to cerebral cortex.

There is some reason to believe that the sensory and affective aspects of pain are subserved in part by separate neural mechanisms. The spinothalamic projection to ventrobasal thalamus and its projection to the somatosensory cortex are required for the discriminative sensory aspect of pain. This is the means whereby the nature and source of the pain are determined. However, projections to the medial thalamus and from the medial thalamus to the frontal cortex seem to be concerned with the affective aspect of pain. This view is supported by observation of patients who have undergone frontal lobotomy. Interestingly, these patients usually obtained striking relief of their pain problem but with no impairment in their ability to detect and identify noxious stimuli as painful. The suffering was thus eliminated with no effect on the purely sensory aspect of their pain.

Pain modulation

The concept of pain modulation is based on the evidence that neural impulses are altered as they travel up to the higher centres. It has been traditional to divide pain into organic and psychogenic; organic being pain which has resulted from an identifiable structural lesion, while psychogenic not only labelled those pains without such a lesion but also implied a 'supratentorial' psychiatric disturbance. This view is no longer held. The emphasis is now on the many factors that influence the excitation and inhibition of the painful experience. Whether the pain is generated by a noxious stimulation of the tissues or occurs as a result of a central
Since their endogenous opioid peptides discovered were leucine nearly identical to those of narcotic analgesic drugs. The first synthesised by nerve cells and have pharmacologic properties mediated by endogenous opioid substances which are produced by the pain-modulating network is believed to be modulation are progressively being unravelled. Analgesia control or the activation of excitatory modulating neurones.

...incorporated in the new model of the gate-control theory (fig. 2). The endogenous opioid system and the monoaminergic network interact and recent studies have shown that morphine may activate the descending serotoninergic pathway and so modulate dental pain transmission. There appears to be a specific central nervous system network for pain control. Analgesia may be demonstrated by the stimulation of brain sites such as the periaqueductal grey (PAG) and nucleus raphe magnus (NRM) (fig. 2) in animals and man. This has provided powerful evidence for a highly selective brainstem control of nociceptive transmission. This system is also involved in emotional and motivational functions and other complex behaviour.

Although there has been much emphasis on the pain-suppressing effect of the modulation system, the brainstem modulating neurones have a bidirectional control of pain transmission in that the network has both excitatory and inhibitory actions on pain conduction. These concepts are incorporated in the new model of the gate-control theory (fig. 3); therefore pain can result from either the loss of inhibitory control or the activation of excitatory modulating neurones.

The anatomical, chemical and physiological bases of pain modulation are progressively being unravelled. Analgesia produced by the pain-modulating network is believed to be mediated by endogenous opioid substances which are synthesised by nerve cells and have pharmacologic properties nearly identical to those of narcotic analgesic drugs. The first endogenous opioid peptides discovered were leucine-enkephalin and methionine-enkephalin. Since their throughout the body. One of the most potent of these is beta-endorphin ("endogenous morphine"). Its precursor, pro-opiomelanocortin, which originates in the infundibular nucleus of the basal hypothalamus, also gives rise to adrenocorticotropic hormone (ACTH). However, it is interesting that ACTH antagonises the analgesic effects of beta-endorphin. This may also be of some relevance to chronic pain conditions, as patients with chronic idiopathic pain syndromes are reported to have higher levels of cortisol in their blood.

It has been a widely held view that the endogenous opioids are involved in anaesthesia or analgesia produced by hypnotic and acupuncture. However, recent controlled studies question this view.  

Cytotoxic studies of the pain-modulating networks have also revealed that, in addition to the endogenous opioid peptides, a variety of other neurotransmitters are involved in the control of pain transmission. The monoamines serotonin and noradrenaline are present in the brainstem neurones and both inhibit spinal cord pain transmission cells.

The endogenous opioid system and the monoaminergic network interact and recent studies have shown that morphine may activate the descending serotoninergic pathway and so modulate dental pain transmission. Monoamines have also been implicated in the pathogenesis of chronic pain and are of particular importance as they can be manipulated by a variety of pharmacological agents, thus raising the possibility of new centrally-acting analgesic agents. In fact there is evidence that the well established value of tricyclic antidepressants in chronic pain management relates to their potency as blockers of the re-uptake of both noradrenaline and serotonin. The use of antidepressants as analgesics opens up an entirely new approach to drug treatment for chronic pain. These drugs do not produce tolerance and dependence and their side effects have been considerably diminished with the development of novel agents.

Chronic and recurrent idiopathic orofacial pain

**Psychological and biochemical aspects**

*Facial arthromyalgia (the temporomandibular joint pain dysfunction syndrome)*

This is the second most common cause of facial pain after toothache. The presenting symptoms are of a dull ache with occasional severe episodes affecting the joint and its neighbouring musculature. It may be associated with disturbances of function of the joint such as clicking and limitation of opening. The term 'facial arthromyalgia' is preferred as it is precisely descriptive and avoids the use of the term 'syndrome' with its implication of exclusive symptoms.

*Atypical facial pain (idiopathic facial pain)*

This condition presents as a continuous dull ache with intermittent exacerbating throbbing episodes which is localised to non-joint and non-muscular sites.

*Atypical odontalgia*

This is identical to pulpal or periodontal pain. However, there is no detectable pathology. Some cases appear to have been precipitated by a dental procedure but they are...
Oral dysaesthesia

The most common presentation is the burning tongue (glossodynia). Occasionally the gingiva and lips are also involved, or the denture-bearing areas of the hard palate and lower alveolus, making the wearing of a denture impossible.

Tension headache (muscle contraction headache)

This is described as a steady non-pulsatile tension or ache. It has been assumed for many years that the pain occurred as a result of muscular contraction. However, this has not been confirmed by electromyographic studies of patients during headache and non-headache periods.

Migraine and facial migrainous neuralgia

Migraine is a recurrent unilateral throbbing headache, whereas facial migrainous neuralgia is a variant which affects the mid-facial region mainly supplied by the maxillary branches of the external carotid artery. Although vasoconstriction has been proposed to be the causative factor, results of cerebral blood flow studies question the validity of this assumption and the precise mechanism for the pain of migraine and related headaches remains unknown.

Facial arthromyalgia (FAM), atypical (idiopathic) facial pain, atypical odontalgia and oral dysaesthesia are the most common idiopathic chronic orofacial pain problems that may present individually, sequentially or simultaneously in any patient. Despite popular belief, there is no controlled evidence that they arise from malocclusion. There is a substantial body of work to show that many chronic pains are stress related. However, it is not clear how stress leads to pain or any other psychosomatic illness. Although it has been thought that the underlying psychological activity operates independently of somatic structures, there is increasing evidence that points to an organic basis for all such disturbances. Thus receptors, neurones, synapses, electric charges and neurochemicals are involved in pain disorders, both psychological ("functional") and structural ("organic").

There have been several reports of a higher level of stressful life events in idiopathic facial pain patients, although one recent study did not find any difference between temporomandibular joint patients and controls with regard to their desirable and undesirable life events. However, it was found that these patients had fewer sources of emotional support, thus indicating stress as an imbalance between the demand on the individual and coping ability. It would appear therefore that whether or not the amount of stress endured is higher than normal, chronic pain patients have personalities that are in some way more vulnerable. Despite this, some pain patients have no apparent psychogenic substrate and remain purely idiopathic.

Magna, in a review article, estimated that 30–60% of patients with ‘non-organic’ chronic pain suffer from depression. This incidence seems to be higher not only than the general population but also than those patients with chronic nociceptive pain. More interesting is the finding that a high degree of first degree relatives of subjects with such pain also suffer from affective (emotional) disorders. This has led to the hypothesis that chronic pain and depression might share a common biological pathogenesis. In other words, some underlying biological abnormality makes certain individuals vulnerable to depression or pain or other psychosomatic disorders. This has been difficult to establish because the majority of studies into the relationship of psychiatric disorders to pain have been faced with methodological problems such as a lack of rigorous psychiatric diagnostic criteria, the use of self-reporting questionnaires rather than standarised psychiatric assessments, and a failure to determine the rates of psychopathology preceding the pain.

Atkinson et al., using a structured clinical interview to diagnose mental disorders according to the rigid criteria set by DSM-III-R (Diagnostic and Statistical Manual of Mental Disorders, third edition, revised), found that in 56% of patients with back pain the first episode of major depression generally followed the onset of pain. Furthermore, all the patients who experienced a mood disorder reported recurrent rather than single, episodes, which again highlights the vulnerability of these patients to depression. It should be added that this relationship allows patients to suffer pain without ever having been depressed, as was demonstrated in 49% of Feinmann and Harris’s patients.

The concept of pain vulnerability is supported by the clinical observation of different pains occurring in some patient at different stages of life. In childhood such patients suffer from abdominal or ear pain, in adolescence temporomandibular joint pain or dysmenorrhoea, and later abdominal pain (usually the irritable bowel syndrome), neck and back pain and pruritus. If this concept is not recognised the differential diagnosis of each pain episode may be misleading and clinically unhelpful, with the tendency to attribute the disorder to purely local abnormalities rather than a generalised problem. It has also been suggested that this vulnerability may be genetically transmitted as children of temporomandibular joint pain patients seem to suffer from a higher incidence of illness and injury.

One of the promising fields of study to explain this phenomenon seems to be the CNS serotonin system, which is believed to be involved in the pathogenesis of both pain and depression. Drugs such as reserpine, which deplete CNS monoamines, produce not only depression but also spontaneous pain, including atypical odontalgia (Harris, personal communication).

Patients with idiopathic pain syndromes have low concentrations of serotonin metabolites in their cerebrospinal fluid, but it has been suggested that this abnormality does not alter during the course of the illness and may be a marker of an increased risk of developing a chronic pain syndrome. Besides central serotonin system markers, chronic pain and depression share a number of other biological abnormalities such as: hypercorisolemia, an abnormal deaminoephaene suppression test, and low serum and urine melatonin level.

An important recent finding has been that patients with chronic TMJ and orofacial pain excrete significantly lower amounts of conjugated tyramine sulphate after a standard oral dose of tyramine as compared to controls. This tyramine conjugation deficit, which was formerly believed to be only a trait marker for ‘endogenous depression,’ was significantly more evident in the non-depressed group of idiopathic pain patients. These findings, together with the well established observation that both tricyclic and monoamine oxidase inhibitor antidepressants are highly effective in the treatment...
of chronic pain syndromes, provide further support that a common biological abnormality underlies the pathogenesis of both chronic idiopathic pain and depression.

The search for biological markers of pain syndromes, in addition to providing insight into their pathophysiology, may help in identification of patients at risk and be of assistance in patient management. The high predictive value of a negative tyramine excretion test justifies its use as an investigative means to help the clinician in the differential diagnosis of enigmatic facial pain.** Furthermore, as the tyramine test has been shown to be of value in the prediction of outcome of treatment in depression, its value as a means of predicting the response to treatment with tricyclic antidepressants in pain patients is currently being investigated.

** In summary, pain is multifactorial and when interpreting the psychophysiologic phenomena associated with pain perception, the significance of, and concern for, the consequences as we know it is a unique experience peculiar to the human. Many consider that clinical pain is an entity.

Conclusion

In summary, pain is multifactorial and when interpreting the results of animal research models one must consider the psychophysiological phenomena associated with pain perception. The significance of, and concern for, the consequences of the painful experience in humans makes it different from that experienced by animals. Many consider that clinical pain as we know it is a unique experience peculiar to the human.

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Prevalence of post-traumatic stress disorder in patients with chronic idiopathic pain

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SUMMARY. 34 patients with chronic idiopathic orofacial pain were assessed by a structured clinical interview for diagnosis of mental disorders according to the Diagnostic and Statistical Manual for Mental Disorders (DSM-III-R). Five (15%) had a history of post traumatic stress disorder (PTSD) which coincided with the pain onset. The majority of these PTSD sufferers also had a personality disorder. The implications of these findings in the diagnosis and management of post-traumatic chronic TMJ pain syndromes is discussed.

INTRODUCTION

Chronic idiopathic orofacial pain is a common problem. It consists of facial arthromyalgia, atypical facial pain, oral dysaesthesia and atypical odontalgia. These conditions can occur simultaneously or sequentially in the same patients. The psychological disturbances associated with chronic facial pain have attracted considerable attention. It is known that the psychological state of an individual may produce pain, increase the severity with which it is felt or even diminish the severity. Furthermore, there has been a substantial body of work to show that chronic orofacial pain is stress related (Lefer, 1966; Fine, 1971; Feinmann & Harris, 1984). Therefore, recognition of stressful stimuli responsible for the psychosomatic component operative in a pain condition is essential for establishing both diagnosis and appropriate therapy.

A psychiatric syndrome which has in the last decade become more clearly defined is the "post-traumatic stress disorder" (PTSD) (American Psychiatric Association, 1987). The essential feature of PTSD is the development of characteristic symptoms following a psychologically distressing event that is outside the range of usual human experience. The stressor producing this syndrome would be markedly distressing to almost anyone, and is usually experienced with intense fear, terror and helplessness. Examples would be assault, a road traffic accident or some form of mass accident. The characteristic symptoms involve re-experiencing the traumatic event, the avoidance of stimuli associated with the event or numbing of general responsiveness, and increased arousal (Table 1). It has been shown that patients demonstrating this psychological symptoms carry a concurrent increased risk of physical disorders, such as peptic ulceration, asthma and hypertension, all of which have traditionally been thought of as, in part, stress-related disorders (Davidson et al., 1991).

Table 1 - Diagnostic criteria for post-traumatic stress disorder according to DSM-III-R

A. The person has experienced an event that is outside the range of usual human experience and that would be markedly distressing to almost anyone

B. The traumatic event is persistently re-experienced in at least one of the following ways:
1. Recurrent and intrusive distressing recollections of the event
2. Recurrent distressing dreams of the event
3. Sudden acting or feeling as if the traumatic event was recurring
4. Intense psychological distress at exposure to events that symbolize or resemble an aspect of the traumatic event
5. Markedly diminished interest in significant activities
6. Restricted range of affect
7. Sense of a foreshortened future

C. Persistent avoidance of stimuli associated with the trauma or numbing of general responsiveness, as indicated by at least three of the following:
1. Efforts to avoid thoughts or feelings associated with the trauma
2. Efforts to avoid activities or situations that arouse recollections of the trauma
3. Inability to recall an important aspect of the trauma
4. Markedly diminished interest in significant activities
5. Feeling of detachment or estrangement from others
6. Restrict range of affect
7. Sense of a foreshortened future

D. Persistent symptoms of increased arousal as indicated by at least two of the following:
1. Difficulty falling or staying asleep
2. Irritability or outbursts of anger
3. Difficulty concentrating
4. Hypervigilance
5. Exaggerated startle response
6. Physiologic reactivity upon exposure to events that symbolize or resemble an aspect of the traumatic event

Psychiatric disturbances in patients with chronic idiopathic orofacial pain, a structured clinical interview for the diagnosis of mental disorders (SCID) (Spitzer et al., 1989) based on the diagnostic and statistical manual for mental disorders (DSM-III-R) of the American Psychiatric Association (1987), revealed a number of patients suffering from post-traumatic stress disorder (PTSD). The onset of this coincided with the onset of the pain. A review of the literature
A 52-year old female patient presented to the pain clinic in 1986 with pain affecting the right TMJ, cheek, nose and the right side of the head which started 2 months after a car accident 1 year previously. She had been the driver of a car which was hit from behind and as a result she sustained a whiplash injury. There had been no loss of consciousness or evidence of head injury. She recalls that after the accident she became very depressed and needed counselling. She felt a lot worse when she was in a car or in a situation that reminded her of the accident for a number of months afterwards. She avoided driving for 2 years. Due to persistence of pain after the accident, she consulted an ear, nose and throat specialist and had her sinus examined, and saw a neurologist who could find no abnormality. Initial examination did not reveal any structural pathology and a diagnosis of facial arthralgia and atypical facial pain was made. She was depressed and was treated by the liaison psychiatrist. The psychiatric history revealed that the patient had a deprived childhood. She had suffered from a major depressive episode after her mother's death when she was 19 years old and has been chronically depressed ever since. A diagnosis of stress-related psychogenic facial pain was made and she was put on a tricyclic antidepressant for the relief of her depressive symptoms. This gradually improved her condition. When the patient was last seen 6 years after the accident, her pain and also the distressing symptoms of PTSD had disappeared. Her depression, although in remission had not resolved completely. The structured clinical interview not only confirmed the occurrence of past PTSD and the current dysthymia, but also revealed the diagnosis of a dependent personality disorder.

**Case report 2**

A 31-year old female presented with a 9-year history of a dull ache affecting the alveolar ridge in the region of the upper left lateral incisor which had been treated 5 years previously. Pain was provoked by tiredness and alcohol and there were no relieving factors. She also suffered from migraine, irritable bowel and pruritus. The history revealed that 9 years before presentation she suffered an unprovoked panic attack and was punched in the face which resulted in a coronal fracture of the upper left lateral incisor tooth. She recalls that after this incident she became severely depressed and was unable to return to her work for 4 years. Her dentist restored the tooth by provision of an artificial crown. However, the pain persisted. She underwent three apicectomies and exploration of the affected area during the subsequent 3 years and eventually the tooth was removed in an attempt to cure the pain. A bridge was made to restore the gap, however she was still in pain. A second bridge was made later which did not result in any improvement. At this time, the patient was referred to our pain clinic. Her past medical history was insignificant. She was single, lived with her parents and had no brothers or sisters. She did clerical work. Clinical and radiographic examination did not reveal any significant abnormality and a diagnosis of atypical odontalgia was made. The psychiatric interview confirmed the past PTSD and major depressive episode. It also revealed that she suffered from generalized anxiety disorder and also a panic disorder. The personality assessment was indicative of the presence of a passive-aggressive and a borderline personality disorder. As she was already under care of another centre for stress management, and was reluctant to take medication, a full explanation of her condition was given, and arrangements were made for her to have regular review by the psychiatrist.

**RESULTS**

Figure 1 shows different orofacial pain conditions diagnosed in the patients. The clinical diagnosis of the orofacial conditions in PTSD patients included the full spectrum of psychogenic facial pain conditions including atypical odontalgia, atypical facial pain and facial arthralgia. Two of the subjects diagnosed as suffering from PTSD had suffered a whiplash injury which was believed to be responsible for the temporomandibular joint pain. Some patients suffered from more than one clinical or psychiatric condition. Among the psychiatric diagnoses (Table 2) a lifetime history of major depression was the commonest (50%). This was followed by PTSD (15%).
Vietnam Veterans with PTSD from normal controls (Jackson, 1991). For instance, the startle response has been shown to be different in depression and pathological grief which argues for a differentiation of PTSD from other anxiety syndromes. The biological response to specific stimuli can accurately reflect traumatic events. Some data suggest that tests of the predominant one being the re-experiencing of the traumatic concept has been recognized for a long time. Records of combat, transport accidents and reports after assault or rape describe a common core of symptoms, the predominant one being the re-experiencing of the traumatic events. Some data suggest that tests of biological response to specific stimuli can accurately differentiate PTSD from other anxiety syndromes, depression and pathological grief which argues for a distinct syndrome (Jackson, 1991). For instance, the startle response has been shown to be different in Vietnam Veterans with PTSD from normal controls (Butler et al., 1990). Other psychiatric conditions, however, often coexist with PTSD and may themselves be brought on by life events. So the notion of a number of inter-relating post-traumatic stress disorders emerges.

Due to the traumatic etiology of many pain conditions, a substantial proportion of patients seen in any pain clinic may suffer from post-traumatic stress disorders. However, this has not been recognized until recently due to the absence of well defined diagnostic criteria and a lack of objective methods of assessment of psychiatric morbidity. PTSD is still a diagnosis which is frequently missed even by psychiatric professionals (Davidson & Smith, 1990). Davidson (1989) has outlined four main reasons why a diagnosis of PTSD might be overlooked: (a) not asking the patient about experiences of trauma; (b) patients' reluctance to disclose painful material; (c) physicians' discomfort in discussing events which might be gruesome, horrifying or unimaginable; and (d) the fact that chronic PTSD often presents with non-specific symptoms such as headache, insomnia, irritability, depression, tension, substance abuse, interpersonal or professional dysfunction.

The prevalence of PTSD in our study is 15%, this compares to 9.5% reported by Muse (1985) in a study of 64 chronic pain patients. The point prevalence of PTSD in the general population is about 1% (Jackson, 1991). Thus the prevalence of this syndrome in the present study is substantial when a comparison is made of its relative ranking among other psychological disorders associated with chronic pain. Shepherd (1990) in a study of psychiatric morbidity following personal violence, reported behavioural changes in two thirds of victims 6 months after assault, particularly the avoidance of locations of violence which is also frequently observed in PTSD sufferers.

This study raises a number of questions about stress related post-traumatic chronic pain.

1. What is the real frequency of this syndrome in the chronic pain population?

The present study provides some idea on the prevalence of the condition. However, it is possible that patients with chronic facial pain may differ qualitatively from other groups of pain patients. For instance, Atkinson et al., (1991) in a study of patients with chronic low back pain (CLBP) found that 64.9% had a history of alcohol abuse and suggested that alcohol use disorders rather than depression may increase the risk of developing CLBP. Whereas in our study there was only 1 (3%) case of alcohol abuse. Furthermore, departments of maxillofacial surgery may care for a significant percentage of latent cases amongst the patients who have sustained an assault or a road traffic accident, the commonest causes of facial injury.

2. How prevalent is PTSD in a population?
support (Lima et al., 1990). Several studies indicate that pre-existing psychopathological conditions predispose to the development of the PTSD. Davidson et al. (1991) in an epidemiological study showed that patients with PTSD had significantly higher family history of psychiatric illness, parental poverty, child abuse and separation or divorce of parents. In our study the majority (80%) of the PTSD sufferers also had personality disorders. However, the disorder can develop in people without any such pre-existing conditions, particularly if the stress is extreme. Nevertheless, it has been suggested that the psychiatric morbidity following an accident is largely dependent on a subjective response to the event and the amount of distress it engenders, as opposed to the nature of traumatic event itself (Horowitz et al., 1979; Feinstein & Dolan, 1991).

3. What is the relation between PTSD, pain onset and its chronicity?

The association of PTSD and pain, suggests a causal role for PTSD in pain onset. The symptoms of PTSD usually begin immediately or soon after the trauma, although the re-experiencing symptoms may develop after a latency of months or years. It is possible that pain is the result of re-experiencing the pain associated with the traumatic event. Davidson et al. (1991) reported that PTSD sufferers are 90 times more likely to suffer from a somatization disorder than the general population which suggests a connection between PTSD and the process of conversion. Other than the stress disorder, other factors such as 'compensation neurosis' or learned pain behaviour may also contribute to the chronicity in certain cases (Muse, 1985). An important diagnostic problem of medico-legal significance is the neuropathic nature of some post-traumatic psychogenic facial pains which resemble causalgia. That is a persistent burning pain involving the face frequently results in the affected area becoming the focus of the attention of the patient, and if there is a susceptible psychiatric make-up, the affected site may become the focus of unresolved conflicts.

In our study various orofacial structures were involved. The clinical diagnoses included atypical odontalgia, atypical facial pain and also facial arthropathy including that associated with whiplash injury. The last condition has frequently been attributed to a physical internal derangement of the meniscus brought about during the traumatic deceleration of the head (Weinberg & Lapointe, 1987). Two cases of PTSD had been diagnosed as this whiplash TMJ internal derangement. However, there may be a substantial proportion of these patients, in whom a hidden stress element plays a greater role in the chronicity of the condition than the simple mechanical disturbance. In these cases, therapy should always be directed towards the resolution of the stress disorder.

4. What is the significance of the 'stress related post-traumatic chronic pain syndrome' in patient management?

If PTSD contributes to a painful condition then therapy should be oriented towards the resolution of the stress disorder. Effective treatment entails use of psychotropic medication (Davidson, 1992). Tricyclics and monoamine oxidase inhibitors have been the most widely studied drugs, and their effect increases with duration of treatment. Pharmacotherapy is probably most effective when administered as part of a broadly based treatment plan. Behavioural treatment directed at exposure to avoided situations or thoughts is often a critical step in treatment (Richards & Rose, 1991). Muse (1986) demonstrated the efficacy of both pharmacological and non-pharmacological desensitization in alleviating anxiety and depression associated with the syndrome. However, these therapeutic approaches do not relieve the associated pain in all cases. This suggests the contribution of other factors, such as compensation neurosis and a learned pain behaviour. However, elimination of anxiety and depression should be beneficial in promoting an early return to increased levels of psychosocial and vocational functioning and the ability to cope with chronic pain.

5. Is every area of the body capable of becoming involved in a stress related post-traumatic chronic pain syndrome or is the face particularly at risk?

The face is one of the most complex areas of the body in terms of pain presentation. The intricate innervation and the concentration of all the special senses give the area a unique significance. In addition the face has a special significance which arises from its emotional and social importance. A major traumatic incident involving the face frequently results in the affected area becoming the focus of the attention of the patient, and if there is a susceptible psychiatric make-up, the affected site may become the focus of unresolved conflicts.

In conclusion, it is important to consider PTSD in all patients with persistent post-traumatic pain and it may take more than one session to confirm one's early diagnostic suspicion. The assumption that TMJ pain and dysfunction is only of mechanical or neuropathic origin is unhelpful. Furthermore surgical management alone appears to intensify the pain and render it intractable. Clinicians should bear in mind...
that PTSD can be chronic or recurrent in a high proportion of those who develop it (Kluznick et al., 1986; Zeiss & Dickman, 1989). Therefore, management needs to include long term follow up and support of these patients. Shepherd (1990) points out that the more victims who can talk and receive support, the fewer there will be who need longer and more intensive help.

Acknowledgement

The authors would like to thank Dr Gary Jackson, Department of Psychiatry, Middlesex Hospital for reading the manuscript and his constructive comments.

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Paper received 11 March 1992
Accepted 15 June 1992
Tyramine conjugation deficit in patients with
chronic idiopathic temporomandibular joint and orofacial pain

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ABSTRACT

This study was carried out to explore the value of the tyramine conjugation test, an established trait marker for "endogenous unipolar depression", in patients with chronic idiopathic temporomandibular joint and orofacial pain. Our results show that the pain patients excrete significantly lower amounts of tyramine sulphate than controls ($p < 0.0004$). Psychiatric assessment by the structured clinical interview for the diagnosis of mental disorders according to DSM-III-R revealed that 48% of the patients had a history of depression and 10% were currently depressed. However, the never-depressed group of patients had the lowest tyramine sulphate excretion values. These findings suggest that a common biological abnormality underlies the pathogenesis of both chronic idiopathic facial pain and depression.

Key words: chronic pain pathophysiology, facial pain, tyramine, depression
INTRODUCTION

Chronic idiopathic orofacial pain is an important health problem and poses major management difficulties for doctors and dentists alike (Hunter 1992). Bonica (1980) estimated that five to seven million Americans suffered chronic pain in the face and mouth at a cost to society of over $4 billion a year. It includes facial arthromyalgia (the temporomandibular joint dysfunction syndrome), atypical facial pain, atypical odontalgia and glossopyrosis (burning tongue) (Feinmann et al 1984). These conditions may coexist or occur sequentially in the same patient in association with other idiopathic pains. The accumulation of descriptive labels and differing therapeutic opinions reflect the confusion about the pathophysiology of such conditions. Nevertheless, the association of different pains in an individual patient at different stages of life suggests the existence of a pain vulnerable person (Engel 1959).

It has been estimated that 30-60% of patients with "non-organic" chronic pain suffer from depression. This incidence seems to be higher not only than in the general population but also than in those patients with chronic nociceptive pain. It has also been reported that a high proportion of the first degree relatives of subjects with such pain also suffer from emotional disorders (Magni 1987). This has led to the hypothesis that chronic pain and depression may share a common biological predisposition, and this is supported by the finding that both tricyclic and monoamine oxidase inhibitor antidepressants have been found to be effective in the treatment of chronic pain syndromes (Feinmann et al 1984; Feinmann 1985).
The search for biological markers in depression and chronic pain has attracted considerable interest (Gjerris 1989, von Knorring 1989). One of the promising leads in this field is the study of tyramine conjugation. When administered orally, 10-15% of tyramine is normally metabolised by conjugation to tyramine-O-sulphate. Patients with endogenous (melancholic) unipolar depression excrete subnormal amounts of this conjugate after an oral dose of tyramine. This abnormality which is independent of severity of illness and age, persists after recovery (Bonham et al 1978) and is present in some of the patients' first degree relatives who have never been depressed (Hale 1986). Bipolar patients are not found to be low on the tyramine test (Hale et al 1991). Low values have also been reported in migraine patients but only in a subgroup with a history of endogenous depression (Jarman 1990). It has also been shown that the tyramine conjugation deficit in depressed patients is a predictor of positive outcome of treatment with tricyclic antidepressants (Hale et al 1989). The biochemical basis of the tyramine test, or its relationship with the pathogenesis of depression, is at present quite unclear (Harrison et al 1984). However, the finding has proved to be a robust one in several independent studies, and the trait appears stable in particular individuals.

To date, there has been no study of tyramine conjugation in relation to chronic orofacial and temporomandibular pain patients. We have therefore studied a group of such patients to find out if chronic facial pain shares this biological abnormality with depression. A pilot study on 10 patients was encouraging (Aghabeigi et al 1991). The study was therefore extended to confirm the hypothesis at a statistically significant level and in addition to establish whether the abnormality is secondary to a coexisting depression or is independently present in pain patients.
MATERIALS AND METHODS:

Twenty-nine patients (F:M = 25:4, mean age(±SD) = 40.2 ± 15.2 years) and an equal number of age and sex matched controls (F:M = 25:4, mean age(±SD) = 40.1±15.7 years) were included in this study. The patients were recruited from new referrals to the Eastman Dental Hospital pain clinics on a non selected consecutive basis. The inclusion criteria was an orofacial pain of at least 6 months duration (mean(±SD) 6.6±6.5 years, range 0.5 - 25 years) for which no primary structural pathology was found. Those who declined to undergo the somewhat arduous screening process and also those who were subsequently found to have psychotic symptoms were excluded. The controls were age and sex matched volunteers from professional, non-professional staff and patients attending other clinics within the hospital. The essential criteria were that they did not nor had not suffered any form of idiopathic facial pain or chronic pain elsewhere. Additionally, those control subjects who admitted to have suffered from any depressive episode were excluded.

From midday the day before the test, patients and controls followed a low-tyramine diet avoiding foods detailed on a standard monoamine oxidase inhibitor card. At the start of the test subjects voided their bladder and were administered a capsule containing 100mg tyramine (125mg tyramine hydrochloride). All urine was collected for exactly 3h and its volume determined. Aliquots were stored frozen at -20 C before the measurement of tyramine-O-sulphate by gas chromatography with electron capture detection (Walker and Sandler 1988). The test is highly reproducible; the inter-batch reliability when urine samples were divided and analysed on two occasions was 96% (Hale et al 1986).
During the 3 h period of the test the patients underwent a structured clinical interview for the diagnosis of mental disorders (SCID) (Spitzer et al 1989) in order to assess current and previous incidence of psychiatric disorder according to the criteria set by the Diagnostic and Statistical Manual of Mental Disorders of the American Psychiatric Association revised volume III (DSM-III-R) (1987). When there was a positive history of depression, its melancholic/non-melancholic subtype was determined and a further assessment was made using the Newcastle scale (Carney et al 1965) as an aid in distinguishing endogenous from neurotic depression. The SCID interview was not applied to the controls as the essential criterion for a control was freedom from idiopathic pain and not psychiatric illness. However both controls and pain patients were also screened with the Hospital Anxiety and Depression questionnaire (HAD) to detect current anxiety and depression.

RESULTS:

I. Clinical
Twenty three of the 29 patients suffered facial arthromyalgia, 6 atypical facial pain, 6 atypical odontalgia and 3 oral dysaesthesia. Some patients suffered from more than one condition which is a common finding. Fourteen patients were medication free at the time of the test and 15 were on 10-50mg of nortriptyline for their pain. Fourteen patients (48%) had a positive history of depression. Of these only 3 were suffering from a major depressive episod at the time of examination and 11 had experienced at least one major depressive episode sometime in their life. With the exception of 1 patient all those with a history of melancholic depression (n = 5) had a Newcastle score > 6.
2. Biochemical  (fig.1, tables 1 & 2)

The mean urinary 3h tyramine-O-sulphate output was significantly lower in the patients as compared with controls ($p<0.0004$)(table 1, fig.1). When the patients were divided into those with a history of depression and those without, the latter showed a lower value compared to the former with a difference which was close to the level of statistical significance ($p=0.06$)(table 2). There was no significant difference between patients with a history of melancholic as opposed to non-melancholic depression. Half of the patients were on tricyclic antidepressants for their pain problem. These did not differ significantly from the drug free patients (table 2).

The combined effects of age, sex, presence of pain and depression on tyramine output were studied using multiple regression analysis. The presence of pain was found to be the only statistically significant factor ($b=-2.099$, $t=-4.08$, $df=57$ $p<0.0005$).

DISCUSSION:

Several previous studies have shown that there are common biological abnormalities between pain syndromes and depression. In fact there has been a widely held assumption that because chronic pain is relieved by tricyclic antidepressants, the pain represents an underlying depressive illness (Blumer and Heilbronn 1982, Katon 1984). Patients without evidence of classical depression are often described as suffering from 'masked depression' or a 'depressive equivalent' (Lopez Ibor 1972). These diagnostic categories have doubtful validity and there are dangers inherent in equating a lack of response to treatment as an adequate criterion for diagnosis (Feinmann and Bass 1989). For this reason, such patients are often abandoned by appropriate
specialists on the assumption that their regional pain is a psychiatric symptom. This was reflected in a formal audit of 813 new referrals to this department in an 18 month period. Their mean pain duration was 4.2 years (median 1-2 years) despite having been seen previously by an average of two consultants.

Patients with idiopathic pain have been shown to have hypercortisolaemia and abnormal dexamethasone suppression test responses (Blumer et al 1982), as well as low platelet \(^3\)H-imipramine binding (Mellerup et al 1988, Eberhard et al 1989), low cerebrospinal fluid 5-hydroxyindolacetic acid and low platelet monoamine oxidase activity (Almay et al 1987 a,b). Patients with idiopathic pain syndromes have also been demonstrated to have low concentrations of melatonin in serum and urine (Almay et al 1987c). These studies suggest that there may be a common pathogenesis between depression and idiopathic pain, and point particularly to the possible involvement of 5-hydroxytryptamine. However, these markers are state markers, and not all of the studies have specified whether the patients were currently suffering from major depression. In the present study we have investigated a trait marker, and also carried out a structured clinical interview using the rigid diagnostic criteria set by DSM-III-R to assess psychopathology. Recent studies have shown that SCID yields highly reliable DSM-III-R diagnoses (Skre et al., 1991). We did not apply the SCID to the controls as the essential criterion for a control was freedom from idiopathic pain and not psychiatric illness. Current anxiety and depression were excluded by the HAD questionnaire. Had our controls concealed a history of depression, they would have also tended to display reduced tyramine conjugation, and so render the difference between the groups non-significant. The patient group as a whole showed a highly significant reduction in tyramine-sulphate excretion, and this result was most clear cut in the group who had never suffered from depression.
Low tyramine sulphate appears therefore to be a marker for vulnerability to idiopathic orofacial pain, independent of depression. This result is most unlikely to be a drug effect as drug free patients showed similarly low values and tricyclic medication has previously been shown to have no effect on tyramine sulphate excretion (Hale et al, 1989). The underlying mechanism for the deficit in conjugated tyramine excretion is not clear. (Harrison et al, 1984) have hypothesized that the decreased tyramine sulfate excretion level in response to a tyramine load may reflect the organism's attempt to retain tyramine because of a central deficiency of this trace amine. It has been suggested that tyramine may play a role as "synaptic activator" or "neuromodulator". The monoaminergic network has been implicated not only in the pathogenesis of affective disorders but also the pain modulating system. It is possible that abnormalities in monoamine metabolism is the underlying mechanism which predisposes to both pain and depression.

Although pain patients had a significantly lower value, unlike the previous reports (7) we did not find a natural cut-off point in tyramine values which could differentiate between patients and controls with a high degree of sensitivity and specificity. The cut off point of 4.1 mg/3 hours as suggested by Hale et al, (1986) if applied to our data gives a sensitivity of 65% (ie 35% false negative) and a specificity of 73% (ie 27% false positive). Harrison et al, (1984) have also reported a high degree of false negatives (30%) and false positive (25%). However, if we choose a tyramine output of 5mg/3 hrs suggested by Harrison et al as the cut-off point, a high percentage (14 out of 16 ie 88%) of the non-depressed pain patients fall at or below this value. This increases the sensitivity of the test at the expense of a reduction in the specificity. In other words, there will be a higher proportion of false positive tests.
The probability with which a subject who screens positive has the disorder, depends not only on the sensitivity and specificity but also on its prevalence. Unfortunately reliable epidemiological data is not available on the prevalence of the chronic orofacial pain in the general population. The majority of studies have documented the epidemiology of the presence of TMJ dysfunction and facial pain without any mention of the severity and the duration of the problem (Shiffman et al., 1988). Our group of pain patients all had chronic persistent pain (mean duration $6.6 \pm 6.5$ years, range 0.5-25 years) and a considerable proportion suffered from chronic pain elsewhere in their bodies. Therefore it seems more appropriate to compare our group with other chronic pain patients. Fortunately data is available on the prevalence of chronic pain in general population and is estimated to be 14% (Magni et al., 1990). Taking into account this value the proportion of false positives will be 0.33, and the predictive value of a true positive amongst apparent positives will be 0.26. Therefore, the procedure can not be recommended as a positive diagnostic screening test. However the proportion of false negatives will be 0.02, and the predictive value of a true negative amongst apparent negatives will be 0.96. This value is slightly higher for the non-depressed group (0.97).

Based on these findings, a patient with a history of chronic orofacial pain particularly in the absence of a history of depression or other psychopathology, with a tyramine sulphate output value above 5 mg/3 hrs is unlikely to fall in the chronic idiopathic pain group and careful follow up supplemented by appropriate investigations and treatment is necessary. Therefore a tyramine conjugation test may serve as an additional means to help the clinician in the differential diagnosis of difficult pain conditions.
Nevertheless these findings support the hypothesis that there is a common underlying metabolic vulnerability to both idiopathic facial pain and endogenous (melancholic) depression even if both do not occur in the same individual. Such pain therefore should be considered an alternative disturbance rather than 'masked depression'. Thus both are syndromes that respond to antidepressant medication, and as previously reported, the response of the pain patient is independent of any concurrent depression (Eriksson 1989).

Acknowledgements. We would like to thank Dr. John Bullman from the Department of Dental Public Health for his valuable help with the statistical analysis, and Mrs Valerie Cardinali for her many patient drafts of the manuscript.
Table 1. Tyramine-O-sulphate excretion values group data

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No.</th>
<th>Tyramine-O-sulphate excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>I. Controls</td>
<td>29</td>
<td>5.38</td>
</tr>
<tr>
<td>II. Pain patients</td>
<td>29</td>
<td>3.74</td>
</tr>
<tr>
<td>(a) never-depressed</td>
<td>15</td>
<td>3.25</td>
</tr>
<tr>
<td>(b) history of depression</td>
<td>14</td>
<td>4.33</td>
</tr>
<tr>
<td>melancholic</td>
<td>5</td>
<td>4.26</td>
</tr>
<tr>
<td>non-melancholic</td>
<td>9</td>
<td>4.38</td>
</tr>
<tr>
<td>(c) drug free</td>
<td>14</td>
<td>3.59</td>
</tr>
<tr>
<td>(d) on medication</td>
<td>15</td>
<td>3.94</td>
</tr>
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</table>
Table 2. Inter-group comparisons of tyramine-O-sulphate level

<table>
<thead>
<tr>
<th>Patient subgroups</th>
<th>vs</th>
<th>controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td></td>
<td></td>
<td>0.0003</td>
</tr>
<tr>
<td>never-depressed</td>
<td>vs</td>
<td>controls</td>
<td>0.00009</td>
</tr>
<tr>
<td>history of depression</td>
<td>vs</td>
<td>controls</td>
<td>0.07</td>
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<tr>
<td>history of depression</td>
<td>vs</td>
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<tr>
<td>melancholic</td>
<td>vs</td>
<td>non-melancholic</td>
<td>0.88</td>
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<tr>
<td>drug-free</td>
<td>vs</td>
<td>on medication</td>
<td>0.55</td>
</tr>
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LEGEND:

Figure 1. Tyramine excretion values after an oral dose of tyramine (100mg).
Individual values and means are shown for controls and patients as well as the patient subgroups with and without a history of depression.
Short Communication

OBSERVATIONS ON THE MEASUREMENT OF 2,3- AND 2,5-DIHYDROXYBENZOIC ACID USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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*Correspondence


This paper describes some of the methodological problems associated with the measurement of the aromatic compounds 2,3- and 2,5-dihydroxybenzoic acid (DHB), using high performance liquid chromatography (HPLC). Diethyl ether cannot be recommended for the extraction procedure because the pyrogallol (1,2,3-trihydrobenzene) used as a stabilizer, co-chromatographs with that of 2,5-DHB. Ethyl acetate can be employed for the extraction of 2,3- and 2,5-DHB but only the former can be satisfactorily assayed by HPLC.

Keywords: free radicals, hydroxyl radical, salicylate, dihydroxybenzoic acid, HPLC

INTRODUCTION

There has been a growing interest in attempts to use aromatic compounds to detect hydroxyl radical (·OH) generated in vivo. Grootveld and Halliwell [1] described the HPLC separation combined with electrochemical detection to identify and quantify 2,3- and 2,5-DHB in human plasma of subjects who had consumed aspirin. These two compounds are believed to be produced by the attack of hydroxyl radical on salicylic acid. The same authors in a later publication demonstrated the presence of these compounds in the diethyl ether extracts of human urine [2].

Having followed the methodology described above, we were not able to reproduce some of their results. Our findings have led us not to recommend the use of diethyl ether for the extraction of human plasma because of its stabilizer content. Furthermore, a putative catecholamine peak, which runs very close to 2,3-DHB, in some cases overlapped the adjacent peaks. This is possibly due to variations in the catecholamine levels in plasma of different subjects. A modification of the original method was required for obtaining adequate resolution in quantification of 2,3-DHB and 2,5-DHB in the ethyl acetate extracts.
MATERIALS AND METHODS

Reagents

HPLC-grade solvents were obtained from BDH Chemicals Ltd., and aromatic compounds from Sigma. Standard solutions were made up and stored as described by Grootveld and Halliwell [1].

HPLC

HPLC was carried out using an HPLC Spectraphysics pump and a 5 μm ODS reverse-phase column 25 cm × 4.6 cm (Brownlee). The mobile phase and electrochemical detection were identical to that described by Grootveld and Halliwell [1], i.e. sodium citrate (30 mmol/L)–sodium acetate (27.7 mmol/L) buffer pH 4.75 at a flow rate of 1.0 ml/min. The mobile phase was sparged continuously with He gas during elution. Detection was by an EDT LCA15 electrochemical detector equipped with a glassy carbon working electrode and an Ag/AgCl reference electrode. The injection loop (10 μl volume) was cleaned with at least 3 ml each of methanol, water and buffer after each injection, until injection of buffer did not produce any electroactivity as a result of residues in the loop from the previous injection.

Plasma preparation and analysis

Venous blood (10 ml) was collected from medication-free healthy volunteers just before and 2 h after ingestion of 1.2 g of aspirin. The samples were centrifuged at 3000 g for 5 min and the plasma layer was aliquoted and stored at -20°C until analysis.

Extraction procedure

200 μl of plasma was treated with 25 μl of 1 mol/L HCl and 25 μl of 7 μmol/L 3,4-DHB (internal standard) and extracted using either diethyl ether or ethyl acetate.

Diethyl ether

Samples were extracted twice with 10 ml of diethyl ether for chromatography. The combined extracts were evaporated to dryness in a water-bath at 40°C and then reconstituted with 250 μl of 50 mmol/L HCl.

Ethyl acetate

Samples were extracted twice with 8 ml of HPLC-grade ethyl acetate. The combined extracts were evaporated to dryness in a water bath at 55°C under nitrogen and then reconstituted with 250 μl of 50 mmol/L HCl.
Figure 1.
(a) Separation of a standard mixture of 2,3-DHB, 2,5-DHB and 3,4-DHB. Details of separation are given in Materials and Methods.
(b) Separation of a diethyl ether extract of a plasma sample from a healthy volunteer who had not consumed aspirin. There is a peak with exactly the same retention time as 2,5-DHB.
(c) The same plasma sample spiked with standard 2,5-DHB.
Figure 2.
(a) Separation of an ethyl acetate extract of a plasma sample from a healthy volunteer collected 2 h after ingestion of 1.2 g of aspirin. A clear separation of 2,3-DHB and 2,5-DHB from the adjacent peaks (catecholamine and the internal standard 3,4-DHB) is achieved.
(b) The separation of an ethyl acetate extract of a plasma sample from another healthy volunteer collected 2 h after ingestion of 1.2 g of aspirin again proved unsatisfactory. The large catecholamine peak overlapped and obscured the 2,3-DHB peak.

RESULTS

Diethyl ether extracts

Figure 1(a) shows separation of a standard mixture of 2,3-, 2,5- and 3,4-DHB. The relative retention time of 2,3-DHB in relation to 3,4-DHB was 0.78. 2,5-DHB appeared close to internal standard but was clearly separable from 3,4-DHB (relative retention time 0.86, and the resolution from 3,4-DHB was 2.2).
Figure 1(b) shows a chromatography of a diethyl-ether-extracted plasma sample from a subject before ingestion of aspirin. Surprisingly, there was a peak with exactly the same retention time as 2,5-DHB. Figure 3 shows the same sample spiked with 2,5-DHB.

Initially, it was considered that the subject may have consumed other non-steroidal anti-inflammatory drugs which could have metabolites with retention times identical to that of 2,5-DHB. However, samples from nine other controls also showed the same peak with a height equal to that of a standard sample of 500 μmol/L 2,5-DHB.

Eventually, by the process of elimination, diethyl ether itself was found to contain a compound with exactly the same retention time as 2,5-DHB. Although diethyl ether was especially made for chromatographic use, it was found that it contains a stabilizer with an aromatic ring. The stabilizer, pyrogallol (1,2,3-trihydrobenzene), was found to have exactly the same retention time as 2,5-DHB. It not only makes the identification of 2,5-DHB impossible under these conditions, but also, because of the large size of its corresponding peak, it overlaps the peaks related to 2,3-DHB and 3,4-DHB. Thus, it is not possible to measure 2,3-DHB in diethyl extracts of biological fluids.

**Ethyl acetate extracts**

Ethyl acetate is allegedly specific for the extraction of phenolic acids [3]. However, the extraction efficiency for 2,5-DHB was very low (20%) from plasma samples which is in contrast to previous reports of 76% [1]. The explanation for this observation is not clear. The possibility that 2,5-DHB was binding to plasma proteins such as albumin or immunoglobulin was tested. It was possible to extract 2,5-DHB efficiently from buffer, albumin (5 g/dl) or human immunoglobulin (3 g/dl). The possibility that 2,5-DHB binds strongly to some other constituent of plasma is under investigation. The extraction efficiency of 2,3-DHB was similar to previous reports (76%) [1].

Figures 2(a) and 2(b) show chromatographs of plasma extracts from two subjects who had consumed 1.2 g of aspirin 2 h prior to blood collection. Due to the large size of the catecholamine peak which is efficiently extracted by ethyl acetate, in one of the subjects it is not possible to detect the presence of 2,3-DHB.

To improve the resolution a C8 and a C18 column were placed in series. This increased the column efficiency and provided the required resolution of 2,3-DHB and adjacent peaks (Table 1). However, the analysis time was doubled, but as it was below 20 min was considered acceptable.

**DISCUSSION**

Our paper once again brings to light a common problem in chromatographic assays which is frequently overlooked. The appearance of a well-resolved band at the retention time of the analyte (standard) does not guarantee that the band represents a pure compound. It is always possible for a second compound to have the same retention time as the analyte. Therefore during method development it is important to check for any interfering peaks that might overlap analyte bands. Commonly used procedures include (a) chromatographic cross-checks, (b) wavelength-ratioing and diode-array detectors, and (c) other spectroscopic tests, as by LC-mass spectrometry [4].
TABLE 1
Comparison of the resolution of 2,3-DHB, 2,5-DHB and 3,4-DHB as obtained by a single C18 column and the combination of C8 and C18

<table>
<thead>
<tr>
<th>Resolution with a C18 column</th>
<th>Resolution with C18 and C8 columns in series</th>
</tr>
</thead>
<tbody>
<tr>
<td>R&lt;sub&gt;s&lt;/sub&gt; between 2,3-DHB and 2,5-DHB</td>
<td>1.6</td>
</tr>
<tr>
<td>R&lt;sub&gt;s&lt;/sub&gt; between 2,5-DHB and 3,4-DHB</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Grootveld and Halliwell [1] point out that in view of the large number of ether-soluble molecules present in biological fluids, the identity of retention time with that of a standard is inadequate evidence to attribute the peak to 2,3-DHB. The identity of 2,3-DHB was confirmed by electrochemical analysis and by mass spectrometry [2]. However, it is possible that due to low efficiency of the column used, 2,5-DHB was not separable from and overlapped with the internal standard 3,4-DHB. This could lead to inaccuracies in the quantification of 2,5-DHB and also 2,3-DHB if an internal standard method is used.

In our study, blank plasma samples were an additional means of confirming the identity of aspirin metabolites. By comparing the chromatograms of samples from subjects before and after ingestion of aspirin, a clear picture is provided of peaks which are attributable to aspirin metabolites. Using this method we found that measurement of salicylate by the above method (oxidation potential 0.96) is not reliable as blank plasma shows a peak with an identical retention time to that of salicylate.

In conclusion, we have shown that diethyl ether containing 1,2,3-trihydrobenzene should not be used in extraction of samples for measurement of aspirin metabolites. However, with improved resolution, ethyl acetate is satisfactory for extraction of 2,3-but not 2,5-DHB from plasma samples. Furthermore, electrochemical detection cannot be recommended for measurement of plasma salicylate level.

REFERENCES
Temporomandibular joint synovial fluid analysis

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SUMMARY. A method for the estimation of the synovial fluid volume of the temporomandibular joint (TMJ) is described. Patients are administered 1.2 g of aspirin and the concentration of salicylate in plasma and in saline aspirates of the TMJ is measured by a sensitive high performance liquid chromatography assay. The ratio of the concentration of salicylate in the saline aspirate to that in the plasma allows the volume of the synovial fluid to be calculated. The method would also allow the determination of the concentration and the absolute amount of putative mediators of pathology in the upper joint.

INTRODUCTION

Continuous or intermittent pain in the temporomandibular joint (TMJ) is common. The accumulation of descriptive labels and differing opinions about the appropriate treatment, reflect the confusion surrounding the aetiology and pathogenesis of this condition. The most commonly used synonomy to describe this condition are: TMJ pain dysfunction syndrome, myofacial pain dysfunction syndrome and facial arthromyalgia (Harris, 1975). The last term seems more precisely descriptive and avoids the misuse of the term 'syndrome' with its implication of exclusive symptoms. There has been a substantial body of work to show the condition is stress related (Lefer, 1986: Fine, 1971; Feinnmann & Harris, 1984). Nevertheless the end organ changes which may lead to internal derangement (meniscus displacement and adhesions) are still obscure.

Synovial fluid occupies a key position in joint physiology. The term synovial, meaning 'like egg white' (Field & Harrison, 1957) was introduced by early wound surgeons, according to Paracelsus (1493-1541) because the fluid is clear and viscous. It lubricates the joint and also acts as a transport medium for the nutrients to the cartilage.

Aspiration and analysis of synovial fluid is routinely performed in other joints (Cohen, 1967). It is of considerable value in supplementing the clinical and serological investigations in distinguishing between inflammatory, non-inflammatory, septic and haemorrhagic lesions of the joints. Aspiration may also have a therapeutic effect. In some cases arthrocentesis is carried out and followed by instillation of medication. Research into physicochemical, immunologic and cytological aspects of synovial fluid has increased our understanding of the pathophysiologic mechanisms underlying various arthropathies. However, due to difficulties associated with the sample collection and problems associated with interpretation of the results, very few methodologically sound studies of TMJ are available. Toller (1961) was unable to aspirate any free fluid from the TMJ in more than 250 attempts on human subjects. In most normal joints the aspirable volume is very small in relation to the internal area. The normal human knee for example usually contains 0.5 ml aspiratable fluid. This volume if spread evenly throughout the joint, would have a thickness of only 24 µm (Levick, 1984). Furthermore, the aspiration is never total and has been estimated to constitute only 52% of the total fluid volume (Rekonen et al., 1973). Toller (1961) ‘guessed’ the total volume of TMJ synovial fluid to be no more than 50 µl. This small volume does not allow the use of established methods applied to larger joints for accurate estimation of the TMJ synovial fluid volume. These methods include the use of radio-isotope dilution (Rekonen et al., 1973: Ekman et al., 1981), non-isotope dilution using a contrast medium (Pereira et al., 1990) and wash-out technique (Geborek et al., 1988). If one considers the small amount of TMJ-synovial fluid (estimated to be 50 µl), any dilution of injected media by this fluid will be very low (approximately 4%). This is within the range of experimental error when using a radioisotope or contrast medium for larger joint volume measurement and therefore cannot be used accurately for TMJ synovial fluid volume determination.

The importance of estimation of the synovial fluid volume is that it provides an estimate of the joint space. One important parameter which may be central to any disturbance in movement is the volume of synovial fluid in the joint which is also an index of any pathological change in the joint space volume.

During the past decade saline aspirates of the upper joint space of TMJ have increasingly been analysed for the presence of various mediators of pathology (Kopp et al., 1983; Quinn & Bazan, 1990; Holmlund et al., 1991). However, caution is needed when interpreting the results. It is not known whether it is possible to obtain a representative sample of the
synovial fluid. The yield of aspirate is variable and it has not been possible to prove that there is a direct relationship between the volume of the aspirate and its synovial fluid content. Furthermore, contamination of the aspirate with blood is an important source of error in published results of TMJ synovial fluid analysis. When measuring any compound in the synovial fluid, correction should be made for its presence due to plasma or blood cell contamination, particularly if the sample is not centrifuged before being frozen. The lysis of blood cells at the thawing stage could possibly lead to a misleadingly high value of inflammatory mediators in the aspirate.

The aims of this study were two-fold. First, measuring the volume of TMJ synovial fluid, and second determination of its amount in saline aspirates. A marker was needed with the following properties:

1. It should be safe to administer to the patients
2. It should attain a measurable level shortly after administration
3. Low levels should be measurable in highly diluted saline aspirates
4. It should have equal concentrations in both plasma and synovial fluid.

We chose to administer 1.2 g of aspirin to patients prior to aspiration, and the haemoglobin and salicylate levels were estimated both in the plasma and the aspirate. By comparing the salicylate concentration in the aspirate with that of the plasma, it was possible to calculate the volume of synovial fluid. Furthermore, correction and absolute amounts of the mediators of pathology in the joint could be calculated. The technique neither involves radioactivity nor is dependent on the efficiency of the washout. From a clinical standpoint the administration of salicylate also has a therapeutic benefit by reducing postoperative pain and discomfort.

MATERIALS AND METHODS

Patients

Nine patients (age range 15–47 years, mean 28.6 ± 9.8; 6 males, 3 females) with painful temporomandibular joints unresponsive to 12 weeks medical therapy with a tricyclic antidepressant, underwent arthroscopy under general anaesthesia. None of these patients had any history of allergy to aspirin, gastrointestinal disorders or a bleeding tendency. 1-2 h prior to the aspiration, and the haemoglobin and salicylate levels were estimated both in the plasma and the synovial fluid and then it was aspirated. The yield of aspirate was variable ranging from 270 μl to 1050 μl. A blood sample was collected simultaneously. All samples were put into heparinized bottles. The needle was left in place on the symptomatic side to distend the joint space by injecting more saline in order to carry out the arthroscopic cannulation. Routine arthroscopic examination was carried out and pathological changes were assessed and photographed. 50 μl of the aspirate was used for haemoglobin determination and the rest was centrifuged (3000 rpm/5 min) to sediment any cells. The volume of the supernatant was measured and then aliquoted and stored at −70°C until the salicylate assay. Three samples with gross blood contamination (haemoglobin content of more than 10 mg/ml) were not analysed.

Biochemical analysis

a. Haemoglobin. Haemoglobin was measured by a commercial colorimetric assay (Sigma). To increase the sensitivity of the assay to allow measurement of samples with only slight blood contamination (down to 1 mg/ml) the manufacturer’s instruction was modified by increasing the volume of test fluid in the assay from 20 μl to 50 μl.

b. Salicylate. Salicylate measurement was by the high performance liquid chromatography (HPLC) method described by Grootveld & Halliwell (1986) with some modifications. Prior to the assay, the samples were extracted by the following procedure: 200 μl of sample (plasma or saline aspirate) were treated with 25 μl of 1 M-HCl and extracted with 10 ml of diethyl ether on a vortex mixer for 2 min. Pyrogallol (1,2,3-trihydrobenzene) which is present in diethyl ether as a stabilizer served as an internal standard. After separation, the ether layer was evaporated in a water bath at 40°C and the residue was dissolved in 225 μl of the mobile phase containing 5% (v/v) 1 M-HCl. Samples not analysed immediately were stored at −20°C until used. HPLC was carried on a SpectraPhysics chromatography system. The column was a Shandon 5 ODS reverse-phase (25 cm × 4.6 mm). The mobile phase was 30 mM sodium citrate/27.7 mM sodium acetate buffer (pH 4.75)-methanol (94:6) at a flow rate of 1 ml/min. The mobile phase was sparged continuously...
with He gas during elution. Detection was at 254 nm on a u/vis detector.

The injection loop (10 µl volume) was cleaned with at least 3 ml each of methanol, water and buffer after each injection, until injection of buffer did not produce any change in absorbance as a result of residues in the loop from the previous injection.

RESULTS

Out of 9 pairs of saline aspirates, 3 samples from the symptomless sides were excluded from the study because of heavy blood contamination. The Table compares the results between the symptomatic and symptomless joints. A paired t-test was used for statistical analysis of the results from 6 patients from whom bilateral samples were analysed. The results of 9 samples from the symptomatic side against 6 of the symptomless side were compared using an unpaired t-test (Table).

The yield of aspirate was variable ranging from 270 to 1050 µl (mean 840 µl). There was no significant difference between the symptomatic and symptomless sides. Blood contamination was unpredictable. Almost half of the samples showed blood contamination. The retention time of salicylate (20 min) relative to the internal standard (pyrogallol) was 2.5 (Fig. 1). The response of the detector was directly proportional to salicylate concentration in the range 1–800 pM. The extraction efficiency of salicylate was 75%. This was determined by adding standard salicylate to plasma samples from volunteers who had not taken aspirin and carrying them through the extraction procedure. Fig. 2 (A,B,C) shows chromatographs of plasma and joint aspirates from a patient. The sample from the symptomless joint shows a higher concentration which in this case was due to blood contamination. The salicylate concentration was determined by comparing its peak height to that of the standard taking into account the extraction efficiency factor. The ratio of the concentration of salicylate in the plasma sample to that in the saline aspirate.

Table - Comparison of the results between symptomatic and symptomless joints

<table>
<thead>
<tr>
<th></th>
<th>symptomless</th>
<th>(n = 6)</th>
<th>symptomless</th>
<th>(n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirate volume (µl)</td>
<td>865 ± 69</td>
<td>811 ± 303</td>
<td>824 ± 274</td>
<td></td>
</tr>
<tr>
<td>Blood contamination</td>
<td>33%</td>
<td>33%</td>
<td>44%</td>
<td></td>
</tr>
<tr>
<td>% of samples</td>
<td>0.58 ± 0.98</td>
<td>1.43 ± 2.99</td>
<td>1.32 ± 2.43</td>
<td></td>
</tr>
<tr>
<td>Salicylate conc. (µM)</td>
<td>8.57 ± 6.02</td>
<td>11.95 ± 10.53</td>
<td>15.3 ± 16</td>
<td></td>
</tr>
<tr>
<td>(4–19.9)</td>
<td>(3.8–29.95)</td>
<td>(0.4–50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dilution factor*</td>
<td>48.7 ± 21.4</td>
<td>49.3 ± 28.1</td>
<td>41.1 ± 30.35</td>
<td></td>
</tr>
<tr>
<td>SF volume (µl)</td>
<td>20.4 ± 5.5</td>
<td>36.1 ± 26.5</td>
<td>50.4 ± 56.2</td>
<td></td>
</tr>
<tr>
<td>(12.6–53.3)</td>
<td>(14.3–80.9)</td>
<td>(0.98–185)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The ratio of salicylate concentration in the plasma to that in the saline aspirate.
salicylate in the joint relative to that of the plasma (the dilution factor) was not significantly different between symptomatic and symptomless joints. In cases where there was blood contamination, corrections were made to exclude plasma salicylate from the calculation. The volume of synovial fluid was calculated by using a concentration-volume equation (Fig. 3), the mean value of which was found to be 37 µl and not significantly different between the symptomatic and symptomless joints. Using Pearson’s Correlation test, no significant correlation was demonstrable between the volume of the aspirate and the dilution factor (r = 0.44).

DISCUSSION

The yield of aspirate after injection of 1 ml of saline was variable, ranging from 270 to 1050 µl (mean 840 µl). The volume collected is to a large extent dependent on the operator’s technique. Correct positioning of the needle in the joint space is confirmed by the criteria mentioned in the aspiration technique. The mean volume of the aspirate collected in this study is much higher than that in other reports (Kopp et al., 1973) or our own earlier report (Aghabeigi et al., 1990). However, there are instances that despite the correct position of the needle as evidenced by the arthroscopy because of the presence of adhesions or meniscus perforation, the injected saline is unavailable for aspiration. Hence, it is not known whether the saline aspirate is a representative sample of the synovial fluid. Therefore in interpreting the saline aspirate analysis for various compounds, it is crucial to obtain a dilution factor which will allow for correction of the results. In this study there was no demonstrable correlation between the volume of the aspirate and its synovial fluid content as measured by the salicylate concentration. Some investigators report the results of joint assays per volume. This may not be accurate as it is possible to obtain a large amount of highly diluted aspirate which is trapped in a compartment formed by fibrous adhesions. Conversely, it is possible for the injected saline to equilibrate fully, but due to blockage of the needle during the aspiration, only a small volume of aspirate to be collected. Therefore, theoretically there is no direct relation between the volume of the aspirate and its synovial fluid content.

\[
V = \frac{K \cdot Ca}{Cp - Ca}
\]

\[
V = \text{volume of the synovial fluid in TMJ}
\]

\[
Cp = \text{concentration of salicylate in the plasma and synovial fluid}
\]

\[
K = \text{volume of the injected saline} = 1000 \mu l
\]

\[
Ca = \text{concentration of salicylate in the saline aspirate-adjusted when necessary for blood contamination}
\]

The expression of results per protein content of the aspirate is also not satisfactory in making comparisons between diseased and normal joints as there may be significant differences between the symptomatic and symptomless joints.

The simultaneous determination of salicylate concentrations in the plasma and joint washes provides therefore a solution to the problems outlined above. By using a concentration-volume equation, taking into account the volume of the injected saline and the ratio of salicylate concentration in the plasma and the saline aspirate, the volume of the synovial fluid can be calculated. The technique neither involves radioactivity nor is dependent on the efficiency of the washout. Furthermore, its administration has a therapeutic benefit by reducing postoperative pain and discomfort. However, it should be noted that this method is based on the assumption that the salicylate concentration in the synovial fluid is similar to those in plasma. A number of studies support this assumption (Rosenthal et al., 1964; Cieland et al., 1985; Groothedt & Halliwell, 1986). However, Sittar et al. (1985) found that although metabolites of salicylate equilibrate completely between synovial fluid and plasma, salicylate concentration in synovial fluid was approximately 80% of that of the plasma. It has been suggested that this may be secondary to lower albumin concentration in synovial fluid affecting the protein bound portion of the drug (Wallis & Simkin, 1983).

Studies with 14C-labelled acetyl salicylic acid in rabbits suggests that complete equilibration of salicylate between plasma and joint fluid is reached within 60 min (Paul & Routh, 1952). Our study of plasma salicylate level in a healthy volunteer showed that a steady state is reached 1 h after ingestion of a single dose (1.2 g) of aspirin which persisted for up to 3 h (Aghabeigi, unpublished data). This is in agreement with animal studies of salicylate concentration in plasma (Higgs et al., 1987).

Therefore, in order to avoid the pharmacokinetic lag period between the primary absorption into plasma and secondary absorption into synovial fluid we chose to perform the aspirations 1–2 h after ingestion of aspirin.

It is possible that the pathology itself may influence the salicylate level in the joint. Concentrations of the protein bound salicylate may be higher in the inflamed joint which may have a higher total protein and albumin concentration due to increased capillary permeability. Although a symptomless joint cannot be considered as normal, nevertheless, salicylate levels were comparable between symptomatic and symptomless joints. There was also no difference in total protein content between symptomatic and symptomless joints in our previous studies (unpublished data).

The knowledge of the pharmacokinetics of salicylates in synovial fluid is usually derived from serial observations in knee joint effusions of patients with rheumatoid arthritis (Sholhoff et al., 1967; Netter et al., 1989). These data might not be applicable to patients with facial arthromyalgia. The rheumatoid patients studied have usually been long-term recepi-
The film is of a uniform thickness (Levick, 1987) and animal TMJ. However, the dog knee has been estimated from 67 to 270 μm (Levick, 1984). It is unlikely that available relating to other human joints or of an (Davies, 1946). Unfortunately, there is no similar data joint. The TMJ internal surface area has not been form a layer only 121 μm thick. For the human knee synovial fluid per cm² surface. If the fluid were evenly this can be estimated to be 5.45-6.75 cm² given that measured accurately. Nevertheless, as the upper joint space can be distended passively with 1.2 ml of fluid, it is possible that complete mixing of injected saline and synovial fluid may not be achieved in all cases owing to presence of fibrous adhesions. This may result in underestimation of synovial fluid volume. This may explain the large standard deviations for salicylate concentrations and the corresponding synovial fluid volume in our study.

In this study, the average volume of synovial fluid was estimated to be 37 μl (0.98–135). It is possible that complete mixing of injected saline and synovial fluid may not be achieved in all cases owing to presence of fibrous adhesions. This may result in underestimation of synovial fluid volume. This may explain the large standard deviations for salicylate concentrations and the corresponding synovial fluid volume in our study.

Given that 37 μl is the volume of TMJ synovial fluid, as with most normal joints this volume is very small in relation to the internal surface area of the joint. The TMJ internal surface area has not been measured accurately. Nevertheless, as the upper joint space can be distended passively with 1.2 ml of fluid, this can be estimated to be 5.45–6.75 cm² given that 5.43 cm² is the surface area of a sphere with a volume of 1.2 ml and 6.73 cm³ is that of a cube with a similar volume. Therefore, there is approximately 6 μl of synovial fluid per cm² surface. If the fluid were evenly 'sandwiched' between the joint surfaces, it would form a layer only 121 μm thick. For the human knee the mean film thickness is calculated to be 24 μm (Davies, 1946). Unfortunately, there is no similar data available relating to other human joints or of an animal TMJ. However, the dog knee has been estimated to have a thicker film of synovial fluid ranging from 67 to 270 μm (Levick, 1984). It is unlikely that the film is of a uniform thickness (Levick, 1987) and the significance of different thicknesses of the synovial fluid in various joints and the relation between the volume of this fluid and joint function is not clear. An increased synovial fluid volume as a result of effusions or haemarthroses is a potential source of morbidity, giving rise to a sense of tension or even pain if formed rapidly (Jayson & Dixon, 1970a; Myers & Palmer, 1972). Impairment of synovial blood flow above a critical effusion pressure (Jayson & Dixon, 1970b; Lucht et al., 1983) and even direct mechanical limitation of movement (Nade & Newbould, 1984).

However, a clinically detectable effusion in the TMJ dysfunction is not a common finding. In all but one case, we have been unable to directly aspirate any fluid from the TMJ. The effusion occurred in a single symptomatic joint in which the aspirate volume was 50 μl more than the volume of injected saline, indicating the presence of a mild effusion into the joint. The patient had developed TMJ symptoms following a visit to his dentist which had persisted for 5 weeks before he underwent an arthroscopy which revealed severe hyperaemia of the retrodiscl tissues. On the other hand, a reduced volume of the synovial fluid may adversely affect its lubricating property and transport of the nutrients to the articular surfaces. Nevertheless, it has been shown that synovial fluid is capable of forming a permanent though extremely thin (10–50 nm) layer between two static surfaces, even under considerable loads, due to the voluminous trapped hyaluronate particles and os short-range electrical repulsive forces (Ogston & Stanier, 1953). In vivo somewhat thicker films might possibly be maintained between loaded surfaces by movement (Levick, 1987). For obvious ethical reasons, it has not been possible to obtain samples of the synovial fluid from normal TMJs and therefore the normal level of TMJ synovial fluid volume is not known for comparison relative to the values obtained from patients with TMJ pain. It should be noted that the symptomless joint cannot be regarded as a reliable control as it is functionally and anatomically related to the contralateral symptomatic side. Frequently, patients complain of bilateral symptoms either simultaneously or sequentially.

In paired samples the synovial fluid volume was not significantly different between symptomatic and symptomless joints. If the additional three symptomatic samples are taken into analysis, the symptomatic joints had larger volumes of synovial fluid which may be biologically important. However this was not statistically significant from the symptomless group using an unpaired t-test.

In summary, we describe a method for the measurement of TMJ synovial fluid volume and its concentration in the saline aspirates. The method would also allow the determination of the concentration and absolute amount of putative mediators of pathology in the joint. This is particularly important if any comparisons between the levels in various joints are to be meaningful. Although, the diagnosis of the common temporomandibular joint disorders are mainly made by clinical examination, research into underlying pathophysiology of this common problem, is essential to increase our understanding of 'internal
derangement in order to achieve consistently successful treatment.

Acknowledgements

The authors are very grateful to Gemma Harris for the mathematical help.

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Paper received 5 February 1992
Accepted 1 July 1992