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MOTOR NEURONE DISEASE TWIN STUDY USING

DEATH DISCORDANT TWINS

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ABSTRACT

Using a novel methodology termed the death discordant twin method, it has been possible to carry out an epidemiological study into the possible causes of sporadic MND. The study population was the largest twin sample so far collected worldwide for this rare disease, and identified 75 twin pairs - 24 monozygotic and 51 dizygotic. This involved a comprehensive and detailed search of the MND death certificate population for England and Wales between 1979 - 1989 inclusive.

The twin sample was utilised for two different purposes: 1) The estimation of the genetic contribution to sporadic MND; and 2) the formation of matched pairs for a case-control study of environmental factors. An extensive review of germane hypotheses and research was made and is reported with reference to relevant papers.

Following a critique of the methods and problems of many traditional twin studies, the advantages of this new method are discussed. The study results are analysed and detailed together with statistical evaluation, and the genetic contribution estimated.

Four monozygotic probands from two concordant pairs were identified, producing a MZ proband concordance rate of 17.4%. This was reduced to 10% when two probands were determined to have had familial MND. No dizygotic concordant pairs were found, but a "coefficient of genetic determination" ('G') between 0.38 - 0.85 was derived, using the methods of Falconer 1965 and Smith 1974. This supports a multifactorial aetiology for MND, probably involving several genetic factors, ie. a single gene defect is excluded.

The environmental risk factors were assessed using Odds Ratios (OR) with 95% Confidence Intervals (CI). The statistically significant factors which held true during conditional logistic regression modelling were 'regular vehicle maintenance' [OR = 7.0 (CI 1.3 - 89.9)], and 'occupational paint usage' [OR = 3.75 (CI 1.1 - 17.1)]. Other factors were of clinical interest. Many of the environmental factors identified in previous studies to be associated with increased risk for MND were not verified.

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ACKNOWLEDGMENTS

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SECTION   1

LITERATURE REVIEW
AND BACKGROUND

1.1 INTRODUCTION

Motor Neurone Disease aetiology remains enigmatical, despite being recognised as a distinct clinical syndrome since the descriptions of Aran 1850, and Charcot and Joffroy 1869. Research has increased greatly over the last 30 years, but so far no effective treatment has been developed.

1.2 CLASSIFICATION AND TERMINOLOGY

The term "motor neurone disease" unfortunately can be confusing and needs clarification. When used in its broadest sense, it identifies a variety of disorders, whose principal common feature is dysfunction of the anterior horn cells in the spinal cord (Williams & Windebank 1991). Motor neurone diseases can include acute poliomyelitis, the inherited spinal muscular atrophies, and corticospinal degeneration, but "motor neurone disease" (MND) is presently used in the British Isles to describe a particular condition involving progressive motor neurone degeneration, leading to muscular paralysis and eventual death. It appears to occur at random in the population, with an increasing frequency in older adults (Buckley et al 1983).

In North America, amyotrophic lateral sclerosis (ALS) has conventionally been used to distinguish the same pattern of motor neurone dysfunction. This is problematic, because ALS, (first coined by Charot 1874), is a specific pathological diagnosis, but is also used clinically to describe the form of MND presenting with combined upper and lower motor neurone dysfunction signs in either or both bulbar and spinal regions. Sometimes MND can present with lower motor neurone symptoms only, either in the bulbar region - Progressive Bulbar Palsy (PBP), or spinal region - Progressive Muscular Atrophy (PMA). Many consider that amyotrophic lateral sclerosis (ALS), progressive bulbar palsy (PBP), and progressive muscular atrophy (PMA) are clinical variants of the same disorder. Support for this is given by three indications: 1) when patients with PMA and PBP are clinically re-examined, signs of upper motor neurone deterioration are frequently revealed (Williams & Windebank 1991);
2) electrophysiological changes of PMA and ALS are very similar (Hansen & Ballentyne 1978);
3) at autopsy, cases with clinical PMA usually have upper motor neurone degeneration as well (Chou S-M 1979). However, there is controversy regarding whether a rare slowly progressing PMA be classified as Spinal Muscular Atrophy and not MND (WFN 1990).

Sometimes upper motor neurone degeneration symptoms of the spinal or bulbar regions predominate clinically in the early stages. This is known as Primary Lateral Sclerosis and Pseudobulbar Palsy respectively. These variants usually progress to include lower motor neurone signs. There is however, debate surrounding the existence of an exclusive upper motor neurone disease which progresses extremely slowly. It has been advocated that such cases are distinguishable and should be considered a separate disease (Pringle et al 1992).

The embracing definition of motor neurone disease can be seen in the International Classification of Disease (ICD) 9th edition, which encompasses under rubric 335.2 (headed MND), the three conditions - ALS, PBP, and PMA. It is therefore this interpretation of motor neurone disease that will be used for this thesis.

Motor neurone disease is generally classified into 3 groups in view of epidemiological, clinical and pathological findings. These are:-

1. Western Pacific ALS-parkinsonism-dementia complex.
2. Sporadic or Classical MND.
3. Familial MND.

The sporadic and familial forms of MND are the focus of this study, but some information about the Western Pacific MND complex is needed to understand fully the nature and complexity of MND, and research into its aetiology.

1.3 WESTERN PACIFIC ALS-PARKINSONISM-DEMENTIA COMPLEX

The Western Pacific form of MND is now regarded as a entity distinct from the sporadic/classical and familial forms (Kurland & Mulder 1988). It is found on the island of Guam in the Mariana Islands, with pockets of a very similar disorder located on the Kii Peninsula of Japan, and among the tribes of Western New Guinea. The type of ALS found among the Chamorro Indians on Guam appeared to be considerably more frequent than that occurring elsewhere. Annual incidence in 1955-59 was 55 per 100,000 of the population, nearly 300 times that expected in 1955 (Plato et al 1986), and at this stage the disease appeared clinically indistinguishable from classical MND. Kurland and Mulder 1954 also noted a striking family aggregation of ALS patients, with a third having affected family members. Early epidemiological studies found the male:female ratio of ALS to be 1.6:1 and mean age of onset around 45 years. Later it became evident that Guamanian ALS did not occur in isolation, but was associated with signs and symptoms of Parkinson's disease and dementia (Hirano et al 1961(a)). Patients symptoms ranged from exclusive ALS, via a combination, to exclusive Parkinson's disease and dementia (the so-called parkinsonism-dementia [P-D] complex) (Kurland 1988). The mean age onset of the P-D complex was 10 years older, and more frequent in males.

Hirano et al 1961(b) found widespread nerve cell loss, with neurofibrillary and granulovacuolar degeneration of the remainder, especially in the hippocampus, subcortical and brain stem regions. This degeneration correlated with the clinical features of Parkinsonism-

dementia, but the same changes were also detected in patients with ALS symptoms only. Later studies (Anderson 1979, Chen 1981) confirmed that neurofibrillary degeneration is common among Chamorros, and indicates a subclinical form of neurological disease in the population.

Since the aggregation of the disease in families did not seem to follow established patterns of Mendelian inheritance, attention was focused on possible environmental agents. Support for an indigenous environmental factor or factors, whose preponderance has diminished, was given by 1) the apparent long latency of the disease found among Chamorro immigrants, who only lived on Guam during early life (Garruto et al 1980); 2) for control and patient offspring, the increasing age of onset and equal risk of developing the disease (Plato et al 1986); 3) the considerable decline in the reported incidence of both ALS and P-D complex (Garruto et al 1985), which coincided with American influence on the island's economy and social structure.

Two main theories dominate recent thinking: 1) "Cycad circinalis" consumption, and
2) Abnormal metallic metabolism.

1) The consumption of the seeds from the Cycad circinalis or false sago palm became the main constituent of the Chamorro diet during times of privation, and subsequently a staple food source in poorer areas. It was also used for medicinal purposes. This occured in the southern area of Guam where MND/P-D complex was most frequently seen (Steele & Guzmen 1987). The seed was known to be poisonous and varying patterns of soaking to leach out the toxins were carried out by families before transforming them into a flour. Untreated seed kernels were also used as poultices. Thus varying unknown quantities of residual toxins were consumed or absorbed.

Two toxins of interest have been extracted from the cycad circinalis seeds. The most abundant is cycasin, a glycoside which has striking hepatotoxic and carcinogenic qualities, but its neurotoxic effect needs further investigation (Spencer et al 1990). The other, found in much lower concentrations, has proven neurotoxic effects with features similar to Guamanian MND/PD complex in cynomolgus monkeys (Spencer et al 1986(a)). It is a non-protein amino acid called beta-N-methylamino-L-alanine or L-BMAA. A predominantly upper motor neurone disease called Lathyrism is considered to be caused by a related plant non-protein amino acid called beta-N-oxalylamino-L-alanine or L-BOAA. L-BOAA is found in low concentrations in the Lathyrus sativus seed or chickling pea (a legume), and when excessive amounts of chickling peas are consumed Lathyrism develops (Spencer et al 1986(b)).

Spencer et al 1990 argues in favour of cycad being able to initiate a long-latency disease and that the degree of poisoning determines the age of onset and clinical characteristics of the

syndrome displayed; ie ALS symptoms appear in younger people who experienced heavy exposure, and P-D complex in older people who are less susceptible, or have lower exposure.

The hypothesis is criticised because the neurotoxic effects of L-BMAA have only been demonstrated in an acute experimental condition, using high doses of pure L-BMAA, many times greater than that found naturally in cycad flour. A quantitative study of the L-BMAA content of cycad flour found 90% - 99% had been removed by varying washing techniques (Duncan et al 1990). The amounts then eaten had been so small that a strong case against L-BMAA being the cause of Guamian MND was able to be constructed (Duncan et al 1990). So far any chronic low dose effects are unknown. Furthermore, anecdotal comment suggests no difference in current preparation and use of cycad products between families with and without ALS/P-D complex (Parker 1988, cited Steele 1990). Gajdusek (1990) also rejects this hypothesis because "delayed effects" require continuous subclinical progression of a degenerative process, or triggering of a predisposition already set in early life, and no known neurotoxin has yet been demonstrated to have this ability.

2) Garruto et al 1988 demonstrated that neurofibrillary tangles, from Guamanian ALS/P-D complex patients and unaffected subjects, contained enormously high levels of aluminium, calcium and silicon deposits. Based on these findings Gajdusek 1990 and Yase 1988 postulated that this results from compensatory hyperparathyroidism triggered by deficient environmental calcium and magnesium. Low concentrations of these elements, along with high concentrations of aluminium have been detected in local soil and water samples (Garruto et al 1984). Experimental models of metal-induced neurofibrillary degeneration support this hypothesis (Garruto 1988). The decline in the disease occurring after the introduction of imported food stuffs, and less dependence on indigenous products, would support this theory.

There is, however, conflicting evidence about the local metal concentrations in water and indigenous food plants. Other studies on Guam indicate an adequate calcium content in the traditional Chamorro diet, and that ingested aluminium is not excessive (McLachlan et al 1989, Zolan & Ellis-Neill 1986, cited in Steele 1990).

Both theories are complicated because the P-D complex variant of Guamian MND may not have decreased in prevalence as documented earlier (Kurland & Mulder 1988). It is yet to be seen if either of these hypotheses will aid investigation into the causation of Classical MND. The experiences of researching the Western Pacific MND complex demonstrates the difficulties of isolating the cause of a degenerative neurological disease, even when it is common in an isolated area.

1.4 SPORADIC / CLASSICAL AND FAMILIAL MND

(i) CLINICAL FEATURES

The most striking aspect of the clinical characteristics of MND, is variation between patients in expression and progression of the disease (Vejjajiva et al 1967, Gubbay et al 1985, Li et al 1990). The onset of symptoms is insidious. Asymmetrical limb weakness is more common than bulbar dysfunction initially, with the upper limbs most frequently affected, where the small muscles of the hand appear to be most vulnerable (Mulder 1984). Around a fifth of patients present with muscle weakness in the mouth and throat, resulting in difficultly speaking and swallowing (Vejjajiva et al 1967, Li et al 1990). Often the tongue is affected first producing slurring and dysarthria and making food manipulation difficult.

The pattern and rate of muscle deterioration is variable, the disease can remain localised to one small group of muscles for many months, even years. Alternatively, weakness can spread rapidly and soon affect the muscles necessary for breathing. Whether the initial degeneration is restricted to the bulbar or spinal regions of the central nervous system (CNS), the disease rarely stays exclusive and clinical features of motor neurone loss in both regions develop in most patients (Mackay 1963). A reversible syndrome with symptoms resembling ALS has been reported (Tucker et al 1991), but how it relates to MND is unknown. Associated with weakness is muscular atrophy and fasiculations which becomes more pronounced as the weakness increases. Muscle cramps, fatigue, paresthesias, and even pain are reported in the early stages of MND.

Involvement of the pyramidal system produces upper motor neurone signs such as weakness, spasticity, hyperreflexia, snout reflexes or Babinski reflexes. If the pyramidal tracts are affected at the brain stem or above, a pseudobulbar palsy occurs with hyperactive jaw jerk, a sucking reflex and emotional lability (Mulder 1984).

Variation in disease duration implies the rate of anterior horn cell degeneration varies considerably between patients. Munsat et al 1988 assessed the pattern of motor neurone loss in 50 patients with ALS, and found the rate of motor loss was symmetric and linear during the symptomatic phase of the disease, with bulbar functioning deteriorating the slowest. The progress of the disease varied greatly between patients, but it did not correlate with age of onset and region first affected. Previously these variables had been associated with poorer prognosis (Kondo & Hemmi 1984).

Motor neurone disease can affect all striated muscle function, except that the extraocular and sphincter muscles are usually spared. If life is artificially prolonged, oculomotor neurone

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abnormalities eventually occur (Mizutani et al 1990). A few patients experience incontinence (Gubbay et al 1985), but whether this is caused by the disease process is unknown. Autonomic function is preserved even in late stages of the disease.

Controversy surrounds the significance of sensory abnormalities to MND. Some patients report subjective sensory symptoms, but eliciting objective clinical signs is often impossible (Mulder 1984). However, sophisticated measurement techniques will provide evidence of mild peripheral sensory neurone involvement (Dyck et al 1975). Consensus opinion favours great caution in diagnosing MND if clinical objective sensory signs are detectable (WFN 1990).

The association of MND with Parkinson's or Alzheimer's disease is contentious (Calne & Eisen 1989, Hudson 1981). A positive correlation would link sporadic and familial MND with the Western Pacific variety, but evidence gathered only supports coincidental association (Leone et al 1987, Mulder et al 1986).

Diagnosis: There are no specific tests for the disease and the clinical features are varied. Diagnosis is achieved by a process of elimination using a battery of tests and comparing the results with positive clinical findings. Li et al 1991 found neurologists appear to agree about the common clinical features of the disease, but vary in their assessment of the likelihood of specified cases being MND. In an attempt to unify the diagnosis of MND the World Federation of Neurology has drawn up a statement of criteria for diagnosis (WFN 1990).

The most useful laboratory examinations are electromyography and nerve conduction studies, along with muscle biopsy in the early stages, because they can demonstrate the neurogenic nature and distribution of the disease before it is clinically apparent (Kennedy 1980). Four stages of MND have been defined on the basis of clinical and electromyographic criteria (Swash & Schwartz 1984). Magnetic Resonance Imaging may show abnormalities in the internal capsule, due to corticospinal tract degeneration (Sales Luis et al 1990, Iwaski et al 1992).

The skin has been advocated as a potential diagnostic element (Ono et al 1989). It shows signs of premature ageing, with collagen bundles in the dermis becoming less numerous, thinner and more loosely woven compared to patients with spinal muscular atrophy. Widespread usefulness of these observations as a diagnostic tool are yet to be seen.

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(ii) PATHOLOGY

Central Nervous System: Macroscopically, the prominent changes are atrophy of the spinal cord and adjacent ventral roots with gliosis of the lateral columns. At cellular level, loss and degeneration of anterior horn cells is the primary feature, which is often asymmetrical (Brownell at al 1970), at least in the early stages. A great reduction in spinal motor neuron numbers, with shrinkage of those remaining has been demonstrated in quantitative studies (Kiernan and Hudson 1991). The diminishing number of large lumber motor neurones have been shown to correlate with increasing weakness and atrophy in corresponding muscles (Sobue et al 1983).

The degenerative process involves shrinkage and intracytoplasmic lipofusion accumulations leading to cell loss with astrocytic gliosis (Tandan & Bradley 1985(a)). Intracytoplasmic inclusions (Bunina bodies) and ghost cells are commonly seen in the affected anterior horn cells, but vacuolation, central chromatolysis, spheroids and neuronophagia are more rare. Their appearance probably varies with the stage of the disease and their relationship to the disease process is obscure (Hirano 1982). Many cytoskeletal inclusions are bound to ubiquitin, a 'heat-shock' protein activated in cells undergoing stress (Lowe et al 1991, Schiffer et al 1991, Leigh et al 1988).

Upper motor neurone signs are associated with loss of Betz's cells and degeneration of the pyramidal (corticospinal) tracts (Hughes 1982). The destroyed cells are replaced by astrocytic gliosis.   Pyramidal tract degeneration continues in the brain stem with depleted and disintegrating motor nuclei of the cranial nerves. Pathological changes similar to the spinal cord are found in these motor cells.

No lesions are generally located in the posterior columns (Iwata and Hirano 1979), though degeneration of the spinocerebellar tracts and Clarke's columns is recognised (Bownell et al 1970, Averback & Crocker 1982). In familial MND, involvement of the sensory pathways appears more frequent (Engel et al 1959, Emery & Holloway 1982). Inclusions, such as Lewy bodies , Alzheimer type neurofibrillary tangles, and those seen in Pick's disease have been reported (Tandan & Bradley 1985(a)), but in isolated cases, and could be coincidental.

Peripheral Nervous System: It has been confirmed that large myelinated fibres are lost from ventral spinal roots in MND (Sobue et al 1981), and from cranial hypoglossal and phrenic nerves (Atsumi & Miyatake 1987, Bradley et al 1983). The thoracic and sacral ventral spinal roots are relatively spared. (Sobue et al 1981). The pattern of degeneration is compatible with a neuronopathy, rather than an axonopathy or 'dying back' process (Tandan & Bradley 1985(a)). Small-myelinated fibres are well-preserved (Sobue et al 1981) and in the phrenic

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nerve actually increased in number (Bradley et al 1983). This may relate to smaller regenerating fibres. (Tandan & Bradley 1985(a)).

Neuropathologic involvement of peripheral sensory neurones has also been established in some cases of MND (Dyck et al 1975), despite the general lack of clinical objective evidence. A considerable reduction in total myelinated fibre number in sural and peroneal nerve has been found, with evidence of possible degeneration of unmyelinated fibres as well (Bradley et al 1983).

1.5 EPIDEMIOLOGY OF MND

Most studies have been carried out in North America and Europe, so their findings may not reflect the patterns elsewhere. Bobowick and Brody (1973) concluded that MND distribution around the world is uniform, partly because of similar mortality rates for ALS among native born and migrant populations in the United States of America (Edgar et al 1973). This conclusion has recently been challenged (Lancet editorial 1990(a), Chancellor & Warlow 1992), in view of better morbidity studies. Patterns of disease can only be determined in areas where health care is comprehensive and accessible to the whole population, and accurate records are kept. This does not occur in many countries and the true pattern of MND in these populations is unknown, though MND does occur (Srinivas 1992, Osuntokun 1971).

(i) MORBIDITY DATA   (Table 1 refers)

Morbidity studies are appropriate for investigating disease patterns, provided the sample collection is rigorous, and patients are still living so that diagnosis can be confirmed. If a disease is terminal but variable in length, cases of short duration may not be identified because of intervening death and therefore be under-represented. The way to overcome this is to include deceased patients, but then the information available is limited to that recorded in medical notes, or by interviewing relatives. Unfortunately, the methodology used in morbidity studies is very variable. This is certainly so for MND, and though some investigators attempted to ensure the sample is complete, not all explored every possible avenue for case ascertainment (The Scottish MND Research Group 1992, Forsgren et al 1983, Tysnes & Aarli 1991, Granieri et al 1988, Chazot et al 1986, Rosati et al 1977, Kahana & Zilber 1984, Yoshida et al 1986, Hudson et al 1986, Annegars et al 1991, - order as shown in table 1). However, the more thorough the sample trawl, the more expensive and time consuming is the exercise. Many other studies have limited the sample collection to a centre's records and then inappropriately taken it to be representative of a regional population (Serradell & Calvet 1991, Scarpa et al 1988, Breland & Currier 1967, Olivares et al 1972).

Survey Area	Population Base	Numb. Cases	Time Period	Avg. Incidence Rates (Crude) 95% C.I.	Age-Adjusted I.R. (for sexes)	Change in Incidence	Prevalence Rate with 95% C.I.
EUROPE Scotland	5,090,700	114-MND	1989	2.24 (1.85-2.65)	—	—	Not Calcuable
Northern Sweden	646,189	128 - MND	1969 to 1980	1.65 (1.4 - 2.0)	All - 1.67 Male - 1.73 Female - 1.61	Decreased 1.86: 1969-72 1.35: 1977-80	4.80 (not given)
Hordaland County Western Norway	407,926	70 - ALS	1978 to 1988 incl.	1.56 (not given)	All - 1.60 M:- 1.78 / F:- 1.41	Increased over-all but variable	All - 3.67 M:-4.94 / F:-2.43
Ferrara Province Italy	398,405 to 381,118	72 - MND	1964 to 1982 incl.	0.98 (0.8-1.2)	All:- 0.82 M:- 1.2 / F:- 0.7	No Temporal Trend observed	3.93 (2.2-6.5)
Limoges Region France	738,000	54 - ALS	1977 to 1985	0.91 (not given)	—	Increased 0.81: 1977-79 1.05: 1980-84	7.31 (not given)
Sardinia Italy	1,490,000	96 - ALS	1965 to 1974 incl.	0.6 (0.5 - 0.7)			1.5 (not given)
MIDDLE EAST Israel	21,800,000	246 - MND	1959 to 1974 incl.	0.66 (0.6 - 0.8)	0.75 (not given)	Increased 0.61: 1959-63 0.86: 1969-74	Information not given
NORTH AMERICA Rochester Minnesota	16,327 to 58,629	44 - MND	1925 to 1984 incl.	2.0 (not given) Men 2.2 / Women 1.8	All:- 2.4 M:- 3.0 / F:- 2.0	Increased 1.2: 1925-54 2.4: 1970-84	Information not given
Southwestern Ontario Canada	1,715,200	139 - MND	1978 to 1982 incl.	1.63 (not given) Men 1.79 / Women 1.45	M:-2.6 (not given) F:-2.0 (not given)	No Temporal Trend observed	4.90 (not given)
Harris County Texas	2,937,875	97 - ALS	1985 to 1988	1.10 (not given)	A:-1.14 (0.93-1.40) M:-1.27 (0.95-1.69) F:-1.03 (0.75-1.38)	Information not given	A:-3.04 (2.39-3.86) M:-3.33 (2.37-4.53) F:-2.74 (1.86-3.86)

Table 1:   Incidence Rates per 100,000 Population and Point Prevalence Rates per 100,000 Population for Motor Neurone Disease in Different Parts of the World.

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(ii) MORTALITY DATA (Table 2 refers)

The main weakness of mortality data is accuracy of death certificate diagnosis. This depends on the knowledge and thoroughness of the certifying doctor. Understandably, there is need for caution in drawing conclusions about disease patterns in living people from mortality data. Fatal cases may represent a biased selection, because death may arise in some cases from other causes, and the underlying illness ignored at certification (Kurtzke & Lux 1985).

Obviously, if a disease process itself directly causes death, for example myocardial infarction or acute renal failure, then it is highly probable that it will be listed as an underlying cause. However, in the case of many chronic disorders, for example dementia (Martyn & Pippard 1988), no mention of the disease may appear on the death certificate, even as a contributory factor.

Mortality statistics for multiple sclerosis, have been shown to be just as useful for studying epidemiological aspects as morbidity data (Kurtzke & Lux 1985). They suggest that mortality data is worthy of consideration for epidemiological research, especially as it is a near complete and readily available source of information.

MND being a terminal and generally short-term disorder means that mortality data should reflect its real incidence. This has been demonstrated by retrospective validation, with MND being noted on death certificates of known patients in 72% - 91% of cases (Buckley et al 1983, O'Malley et al 1987, Hoffman & Brody 1970, Juergens et al 1980) There is support for the use of mortality data in rare diseases, because morbidity studies are not feasible, or their accuracy questionable because the resources available are limited (Durrleman & Alpérovitch 1989).

Rise in MND Incidence: It is logical that there should be a rise in the diagnoses of MND because of increasing numbers of neurologists and geriatricians and better awareness of the disease by other physicians (Hawkes et al 1992). This phenomenon is reflected in the incidence and mortality statistics (table 1 and 2). There is much argument about whether the MND increase is due entirely to better diagnosis and medical care, or represents a true increase. All the identified mortality studies in table 2 show large increases in the death rates from MND over the last 30 years (Durrleman & Alpérovitch 1989, Buckley et al 1983, Hawkes et al 1992, Holloway & Emery 1982, Gunnarsson et al 1990, Flaten 1989, Lilienfeld et al 1989). Part of the increase in all these studies will be due to changes in the International Classification of Diseases coding for MND over this period, but rises in mortality also occurred in these studies during the years when the classification did not change (Durrleman & Alpérvitch 1989, Buckley et al 1983, Hawkes et al 1992).

Survey Area	Population used for age adjustment	Time Period	Numb. Cases	Sex Ratio M:F	Average Mortality Rate (age adjusted) 95% C.I.	Change in Mortality (age adjusted)	Pattern of Mortality and Age Group of Peak Mortality
EUROPE France	France 1982	1968 to 1982 incl.	8,962	1.60:1	All:- 1.15 (1.12-1.18) Men:- 1.45 (1.41-1.49) Wom:- 0.90 (0.87-0.93)	Increased overall:- 0.84: 1968-71 - 1.46: 1979-82 M:- 1.11 (1.04-1.18) - 1.92 (1.83-2.01) W:- 0.63 (0.59-0.68) - 1.12 (1.06-1.18)	Rates increase with age Peak:- 65-74 yrs M.R.- M: 7.27 (6.94-7.60) M.R.- W: 4.64 (4.44-4.89)
England and Wales	England & Wales 1971	1959 to 1979 incl.	Not given	1.60:1	Not given	Increased overall:- 1.2: 1959-61 - 1.6: 1977-79	Rates increase with age Peak:- M:70-74yrs = 7.00 Peak:- W:65-69yrs = 4.50
England and Wales	England & Wales 1981	1979 to 1989 incl.	10,654	1.18:1	All:- 1.94 (1.80-2.08) Men:- 2.15 (2.00-2.30) Wom:- 1.74 (1.59-1.89)	Increased overall:- 1.68: (1979-81) - 2.18: 1987-89 M:- 1.90 (1.68-2.12) - 2.40 (2.34-2.46) W:- 1.47 (1.39-1.54) - 1.98 (1.95-2.01)	Rates increase with age Peak:- M:75-84/ W:70-79yrs M.R.-M: 12.46 (11.65-13.27) M.R.-W: 7.43 (6.80-8.06)
Scotland	Scotland 1968	1968 to 1977 incl.	725	1.22:1	Men:- 1.57 (not given) Wom:- 1.18 (not given)	Increased overall:- Men:- 1.45:1968 - 1.77:1977 Women:- 1.07:1968 - 1.69:1977	Information not given
Sweden	Sweden 1970	1961 to 1985 incl.	3,871	1.20:1	All :- 1.90 (not given)	Increased overall:- 1.4: 1961-69 - 2.3: 1978-85	Rates increase with age Peak:- 70-79yrs Risen from 4.5:'61-65 - 11.3 '81-85
Norway	Norway 1980	1969 to 1985 incl.	1,225	1.24:1	Men:- 2.23 (not given) Wom:- 1.49 (not given)	Increased overall:- Men:- 1.65: 1970 - 2.81: 1985 Women:- 1.17: 1970 - 1.81: 1985	Rates increase with age Peak:- 70-79yrs Risen by 95% for men, 45% for women
NORTH AMERICA United States of America	U.S.A. 1970 & 1980	1962 to 1984 incl.	47,332	1.21:1	Not given	Increased - both white/non-white races W.M:- 1.67 (1962-64) - 3.00 (1980-84) W.W:- 0.94 (1962-64) - 2.06 (1980-84) N.M:- 0.83 (1962-64) - 1.50 (1980-84) Figures for Non-white women unreliable	Rates increase with age Peak: (1962-64)- W.M.& W.W: 65-74ys. N.M.& N.W: 55-64ys Peak: (1980-84)- W.M.& W.W: 70-79ys. N.M.& N.W: 65-74ys

Table 2:   Mortality Rates per 100,000 Population for Motor Neurone Disease in Different Parts of the World.

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The greatest increase in the Standard Mortality Ratios (SMR) are seen in the older age groups (Buckley et al 1983, Durrleman & Alpérovitch 1989, Lilienfeld et al 1989, Flaten 1989, Gunnarsson et al 1990, Hawkes et al 1992). The increase in MND diagnosis in the very old (> 70 years) could easily be explained by improved diagnosis, but in the younger age groups where mortality rates have risen this is not such a satisfactory answer. Lilienfeld et al 1989 strongly advocate that the increase is real because it is seen in most age cohorts, during a time when there have been no radical changes in treatment and diagnosis of MND that would affect ascertainment. Over the period of their study, 1962-84 however, the number of neurologists in the USA increased ten-fold, therefore, the reality of the increase is questioned (Swash et al 1989). Support for the view that at least part of the increase reflects a true rise in MND incidence is indicated by the authors of other mortality studies quoted in table 2, and in a recent review (Chancellor & Warlow 1992). In addition, a study tracing mortality patterns in successive birth cohorts, detected small rises in age-specific mortality rates for both sexes over 65 years old and born after 1900 (Li et al 1985). As these trends did not correspond with changes in ICD coding, and were different for males and females, the authors suggest the rises are unlikely to be artifacts of coding or different diagnostic practises over the years. The only reports of decreased mortality have been from Japan (Kondo & Minowa 1988).

Referring to table 1, it can be seen that the increased SMR with time is not universally supported by the incidence rates quoted. This is probably because the time period over which several studies were conducted was too short (< 6 years), for detecting any similar change in incidence. The studies covering longer time periods however, reported increased incidence rates. In the two taken over 10 years the increase was small, but in Rochester Minnesota where great care was taken to clearly define the MND sample and its reference population, the incidence doubled over the 50 years covered. The decreased incidence reported from the study in Northern Sweden is puzzling as mortality rates in the whole country have increased.

Caution must be taken when comparing incidence and mortality rates across the studies because of the different populations and methodologies utilised. Nonetheless, there does appear to be reasonable homogeneity, with relatively small variations in the incidence and mortality rates quoted (0.6-2.0 x 10^{-5} incidence and 1.5-2.5 x 10^{-5} mortality). Chancellor and Warlow 1992 propose the dissimilarity is great enough between the more complete studies, to reflect a non random distribution of MND. The differences in incidence rates appear to possibly follow a latitudinal gradient (Annegers et al 1991, Chancellor & Warlow 1992), because across North America and Europe the incidence rate is higher in the northern countries/states compared to the southern. The fact that MND is rare, implies that methodological variation is a more likely explanation. As MND is a terminal condition it would be expected that the mortality and incidence rates would be similar. The slightly higher mortality rates overall either suggests: 1) greater ascertainment of cases in mortality studies

(Durrleman & Alpérovitch 1989); 2) a certain percentage of MND sufferers are only diagnosed shortly before death; or 3) there are more false positive MND diagnoses at death certification.

Racial Distribution: Any racial variability in susceptibility to MND is impossible to evaluate, via epidemiological studies at present. In Texas USA similar incidence rates to the white population were found in Hispanic and Black peoples (Annegers et al 1991). Yet smaller mortality rates are recorded for the non-white population in the USA (Lilienfeld et al 1989). In Britain also, smaller incidence rates have been calculated for Asian and West Indian immigrants compared to the indigenous population (Elian & Dean 1990). The factors affecting these figures are so complex, that it is impossible to draw any conclusions from them.

Sex Variation: (see tables 1 & 3) Both the mortality and morbidity studies confirm male preponderance in MND. The male:female ratio ranges from 1.6:1 to 1.1:1, with a possible slight convergence of the sexes in the more recent time periods. This trend could either be due to diagnostic factors, particularly in elderly ladies, or a genuine increase in female MND.

Age of Onset: The mean or median age of MND onset has risen from around 50-55 to 60-65 years over the last 30 years. This reflects the greater incidence in older age cohorts, whereas in the young (30-50 years old) incidence appears more stable (Durrleman & Alpérovitch 1989, Buckley et al 1983), or even slightly reduced (Hawkes et al 1992).

Survival: (see table 3) Average or median duration of survival is around 30 months, but figures on the percentage of sufferers living over 5 years vary from 0% to 40%. Analysis of the survival patterns for the different forms of MND is also vague. From the data presented, the mean age of onset with ALS is slightly lower than PBP, but a much smaller percentage of PBP patients survive for 5 years. In a Danish study of survival, prognosis of patients over 60 at diagnosis was significantly worse than for younger patients (Christensen et al 1990).

If the trends seen in the incidence and mortality rates of MND are genuine and not just due to methodological vagaries, then this pattern reflects the changing influence of some unknown endogenous or exogenous risk factors. If so, the hypothesis that environmental factors play a big role in sporadic MND aetiology would be supported.

(iii) GEOGRAPHICAL CLUSTERS

Apart from the high-incidence foci of MND discovered on Guam and the Ki peninsula, the distribution of MND in the western world is fairly even. The conclusion that Western Pacific

Survey Area	Time Period	No. of Cases	Sex Ratio M:F	Overall Median or Mean Age & Range of Onset	Peak Age Group for Onset	Median or Mean Survial % > 5 yr	Median or Mean Age & Survival for A.L.S.	Median or Mean Age & Survival for P.M.A.	Median or Mean Age & Survival for P.B.P.
Scotland	1989	114-MND	1.2:1	Not given	Men 65-74 yrs Wom.75-84 yrs	Not given	Not given	Not given	Not given
Northern Sweden	1969 to 1980 incl.	128-MND	1.1:1	Median age 61.0 yrs (20 - 82)	Not given	Median 34 mths (not given)	M.59ys (20-82) 32 mths	M.60ys (22-80) 70 mths	M.65ys (46-75) 30 mths
Hordaland County Western Norway	1979 to 1988 incl.	70 - ALS & PBP	1.3:1	Mean age 61.2 yrs (34 - 82)	Not given	Mean 30 mths > 5yrs 25.0%	A.59.9ys SD 8.8 37 mths > 5yrs 42.3%	Not given	A.62.8ys SD 10.8 22 mths > 5yrs 7.6%
Catalonia Spain	1984 to 1989 incl.	70 - MND	1.2:1	Mean age 54.6 yrs (S.D. 12.5)	All 65-74 yrs	Mean 30 mths > 5yrs 0%	Not given	Not given	Not given
Ferrara Province Italy	1964 to 1982 incl.	72 - MND	1.6:1	Mean age 59.4 yrs (30 - 76)	Men 60-69 yrs Wom.50-59 yrs	Not given > 5yrs 39.8%	Median 50-59ys > 5yrs 36.8%	Median 60-69ys > 5yrs 63.7%	Median 50-69ys > 5yrs 6.7%
Limoges Area France	1977 to 1985	54-ALS	1.2:1	Not applicable	Not applicable	Not applicable	Median 65.0yrs 20.7 mths	Not applicable	Not applicable
Modena Province Italy	1976 to 1986 incl.	51 - ALS & PBP	2.9:1	Mean age 61.43 yrs (35 - 81)	Men 60-69 yrs Wom.50-59 yrs	Mean 28.85 mths Med. 24.5 mths > 5yrs 24.4%	Mean 59.27yrs (SD 8.94) A.30/M.22 mths	Not applicable	Mean 63.38 yrs (SD 10.44) A.21.5/M.20.5m
Rochester Minnestota	1925 to 1984 incl.	44 - MND	1.5:1	Median age 67.5 yrs (48 - 88)	All > 65 yrs	Median 23.8 mths > 2yrs = 48% > 5yrs = 14%	No difference to overall figures	No difference to overall figures	No difference to overall figures
Southwestern Ontario Canada	1978 to 1982 incl.	139 - MND	1.2:1	Mean age Men 62.4 yrs (29-86) Wom.62.7 yrs (21-89)	Men 70-79 yrs Wom.60-69 yrs	Mean in mths M:33.6 (SD 28.8) W:24.0 (SD 15.6)	Not given	Not given	Not given
Harris Co.Texas	1985-88 inc	97-ALS	1.1:1	Not applicable	M.55-64 W.65-74	Not applicable	Not given	Not applicable	Not applicable
Cen.Mississippi	1959-64 inc	45-UK.	4.0:1	Mean 54.0 yrs (25-76)	Not given	Not given	Not given	Not given	Not given
Mexico City	1962-69 inc	16-MND	1:2.2	Mean 50.6 yrs (33-70)	Not given	Not given	Not given	Not given	Not given
Israel	1959 to 1970 incl.	318-MND	1.7:1	Mean age 56.6 yrs (20 - 88)	All 60-64 yrs	Median 36.0 mths > 5yrs = 29%	Not given	Not given	Not given
Japan	1958 to 1966 incl.	515-MND	1.4:1	Mean age 50.8 yrs (9 - 78)	All 55-59 yrs	Not given	Mean 41.0 mths Rang.4-464 mths	Mean 57.0 mths Rang.3-389 mth	Too few cases

Table 3:   The Characteristics of Motor Neurone Disease Within Different Population.

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MND must be linked to some local environmental factors (Garruto 1980), makes identification of MND clusters in other countries appealing, but of questionable significance (editorial Lancet 1990(b)). Even though disease clusters can be epidemiologically valuable (Taylor & Davis 1989), as illustrated by the identification of AIDS, they can also be very misleading (editorial Lancet 1990(b)). The problems associated with determining whether a cluster is epidemiologically significant, has resulted in a modified approach to cluster investigation of neurologic disease being proposed (Armon et al 1991(a)). The probability that chance alone may account for the findings is calculated before fieldwork is instigated. This calculation should also take into account similar communities that have not reported clusters. The traditional method only involves the study community. In this way the threshold for significance is raised by a factor of 1.3 to 2.0, and by using this technique, effort is hopefully only put into researching genuine disease clusters, without reducing the sensitivity too greatly (Armon et al 1991(a)).

Most reported geographical clusterings have been based on anecdotal evidence, for example MND diagnosed in 3 people linked by residence (Melmed & Krieger 1982) and location of occupation (Kilnes & Hochberg 1977). These observations however, could just reflect random groupings. Population-based studies looking for geographical distribution are more objective and these have been carried out in various parts of Europe and North America.

Mortality data from England and Wales revealed MND clusters occur in southern and western counties, but the variation was not statistically significant (Buckley et al 1983). In a study of south-western Ontario Canada, no significant geographical variation in MND incidence was identified (Hudson et al 1986).

In Sweden during a study of mortality data, a substantially larger mortality ratio was found among males in one county. In a later study it was discovered that a much higher proportion of males from that county, who died from MND, had worked in agriculture, compared to the county male population as a whole (Gunnarsson et al 1992).

A geographical study of ALS in a region of North-west England showed that ALS incidence can be significantly raised in small areas, even if the numbers in such areas are no greater than would be expected on a chance basis (Mitchell et al 1990). The small numbers involved caused this observation to be spurious, since the clusters appeared to move around the region depending on how the geographical boundaries were set and age group considered.

Collection of mortality data for a ten-year period (1973-82) in Wisconsin USA, analysed using computer simulation, revealed a significantly non-homogenous distribution of MND across the state (Taylor & Davis 1989), with a cluster detected in three adjacent counties situated in

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the northeast. The authors concluded that cluster identification could prove more successful than traditional methods in identifying populations for case-control studies. Unfortunately, such studies (Sienko et al 1990) are of limited value because small sample size prevents comparison or generalisation.

1.6   EXOGENOUS NEUROTOXIC FACTORS

(i)   VIRUSES

There is little evidence to support a viral cause (Williams & Windebank 1991). Attempts to demonstrate virus material in motor neurones have met with no success. There is no difference in frequency with which viral nucleic acid sequences are seen in patients and controls (Brahic et al 1985, Weiner et al 1980). Likewise, viral antibodies in cerebrospinal fluid of patients with MND are normal (Kurent et al 1979), and antibody titres are similar to controls for a selection of viruses (Kascsak et al 1978, Cremer et al 1973). This does not rule out the possibility that MND is a consequence of a previous infection, and there is still much interest in this area in relation to specific infectious agents (Maurizi 1987).

Poliovirus: MND was first suggested to be the delayed consequence of acute poliomyelitis by Zilkha 1962, though this idea has been widely debated. More recently it has been proposed that MND results from a latent poliovirus infection that affects the CNS, but does not cause motor symptoms during the acute illness. Only later after loss of further motor neurons, perhaps through ageing, do symptoms become apparent (Martyn et al 1988). Survivors of paralytic poliomyelitis can experience new symptoms of muscle pain, fatigue and weakness after a prolonged period of stability (Windebank et al 1991), but it is uncertain if this truly represents MND or simply the effects of nerve problems due to skeletal deformity.

An interesting epidemiological approach revealed that recent geographical mortality patterns of MND (1968-78), in 55-74 year olds in England and Wales, were moderately correlated with the distribution of polio notifications forty years earlier, along with social class (Martyn et al 1988). However, this observation was unconfirmed by a similar study in Scotland (Swingler et al 1992). There have been case reports of people developing MND in late adult life who previously suffered poliomyelitis in childhood (Roos et al 1980, Moriwaka et al 1991), but no serological evidence of present infection or poliovirus in the CNS were detected at autopsy, and the relationship could just be coincidental. Two case-control studies reported a higher incidence of polio infection in MND patients compared to controls (Pierce-Ruhland & Patten 1981, Chio et al 1991), the difference being statistically significant in one study. However, this has not be substantiated in other similar studies (Deapen & Henderson 1986, Provinciali & Giovagnoli 1990, den Hartog Jager et al 1987).

Prions: Some neurodegenerative dementing diseases, Creutzfeldt- Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, and Kuru can be experimentally transmitted to animals (Prusiner 1987). These conditions share many features with sheep and goat scrapie, bovine spongiform encephalopathy and other related animal diseases. All primarily affect the CNS, have prolonged incubation periods and are fatal (Griffin 1985). The causative agents are suggested to be unconventional proteinaceous infectious particles (prions). Unlike viruses, nucleic acids have not been detectable in the scrapie agent (Prusiner 1987). The similarity between scrapie brain inclusions and the amyloid plaques of Alzheimer disease (AD), implies that a prion-like agent may trigger this, and possibly other neurodegenerative disorders. However, at present no positive identification of prion protein has been made in brain tissue from AD patients (Roberts et al 1986).

Human Immunodeficiency Virus (HIV): In a review, Rowland 1987 discussed several clinical reports of motor neurone involvement in patients suffering from auto-immune deficiency syndrome (AIDS). He concluded that HIV may cause motor neurone type disorders, but it is debatable whether this can be equated with MND. Certainly the pathological changes to the nervous system in patients with AIDS and neuromuscular features are not typical of MND (Verma et al 1990).

Exposure to Animals and Animal Carcases: Connected with viral exposure is contact with animals, which could be potential sources of contamination. In epidemiological case-control studies, patients with Western Pacific MND reported increased exposure to farm animals (Reed & Brody 1975). Contact with animal carcases and hides was more frequent in one group of ALS patients (Hanisch et al 1976), as was living with domestic pets, particularly small dogs, in another (Tarras et al 1985). Conversely, in two other case-control studies no excess of animal contact was found among patients (Mulder et al 1983, Norris & Padia 1989). Thus any contamination from this source looks very questionable.

(ii)   METALLIC TOXINS

Maintenance of normal tissue levels is essential for many metabolic processes. The precise role of heavy metals in MND is controversial because they are present as trace elements in several tissues, but their physiological role is often unclear.

Both inorganic and organic compounds of a number of metals, particularly those of heavier atomic weight, are known to produce peripheral nerve disease in humans (Windebank et al 1984), for example lead, arsenic, mercury and thallium. Other neurological effects are known to occur with different heavy metals (Raffle et al 1987, Polites et al 1980) for example

manganese, aluminium, zinc and cobalt. As a general group heavy metals have been implicated in MND aetiology by case-control studies of life-style (Roelofs-Iverson et al 1984, Provinciali & Giovagnoli 1990).

Lead: A widely used metal that resulted in epidemics of lead poisoning in the 19th century (Raffle et al 1987). The most severe cases suffered a lower motor neuron disorder or encephalopathy. It is claimed to cause an ALS-like syndrome (Campbell et al 1970) which can be reversible if treated with chelating agents (Boothby et al 1974). Several case-control studies have found MND patients report higher lead exposure than controls (Campbell et al 1970, Felmus et al 1976, Pierce-Ruhland & Patten 1981), and in one, the differences were statistically significant (Armon et al 1991(b)). This is not supported by other studies (Kurtzke & Beebe 1980, den Hartog Jager et al 1987).

Evidence of abnormal lead accumulation in specific tissues remains inconclusive or difficult to interpret. Increased levels of lead have been reported in serum, erythrocytes and cerebrospinal fluid (CSF) of ALS patients compared to controls in some studies (Conradi et al 1980, Conradi et al 1978), but these findings are disputed (Kapaki et al 1989, Stober et al 1983). Spinal cord tissue from patients with MND has also been found with increased lead levels, which correlated with disease duration, but not lead exposure (Kurlander & Patten 1989). The precise mechanism causing lead induced neurotoxicity, or how it enters the nervous system is unclear (Tandan & Bradley 1985(b)). Also most MND patients do not benefit from chelating agents (Currier & Haerer 1968). If any relationship exists between lead and MND it cannot be a simple one.

Mercury: Acute mercury poisoning causes, tremor and irritability (Raffle et al 1987). There have been reports of mercury poisoning inducing MND after brief, but intense exposure to elemental mercury (Adams et al 1983), which reduced with time. In some case-control studies, MND patients were found to have higher exposure to mercury than controls (Pierce-Ruhland & Patten 1981, Roelofs-Iverson et al 1984), but this factor has not been investigated in others, and gender variance makes the findings debatable.

Aluminium: This is the world's most abundant metal, generally considered non-toxic, until recently. Aluminium has been identified as the possible cause of progressive encephalopathy in chronic renal patients being treated with aluminium salts (Alfrey et al 1976). It appears to be a constituent of the senile plaques associated with Alzheimer disease (Crapper et al 1976), though the histopathological changes in both cases are different (Crapper & De Boni 1980). Evidence for aluminium poisoning and accumulation associated with hyperparathyroidism is the basis of a hypothesis for the Western Pacific MND/P-D complex (Yase 1988, Garruto et al 1986). Support for this has come from animal models of aluminium induced neurofibrillary

degeneration (Griffin et al 1984). A link between aluminium and sporadic ALS has been presented in the form of a case study of a man with ALS whose symptoms improved with chelating therapy for aluminium (Patten 1988). There are however, many facets of these observations that remain unexplained and even Yase 1988 admits aluminium as a simple cause of ALS seems remote.

Manganese: It has long been recognised that mining manganese can cause chronic poisoning resulting in psychosis and a movement disorder with Parkinsonian features (Politis et al 1980). Implication of manganese in MND pathogenesis has come from the observation that manganese miners on Guam succumb frequently to Western Pacific MND (Yase 1972). There has also been one case of an ALS-type syndrome in a manganese miner in Europe (Voss et al 1939). The determination of manganese in spinal cord tissue, and blood cells however, has produced conflicting results (Kurlander & Pattern 1979, Nagata et al 1985, Mitchell et al 1986).

Selenium: Interest in a possible link between selenium and MND was initiated by the discovery of increased incidence of ALS in a small rural community (Kilness & Hochberg 1977). Environmental selenium levels were known to be high, and subsequently abnormal amounts were found in the patients' urine. However, this has not been confirmed (Norris & U 1978). Selenium levels in blood cells, spinal cord and liver tissue are reported to be significantly higher in MND patients compared to controls (Nagata et al 1985, Mitchell et al 1986). Mitchell et al 1986 suggests the abnormally high levels of selenium and manganese indicate increased activity of enzymes containing these metals which are involved in free radical degradation. Free radicals have been implicated in a number of degenerative disorders and are thought to be important to the ageing process. Unfortunately, indices of free radical activity in the CSF from patients with MND, were no different to those of controls (Mitchell et al 1987). The wide range of selenium and manganese levels in the tissues of patients and controls more likely represent a difference in dietary intake.

Measures of other essential metallic trace elements known to have neurotoxic effects when imbalanced, for example, zinc, copper, cobalt, and arsenic have been in measured in various tissues (Currier & Haerer 1968, Mitchell et al 1984, Kurlander & Patten 1979). The amounts recorded from both patients with MND and controls are variable and provide conflicting data.

Attribution of an aetiological role to metals in MND is difficult in view of the contradictory evidence. Control levels of tissue metals are inconsistent between studies. This questions the data reliability, and may reflect the analytical techniques employed (Tandan & Bradley 1985(b)). Abnormal metal concentrations in MND could be the consequence of atrophy, or represent a phenomenon secondary to cellular damage or blood-brain barrier changes (Mandybur & Cooper 1979).

(iii) MISCELLANOUS EXOGENOUS ENVIRONMENTAL NEUROTOXINS

Some exogenous neurotoxic chemical may be involved in the aetiology of MND. This is supported by the discovery of a parkinsonian syndrome induced by methyl-phenyl-tetra-hydropyridine (MPTP) (Langston et al 1983), and a hypothesis regarding the role of plant amino acids with excitotoxic actions in Western Pacific MND and Lathyrism (Spencer et al 1986(a), Spencer et al 1986(b)).

Many chemicals are toxic to the human nervous system. Peripheral neuropathy is a common outcome, usually taking the form of a distal axonopathy (Schaumburg & Spencer 1984). Chemicals which cause peripheral neuropathy include acrylamide, carbon disulphide, triorthocresylphosphate and hexacarbons (Schaumburg & Spencer 1984). Some data implicates certain chemical compounds in the development of MND. A case study of a man who developed a rapidly progressing MND after acute toxification with a pesticide (ant killer) has been documented (Pall et al 1987). An investigation into the chronic effects of organophosphate pesticide toxification found neuropsychological performance in the victim to be markedly impaired, compared to controls, two years after a single exposure (Rosenstock et al 1991). Therefore, motor neurone pathology is more likely during initial stages following acute poisoning, rather than the result of chronic small dose exposure.

Solvents are used widely in industry and are also an important constituent of many products found in the home. Occupational epidemiological data has implicated solvents in MND aetiology. It has been proposed that MND may be secondary to the effects of solvent exposure (Hawkes et al 1989). Inspection of occupational mortality figures for England and Wales over the period 1959-83, revealed a significantly higher than expected death rate for leather workers from MND in men aged 15-64 (Hawkes et al 1981). Solvents are essential elements in the shoemaking process, and many of these are neurotoxic (Hawkes et al 1989). There is a sensory-motor polyneuropathy which has been well documented in Italian shoemakers (Muzi et al 1990), and attributed to the solvent n-hexane. Unfortunately, the occupational mortality figures for England and Wales over the period 1979-89 have altered quite considerably (Hawkes & Fox 1992). Leather workers no longer appear to have greater than expected mortality from MND, and have fallen well down the proportional mortality league table. Hawkes and Fox 1992 suggest the explanation for this could be changes in solvent utilisation within the leather industry.

Other occupations that probably involve solvent exposure, have also been identified. Swedish occupational mortality data for MND show that agricultural labourers have an increased risk of MND (as indicated by a raised odds ratio (OR), ie, cases to controls ratio >1) (Gunnarsson et al 1991). This excess pertained to one region of the country only. When cases with definite

solvent exposure were compared to controls, persons working in printing works or as petrol station attendants had the greatest increased risk (Gunnarsson & Lindberg 1989). In a case-control study in Italy (Chio et al 1991), a significant excess of farmers and breeders were found among MND sufferers. Increased risk was also found for hairdressers, tanners, carpenters and house painters. A similar study in the USA found the only difference between patients and controls on occupational exposure to chemicals, was in the manufacture of plastics (Deapen & Henderson 1986). Unfortunately, all these data are very weak because of the small numbers involved. The figures are also unstable, as the occupations with peak mortality change over time (Hawkes & Fox 1992), making such data unreliable. The results from a recent case-control study in Sweden imply solvent exposure may be a factor linked to MND aetiology, particularly in males of younger age groups (Gunnarsson et al 1992).

Obviously the results of chronic exposure to solvents is very difficult to investigate. A method which achieved this was a study of "healthy" painters (Spurgeon et al 1990). During psychological assessment the painters exhibited greater vulnerability to stress than the controls, but not to psychiatric illness. Identifying neurophysiological changes that can be related to a toxin is very difficult. Symptoms often only appear when pathological changes are severe, and the instigating toxin may have been rendered harmless or excreted some time beforehand.

1.7   ENDOGENOUS PHENOMENA

(i)   PATHOLOGICAL CHANGES WITHIN THE NERVOUS SYSTEM

Several hypotheses have been proposed in relation to the mechanism of neuronal degeneration in MND. These hypotheses are concerned with possible pathological processes that result in anterior horn cell death, which could either be the result of exogenous or endogenous triggers.

Abnormal Axonal Transport: This is an essential neuronal function, which if defective could possibly cause neuronal degeneration in MND. Proximal axonal swellings have been detected in some patients with MND (Delisle & Carpenter 1984), and abnormalities in fast axonal transport in others (Breuer & Atkinson 1988), but changes in slow axonal transport have only been demonstrated in animal models. These changes are probably secondary to underlying biochemical abnormalities (Tandan & Bradley 1985(b)).

Disrupted Membrane Properties: Defects in membrane structure or function may be relevant. Erythrocytes from patients with MND exhibit greater fragility in the presence of lead than those from controls (Ronnevi et al 1982). Nevertheless, no such abnormalities have been demonstrated by others using various methods (Butterfield & Markesbery 1980). Currently there is no available evidence to support this hypothesis (Williams & Windebank 1991).

Alterations in Nucleic Acid Metabolism: There is conflicting evidence for the DNA (deoxyribonucleic acid) hypothesis proposed by Bradley and Krasin 1982. It is suggested that ALS results from defective DNA repair enzymes leading to accumulation of damaged DNA, and disruption of protein synthesis in affected motor neurones, causing premature cell death. This is supported by reports of deficient DNA repair mechanisms in skin fibroblasts (Tandan et al 1987) and increased sensitivity of cultured ALS cells to ionising radiation (Kidson et al 1983). Contradicting this hypothesis, is data showing that irradiated lymphoblastoid cell lines from patients with ALS have similar cell survival to controls (Robbins et al 1984), and no difference was found in spontaneous and mutagen-induced sister chromatid exchange frequencies when comparing the lymphocytes of patients and controls (Pentland et al 1985). If the DNA hypothesis was correct, it might represent a biochemical abnormality through which other factors produce their effects.

Trophic Factors: These are polypeptides affiliated with assimilation and growth, and several neurotrophic factors have now been discovered. Like hormones, the action of growth factors is via receptor-mediated mechanisms, but dissimilar, in that they are synthesised by a variety of tissues and act locally at their site of release (Logan 1990). One of the most important of these growth factors is nerve growth factor (NGF), which is synthesised and secreted by tissues innervated by sympathetic and some sensory neurones (Cholinergic). A review by Logan presents data that implicates NGF in the regulation of normal cellular function, including development and maintenance of neurone viability, and a possible role for NGF in some proliferative, degenerative and injury-related conditions of the CNS. Therefore, disruption of similar agents associated with motor neurones and muscles is an appealing hypothesis for MND (Antel et al 1990). Unfortunately, no comparable trophic factors with NGF specific to motor neurones have yet been identified (Sendtner 1992(a), Gibson & Polak 1991). So far, disruption to any of the known motor neurone orientated growth factors, such as ciliary neurotrophic factor (CNTF) and brain-derived neurotrophic factor (BDNF), does not appear to be associated with motor degenerative diseases (Sendtner 1992(b)). Nonetheless, the potential of CNTF and BDNF in the treatment of MND is presently being investigated (Oppenheim 1992, Sendtner 1992b).

Androgen Hypothesis: The discovery that androgen receptors in rats are concentrated on neurones primarily affected in ALS, has led to the hypothesis that loss of these receptors is a factor in ALS aetiology (Weiner 1980). Relevant to this theory, is the discovery that the gene for X-linked bulbospinal neuropathy has been located in the same chromosome portion as the androgen-receptor gene (Warner et al 1990). There is little direct evidence to support this theory.

Ageing: The process of ageing is fundamental to all living systems. The similarity between neurodegenerative diseases and ageing makes hypothesis associated with accelerated or premature ageing, (abiotrophy) attractive for these disorders including MND (McComes et al 1973, Calne et al 1986). Likewise, disruption of "apoptosis", which ensures the programmed removal of abnormal, damaged or senescent cells, via a controlled cell death pathway (Carson & Ribeiro 1993), could be aetiologically significant. Epidemiological data on MND has shown increasing incidence and mortality with increasing age (Yoshida et al 1986, Lilienfeld et al 1989 Hawkes et al 1992), though this has only become evident recently.

Electrophysiological data has demonstrated that continuous anterior horn cell loss, particularly after the age of 60, occurs during normal ageing (McComas et al 1973). It is also probable that compensatory processes, such as axonal sprouting, become less efficient at the same time. Proposed mechanisms of premature ageing in MND are similar to those suggested for normal ageing. For example, accumulation of damaged DNA (Bradley & Krasin 1982) (links with DNA hypothesis), and loss of postulated muscle-derived trophic factor essential for motor neurone health (Appel 1981). Premature motor neurone degeneration in MND could represent an age-related phenomenon that is accelerated by exogenous factors, possibly remaining subclinical for decades (Calne et al 1986). This could even be influenced by a genetic predisposition to neuronal attrition. Unfortunately, specific evidence supporting this hypothesis is sparse.

Akin to the ageing hypothesis, have been investigations into the possible effects of parental age and birth order in MND (Hawkes et al 1989, Kondo & Fujiki 1984). The idea is that children of older mothers are at higher risk, in a similar way to the relationship between maternal age and the incidence of Down's syndrome (Hawkes et al 1989). However, parental age and birth order were very similar for patients with MND and matched controls.

Synaptic Receptors and Transmitters: The selective destruction of anterior horn cells in MND suggests defects in discreet neurological processes. Ideal candidates are the highly specific neurotransmitters and their receptors. Whether any changes identified in these are primary or secondary effects is impossible to determine. This is because receptor and transmitter concentration decrease concurrently as anterior horn cells degenerate, with unknown reactive consequences. The effect of this phenomenon is reflected in the contradictory findings of several studies looking for changes in various CNS receptors and transmitters (reviewed by Williams & Windebank 1991, Tandan & Bradley 1985(b)).

More enlightening is the possible role of excitatory and inhibitory amino acid neurotransmitters and their receptors. There is evidence implicating over stimulation of specific receptors by excitatory amino acids (excitotoxicity) in acute disease processes, such

as hypoxia/ischaemia and hypoglycaemia (Albin and Greenamyre 1992). In addition, animal studies have shown potent anticonvulsant actions of pharmacological agents that either enhance inhibitory or decrease excitatory amino acids (Meldrum 1984). The possible role of excitotoxicity in MND is presently being explored (discussed in following sections).

(ii) DEFECTIVE METABOLIC PROCESSES

Alterations seen in metabolic processes in MND, are compatible with the concept of an exogenous or endogenous neurotoxin interacting with protective metabolic mechanisms and causing MND. Genetic variation of these mechanisms means some people have increased susceptibility to certain environmental insults (Williams 1991). A good example of this is the development of Parkinsonian symptoms after ingestion of MPTP in some drug addicts because of defective metabolism (Williams 1991).

Data supporting this concept has been produced which demonstrates that patients with MND have a substantially slower and reduced capacity for detoxifying exogenous agents by sulphation or sulphoxidation (Steventon et al 1988), but increased ability for S-methylation reactions compared to control subjects (Waring et al 1989). Poor sulphoxidation could lead to; 1) the accumulation of cysteine, which in turn can have toxic effects via disturbance of metal metabolism resulting in free radical formation, or by its neuroexcitatory action; 2) very low levels of sulphate which will impede detoxification of a variety of compounds, such as metals (Williams 1991). Measurement of plasma and brain concentrations of cysteine and inorganic sulphate in patients with MND however, have been found to be normal (Perry et al 1991).

The present hypothesis regarding neurotoxic chemicals in the aetiology of Western Pacific MND, ie BMAA explained earlier, could also exert its toxic actions in some Chamorro natives because of inefficient metabolic detoxification processes.

Alterations in Calcium Metabolism: Apart from this being a prominent theory regarding Western Pacific MND aetiology, disrupted calcium metabolism is also implicated in sporadic MND (Patten and Engel 1982). Hyperparathyroidism has similar clinical features to MND. Skeletal fractures (Felmus et al 1976, Kurtzke & Beebe 1980, Provinciali & Giovagnoli 1990) and abnormalities seen on X-ray (Patten & Engel 1982) reportedly occur more frequently in patients with MND compared to controls, though this is not confirmed by other studies (Deapen & Henderson 1986, Pierce-Ruhland & Patten 1981, Armon et al 1991(c)). Some patients have been found to have abnormal calcium levels, elevated parathyroid hormone levels, and deranged intestinal calcium absorption (reviewed Patten & Engel 1982). Yet no

disturbance of calcium and vitamin D metabolism could be detected in the Chamorros with MND/P-D complex, so if this is a cause of Western Pacific MND it must have been resolved before the disease appeared (Yanagihara et al 1984).

Carbohydrate Metabolism Disturbance: Glucose intolerance in conjunction with MND has been demonstrated (Saffer et al 1977, Reyes et al 1984), but other data shows that only insulin sensitivity appears inversely related to disease severity (Harris et al 1986). This suggests the changes reflect diminished muscle activity, and not a primary abnormality (Harris et al 1986).

Derangement of Amino Acid Homeostasis: Perhaps most interesting at present is the apparent abnormalities of amino acid homeostasis in MND (de Belleroche et al 1984) and the link with an excitotoxic mechanism hypothesis. Attention has centred on the amino acids that are known neuronal transmitter substances: excitatory – glutamate and aspartate; and inhibitory – glycine, gamma-aminobutyric acid (GABA) and taurine.

Much work has centred on the physiological roles of glutamate in the CNS and its possible contribution to several neurological disorders when its action is abnormally enhanced (Greenamyre 1986). After several investigations into glutamate distribution and homeostasis (Plaitakis & Caroscio 1985 & 1987, Rothstein et al 1990, Perry et al 1987), Plaitakis et al (1988(a),1990(a)) proposed that the increased plasma and decreased CNS glutamate levels indicate an altered distribution of glutamate between intracellular and extracelluar pools in MND patients. This could result from a defect affecting the high affinity uptake system or processing of synaptic glutamate by glial cells (Plaitakis 1990(a)). Alternatively, increased glutamate release at nerve terminals, or leakage from cells could be responsible for the low intracellular concentration in nerve tissues (Plaitakis 1990(b)).

Confirmation of abnormal glutamate metabolism is seen in changes to the activity of a key enzyme, glutamate dehydrogenase (GDH), which mainly controls glutamate synthesis. Reduced levels have been found in the leucocytes of a section of people with MND, which suggests altered cellular metabolism (Hugon et al 1989). More convincingly, spinal cord GDH activity appears to be increased in certain areas of the spinal cord, particularly in the cervical region (Malessa et al 1991). This does not however, uniformly correspond with areas of major pathological change in MND, and it is impossible to discern whether this could be a primary or secondary phenomenon.

In association with glutamate abnormalities, is evidence of decreased aspartate levels in the spinal cord (Plaitakis et al 1988(a), Malessa et al 1991) and increased levels in CSF from patients with MND (Rothstein et al 1990). Inhibitory amino acid levels also appear disturbed in the spinal cord with reported excess of taurine and significantly reduced levels of glycine

(Malessa et al 1991). Conversely, CSF glycine levels appear abnormally elevated in some patients (de Belleroche et al 1984) and their ability to restore glycine homeostasis is relatively impaired when compared to controls (Lane et al 1991), but this data is unconfirmed.

Not all studies support these amino acid changes, with normal levels of glutamate, aspartate and glycine being measured in CSF from patients with MND (Perry et al 1990). However, methodological variations may account for the vast discrepancies in values obtained between this and other studies (Young 1990). Instability in amino acid storage within atrophied muscle could also be a factor.

(iii) AMINO ACIDS AS ENDOGENOUS EXCITATORY NEUROTOXINS

Based on the documented evidence of abnormal amino acid metabolism, Plaitakis (1990) proposed a central role for excitatory amino acids in MND. The hypothesis suggests that defective presynaptic glutamate metabolic processes may render certain groups of neurones vulnerable to neuroexcitotoxic damage. The selectivity of nerve cell loss in MND is possibly explained by the postulated changes in glycine metabolism described above. Glycine is an inhibitory transmitter of interneurons in spinal cord and brain stem, where it is abundant, but it is also a positive modulator of one type of glutamate receptor (N-methyl-D-aspartate or NMDA), enhancing glutamate action (Young 1990). A high density of this receptor has been located around lower motor neurone perikarya in the spinal cord (Shaw et al 1991(a)).

This hypothesis, though intriguing, requires further evidence before concluding that excitatory amino acids play a role in MND (Young 1990). Yet because this theory holds great potential for therapeutic manipulation, drug trials in this area have already commenced (Plaitakis 1988(b), Steiner 1991).

Exogenous Excitatory Neurotoxins: Basically the mechanism of excitotoxic neuronal death is initiated by over stimulation of excitatory post-synaptic receptors. Excessive stimulation stretches the nerve cells' biochemical homeostatic mechanisms, leading to exhaustion and inability to maintain the cells integrity. There are three main types of excitatory amino acids receptors identified in the CNS, namely N methyl-D-aspartate (NMDA), (alpha)-amino-3-hydroxy-5-methyl-isoxazlo-4-propionic acid (AMPA) and Kainate. All are sensitive to glutamate, but selective to other excitant and inhibitory compounds (Nunn & O'Brien 1991). Apart from glutamate and aspartate, other naturally occurring exogenous food-borne amino acids have been shown to generate characteristic excitotoxic neuropathology in test systems (Nunn & O'Brien 1991).

B-Oxalyamino-L-alanine (BOAA): The effects of this non-protein dicarboxylic amino acid has already been described earlier. Laboratory data into the precise action of BOAA has demonstrated that it has high affinity for the AMPA receptor at low concentrations, and both AMPA and kainate receptors at high concentrations. BOAA probably produces its neurotoxicity by interacting with AMPA receptors and symptoms appear after daily consumption of Lathyrus sativus for 2-4 months (Allen et al 1990).

Domoic Acid: A known constituent of marine algae of the genus Chondria. Consumption of the algae results in an acute reaction, which can include loss of short term memory and seizures. Neuropathological studies of patients dying from the toxins effects show neuronal death particularly in the hippocampus and amygdala (Nunn and O'Brien 1991). Domoic acid appears to produce an excitoxic action with kainate receptors only (Nunn and O'Brien 1991), but chronic exposure effects are unknown.

B-N-Methylamino-L-alanine (BMAA): This is also a non-protein dicarboxylic amino acid and the reasons for this being a candidate in the aetiology of Western Pacific MND were explained earlier. BMAA has been shown to cause neuronal death through activation of both NMDA and non-NMDA receptors, though its characteristics are confusing, and there is still no firm evidence that it is the culpable agent in cycad seed (Allen et al 1990). Since Western Pacific MND is a long-latency progressive disease, it is unlikely to be triggered by a straightforward excitotoxic mechanism. However, because BMAA does not behave like a conventional excitatory amino acid and accumulates in neurones, a possible chronic pathological role may be elicited in the future (Allen et al 1990).

No such agents have been identified so far in relation to sporadic MND, but many researchers feel that an exogenous neuroexcitatory neurotoxic mechanism is one of the more plausible hypothesis for MND aetiology. This is reflected in the development of two modified forms of the excitotoxic hypothesis, more compatible with chronic degenerative neurological diseases (Albin & Greenamyre 1992).

1.8 ASSOCIATION BETWEEN PHYSICAL STATUS AND MND

Epidemiological studies have detected possible associations between MND and previous disturbances to the neuromuscular system. For example, acute injury to muscles and nerves caused by trauma and surgery, and more chronic damage incurred during hard physical exercise either occupational or sports orientated.

Back and limb trauma (Kurtzke & Beebe 1980, Gawel et al 1983, Gallagher & Sanders 1987), "mechanical injuries" (Kondo & Tsubaki 1981, Chio et al 1991), and experience of severe electric shocks (Gawel et al 1983, Deapen & Henderson 1986) before onset of MND, have all been reported more frequently by cases compared to controls, though other studies have not found any difference in the occurrence of these factors (Armon et al 1991(b)). The relationship between acute injuries and MND is unknown, and does not follow a particular pattern (Chio et al 1991) or correspond to the initial site where MND manifests itself (Kondo & Tsubaki 1981). It could represent a greater vulnerability to trauma because of pre-clinical MND muscle weakness, or in some way predispose to or precipitate the disease. Alternatively it could simply reflect bias recall. Negative results for increased trauma suggest MND is not a direct consequence of a mechanical injury (Kondo & Tsubaki 1981).

Greater incidence of surgical operations has been documented for patients with MND compared to controls (Kurtzke & Beebe 1980), but many other researchers could not confirm this.

In favour of MND being the result of 'wearing out' the motor neurones from excessive use, is documentation showing patients with MND having participated more intensely in sports activities (Felmus et al 1976, Kurtzke & Beebe 1980), or been employed more frequently in hard manual occupations (Provinciali & Giovagnoli 1990, Gunnarsson & Palm 1984) compared to control subjects. The focus of this theory centres on the famous baseball player Lou Gehrig, whose pre-diagnostic performance started declining 18 months before he retired from the game (Kasarskis & Winslow 1989). The use of pneumatic tools has also been suggested as a possible contributory factor (Gallagher & Sanders 1983). However, again these are not constant findings in other case-control studies (Armon et al 1991(b), Kondo & Tsubaki 1981), and therefore cannot have a simple relationship with MND.

(i)   MND ASSOCIATED WITH OTHER DISEASES/DISORDERS

Other Neurological Diseases: Case-control studies have identified increased frequency of other neurological diseases in relatives of patients with MND compared to controls (Pierce-Ruhland & Patten 1981, Deapen & Henderson 1986, Armon et al 1991). Different approaches to assessing this area however, makes any conclusions impossible. Patients themselves do not appear to suffer other neurological diseases more frequently than control subjects (Armon et al 1991(c), Leone et al 1987). Nevertheless, the occurrence of dementia in conjunction with sporadic MND is increasingly recognised in a small proportion of sufferers (Myrinathopoulos & Smith 1962, Editorial Lancet 1990(c)). There are also case reports of MND in patients with both Parkinson's disease and Multiple Sclerosis (Hader et al 1986, Hudson 1981).

Neoplasms: The discovery that a considerable number of patients with MND had coexisting carcinoma led to the proposal that MND was attributable to the carcinoma (Brain et al 1965). This trend has been reported elsewhere (Chio et al 1991), but other studies have not confirmed this relationship (Leone et al 1987, Barron & Rodichok 1982, Provinciali & Giovagnoli 1990). MND in patients with lymphoma has been recognised for some time (Younger et al 1991) and the relationship could indicate a common cause (see section on Immunological Hypothesis). A link between inherited colorectal cancer (Editorial Lancet 1991) and lower motor neurone degeneration in adults has been postulated (Shaw et al 1991(b)). It remains to be seen whether the location of the genes responsible for Spinal Muscular Atrophies and a specific colorectal cancer to chromosome 5q will be significant for other inherited motor neurone disorders.

Thyroid Diseases: Exophthalmic goitre can produce symptoms that simulate progressive muscular atrophy which resolve after partial thyroidectomy (Ayer et al 1934). There are however, indications that a history of thyroid disease may occur in patients with MND more often than expected by chance (Appel et al 1986, Chio et al 1991, Armon et al 1991(c)). The heterogeneity of antecedent thyroid disease makes it highly unlikely that a causal relationship with MND exists (Armon et al 1991(c)). Interestingly, there does appear to be a physiological relationship between thyrotropin releasing hormone (TRH) and motor neurone function (reviewed Guiloff 1987), which could include an excitatory and "trophic" role. This has led to experimental treatments with TRH using MND patients, but unfortunately improvement in symptoms is slight and transitory (Modarres-Sadeghi et al 1988).

Gastro-intestinal Problems: An association between gastric disorders or peptic ulceration and MND has been considered because of the increased frequency reported in some studies (Chio et al 1991, den Hartog Jager 1987). This has not been confirmed elsewhere (Kondo 1979), and could be a chance association.

Any positive relationship between MND and other diseases is tenuous considering that many epidemiological studies have investigated medical history and found no links (Roelofs-Iverson et al 1984, Kurtzke & Beebe 1980, Pierce-Ruhland & Patten 1981). Different methodology and populations could account for some of the discrepancies.

1.9 IMMUNOLOGICAL HYPOTHESES

When the aetiology of a disease remains elusive, researchers are attracted by the idea of autoimmunity. Considerable effort is presently being expanded to support this concept, but much scepticism abounds because evidence for conventional immunological dysfunction in patients with MND has been unobtainable (reviewed Williams & Windebank 1991, Antel et

al 1990). In addition, treatment with immunosuppressive drugs (Appel et al 1988) produces no benefit in double-blind placebo trials. Consequently, an unconventional autoimmune hypothesis has been proposed (Drachman & Kuncl 1989), though it is based on incomplete evidence and is yet to be tested.

Evidence for an immunological cause of MND comes from various sources. A greater than expected incidence of thyroid diseases and autoimmune disorders has been identified among patients with MND and their blood relatives in one series (Appel et al 1986). There have also been case reports indicating a possible association between rheumatoid arthritis and MND (Schady et al 1989), but this has been argued as a chance occurrence (Outhwaite et al 1989).

In a small percentage (4.8% & 9.0%) of patients with MND, plasma cell dyscrasia has been detected, consisting of IgG, IgM, or IgA paraproteinaemias (Shy et al 1986, Younger et al 1990). Most patients in a series with high CSF protein content (especially > 75 mg/dl) were found to have a paraproteinaemia (Younger et al 1990). The incidence of plasma cell dyscrasia in a population rises with age, but a higher incidence was found in all age groups of MND cases, compared to general population samples and neurological controls (Shy et al 1986). The significance of this association is unclear, and therapeutic lowering of raised IgG does not improve the disease.

Immune complex disposition has been demonstrated within organs of patients with MND (Oldstone et al 1976). Circulating immune complexes can also be identified in a small proportion of cases, but the levels are minor compared to those in recognised autoimmune diseases such as systemic lupus erythematous (Williams & Windebank 1991).

Inherited HLA antigens partly determine the immune responses of an individual, and specific HLA allotypes and haplotypes are associated with particular autoimmune diseases. Results from HLA-linkage studies in patients with MND are equivocal. Different HLA antigens were over-represented in separate groups of patients, for example HLA-A3 and B12 in classical ALS (Antel et al 1976), and Bw35 in Western Pacific MND/PD-complex (Hoffman et al 1978), but the findings have not been replicated (Bartfeld et al 1982). The low levels of HLA-A3 in Mexicans has been suggested to be the possible reason for their apparent "resistance" to MND, but other communities with low HLA-A3 levels have average or higher incidence of MND (Antel et al 1976).

Target-directed immunity has been explored by several methods assessing the cytotoxic activity of MND sera. MND plasma has been shown to cause haemolysis of normal erythrocytes, and this effect attenuated by treating the patients with immunosuppressive drugs (Conradi & Ronnevi 1985). On the other hand, evidence for cytotoxic activity of MND sera

on neural tissue is mixed (reviewed Antel et al 1990), so no conclusions can been drawn. Antel et al 1990 concluded autoantibodies do not have a pathogenic role in MND, but they could still participate in the pathogenesis of individual cases of MND.

Recently there have been reports linking MND with the presence of IgM antibodies to GM_1, GD_{1a} and GD_{1b} gangliosides found in the serum of some patients (Shy et al 1989, Pestronk 1989, Salazar-Gruesco et al 1990). However, in patients with other neurological diseases (Sadazar-Gruesco et al 1990), non-neurological immune disorders and normal controls (Pestronk 1989), a comparable frequency of these antibodies has also been detected, implying these are part of the normal human antibody collection. The successful treatment with immunotherapy of pure lower motor neurone disorders associated with specific antiganglioside antibodies (Shy et al 1990, Salazar-Gruesco et al 1990) has generated interest in the possible relationship between these and MND.

Immunoactivity against muscle-derived neurological growth (trophic) factors have been demonstrated. Compared to controls, sera from MND patients significantly inhibited terminal axonal sprouting in experimental conditions (Gurney et al 1984). Conversely, sera from patients with MND and an IgG paraproteinaemia does not appear to have the same effect (Donaghy & Duchen 1986) and challenges the idea of a causative relationship.

It seems that the immunological hypothesis is equally supported and refuted by the research undertaken so far. It remains to be seen whether immunological animal models of ALS (Appel et al 1991) will produce any useful data.

1.10   GENETIC HYPOTHESES

The influence of genetic determinants on MND pathogenesis were initially postulated soon after MND was first identified and described. Yet the degree to which genetics contributes to the disease is still being debated.

Familial aggregation of MND does happen, but was only acknowledged widely after the investigations of Kurland and Mulder in 1955 among their own patients. Occurrence of familial aggregations is estimated to range from 5% to 10% of the total MND incidence (Li et al 1988, Tandan & Bradley 1985(a)). Similar familial patterns are seen in Alzheimer's disease (5-10% of cases) (Fitch et al 1988), and Parkinson's disease (15-20% of cases) (Duvoisin 1986), and unravelling the genetic mechanism of one disease may provide clues for understanding that of the others. Familial aggregations however, do not necessarily imply a hereditary source, and a shared environment could be just as important. Familial studies however, strongly allude to genetic mechanisms.

The pattern of inheritance exhibited among afflicted families is not straightforward, although many of the families exhibit autosomal dominance (Mulder et al 1986, Emery & Holloway 1982), with an equal male:female ratio (Strong et al 1991). Within other families this line of inheritance is broken, as obligate carriers of the offending gene fail to develop MND (Williams et al 1988, Horton et al 1976, Kurland & Mulder 1955). Reasons for this could be either: 1) the gene carrier dies before this late-onset disease could develop; or 2) the disorder is not always recognised or is misdiagnosed in an elderly person. Dominant inheritance with incomplete penetrance has been described in other pedigrees of FMND (Mulder et al 1986, Chio et al 1987), and is more likely to occur in families with a wide age range of disease onset, or predisposition to early death from another cause (Williams et al 1988). The consequence of low penetrance FMND is that cases may be diagnosed sporadic MND, resulting in an underestimation of FMND incidence (Williams et al 1988).

Unusual patterns of inheritance found within families have also been explained by: 1) recessive inheritance (Emery & Holloway 1982); 2) vertical transmission of an infective agent (Li et al 1988); 3) polygenic inheritance; 4) genetic predisposition; and 5) multifactorial causation (Kondo 1979). A common environmental exposure is the other obvious possibility.

There have been many case reports of families describing the clinical and pathological features of FMND. A striking aspect is the great variation in disease presentation within some families (Appelbaum et al 1992, Gardner & Feldmahn 1966, Pinsky et al 1975, Gimenez-Roldan & Esteban 1977, Strong et al 1991), and also between families (Horton et al 1976), suggesting genetic heterogeneity. Interestingly, this is also a cardinal characteristic of sporadic MND.

The most obvious differences between FMND and sporadic MND is the apparent younger mean age of onset 45.7 years for FMND (SD +/- 11.3 - range 20-72 years), and the even sex ratio (Strong et al 1991). Overall, clinical presentation, progression and duration however, are very similar (Mulder et al 1986, Strong et al 1991), despite some case reports which emphasise the extreme and unusual. A small proportion of families are known to develop concurrent dementia (Pinsky et al 1975, Finlayson et al 1973). Pathologically, FMND generally produces the same degenerative changes, though again there are reports describing pathology of the posterior columns and spinocerebellar tract, and slight differences in the structure of the cytoplasmic inclusions (Hirano et al 1967, Engel et al 1959). These changes are also known to occur in sporadic MND (see section on pathology).

The similarities between familial and sporadic MND make a genetic hypothesis very attractive. At this time, a large coordinated FMND linkage study is under way, though the nature of the disease makes this a difficult task (Figlewicz et al 1992). Many chromosome regions have been excluded as the likely locus of the FMND gene (Siddique et al 1989). Encouraging results

have recently been obtained for chromosome 21 (Siddique et al 1991), but so far no marker has produced a high enough Lod score to demonstrate linkage (Conneally 1991), and other researchers are unable to confirm this particular linkage (de Belleroche personal communication). A significant probability of genetic locus heterogeneity has been established (Siddique et al 1991), meaning more than one gene mutation could be involved.

Further progress by the FMND linkage study collaborators has just been reported (Rosen et al 1993). They have demonstrated tight genetic linkage between a FMND marker and a gene that encodes a cytosolic CU/ZN-binding superoxide dismutase (SOD1) found on chromosome 21q. This is a homodimeric metalloenzyme that catalyses the dismutation of the toxic superoxide anion O_2 to O_2 and H_2O_2. Superoxide is a byproduct of numerous physiological and pathological processes and therefore a common free radical (McNamara & Fridovich 1993). Rosen et al 1993 investigated the SOD1-gene as a candidate gene in FMND and identified 11 different single amino acid mutations in SOD1 within the DNA from 13 different FMND pedigrees. Consequently, they hypothesised that alterations in the activity of SOD1 results in the progressive accumulation of neurotoxic free radicals, but how this could cause selective damage is unclear. A decrease in SOD1 activity leading to an excitotoxic mechanism of motor neuronal death is one suggestion (McNamara & Fridovich 1993). Unfortunately so far, these findings only relate to a minority of FMND families.

1.11   TWIN STUDIES

In about 5% to 10% of cases, MND does appear to be genetic in origin (Li et al 1988, Tandan & Bradley 1985(a)). A similar percentage of patients with Parkinson's disease and Alzheimer's disease also seem to inherit their disorder. The fundamental question is whether genetics plays a role in the apparent sporadic cases of these degenerative diseases, and if so to what degree. Sporadic cases of these diseases could result from a genetic susceptibility to the effects of a common environmental assault, or by the overwhelming of a healthy defence mechanism by a rare, but highly neurotoxic factor.

Types of Twin Study: A potentially powerful and well-recognised method for determining the relative importance of genetic and environmental factors in disease aetiology is the classical twin study (Hrubec & Robinette 1984). The purpose of a classical twin study is to measure the frequency of the relevant disease occurring in both members of a twin pair, which is known as "concordance", and compare the rates found in identical and fraternal twins. If the concordance rate for the study disease is high in pairs of identical (monozygotic) twins, and much lower for fraternal (dizygotic) twins, then this would imply a strong genetic influence. Equal concordance between monozygotic and dizygotic twins, infers that environmental influences are more important.

A good illustration of this principle can be seen in an epilepsy twin study (Lennox & Lennox 1960). This found that when the epilepsy was associated with a suspected brain lesion, and therefore an environmental influence, the concordance rate in both monozygotic (MZ) and dizygotic (DZ) twins was similar. Conversely, when no brain lesion was detectable the concordance rate for epilepsy in MZ pairs was very high (70.2%), but in DZ it was considerably lower (5.6%), thus strongly supporting the role of genetics.

Traditionally, twin studies have been used to assess either the genetic contribution to human physical / mental characteristics, or the twins are taken as matched pairs for control studies of a particular phenomenon (Hrubec & Robinette 1984). Recently, the value of twin studies has been seriously questioned (Bundey 1991, Phillips 1993). Much criticism is a consequence of the failure of many twin studies to produce meaningful results. The main reasons are that when a twin study is designed to determine heritability of a trait, the methodological process needs to be rigorous, and care taken when interpreting the findings. Many neurological twin studies have serious methodological flaws, which are reflected in their results. Important factors to be considered include:

Zygosity Ratio: Determination of twin zygosity is imperative, so comparisons can be made of the intra-pair differences between MZ and DZ twins.

Estimation of the expected number of MZ and DZ twins in a random sample can be calculated using Weinberg's Method (Weinberg 1901).

$$\text{Proportion of MZ twins} = \frac{\text{The number of like-sexed pairs} - \text{the number of unlike-sexed pairs}}{\text{Total number of twin pairs}}$$

$$\text{Proportion of DZ twins} = \frac{2 \times \text{number of unlike-sexed pairs}}{\text{Total number of twins pairs}}$$

This estimation is sufficiently accurate for most purposes (Emery 1986), and provides a means of judging whether a collected twin sample is representative or unbalanced.

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It is now recognised that the arrangement of foetal membranes does not relate to twin zygosity, as 15% of MZ pairs are known to be dichorionic, and another 15% have separate placentas (Emery 1986). This is a poor way of verifying zygosity.

Verifying Zygosity: Reliable methods of determining zygosity of like-sexed pairs are tedious and expensive (Hrubec & Robinette 1984). Commonly, blood is used for immunological typing of genetic systems, for example ABO, MNS and Rhesus blood-group systems, sometimes in conjunction with secretor status and dermatoglyphics. Discordance of any one trait tested is sufficient to classify a pair dizygotic. The probability of misclassifying a DZ pair as MZ can be calculated using independently estimated gene frequencies (Maynard-Smith & Penrose 1955). This probability becomes progressively smaller as the number of systems tested increases, or if information regarding the parents immune systems is available. Today, DNA fingerprinting using microsatellite probes can be utilised for zygosity verification, which has negligible error.

A much more accessible and cheaper approach for establishing zygosity is assessment of physical similarities in conjunction with the twins own judgement. The validity of this method has been explored and tested on different populations of twins (Cederlöf et al 1961, Nichols & Bilbro 1966, Magnus et al 1983). All obtained > 90% agreement in the zygosity classification indicated by a physical appearance questionnaire and that obtained by blood analysis. A refined set of simple questions developed by Magnus et al 1983, was found to be accurate in establishing zygosity in **97.6%** cases, if **both** members of a pair answered the questionnaire. This level of accuracy was only slightly reduced to **96.1%** if one twin was used. The single most discriminative question was "were you and your twin alike as 'two drops of water' in childhood...". A slightly different version of this question substituting in 'two peas in a pod' was the most informative in a similar evaluation (Cederöff et al 1961).

Clearly this method has sufficient validity and reliability to be used for zygosity classification and may be more suitable in some research situations, for example, when one twin has died. Likewise it avoids the problem of a diminished and biased sample when volunteers refuse a blood test (Nichols & Bilbro 1966).

Twin Type Apportionment: It is important when using twin studies to estimate heritability, that the sample contains the correct ratio of MZ:DZ twins for the population being used. The rates of the two twin types varies between different races. The frequency of MZ twinning is constant throughout the world, but DZ twinning rates vary (Emery 1986). If the ratio is skewed, the proportion of concordant MZ to DZ pairs will be adversely affected, and thus any heritability calculation will be erroneous. The ages of the twins in the sample also influences the MZ:DZ ratio.

Since records were kept in England and Wales of the number of twin births, and the frequency of unlike and like-sexed twins, the "twinning rate" has fallen. This was from approximately 12 twin births per 1,000 maternities in 1938, to 10 per 1,000 maternities in 1983 (OPCS birth statistics 1984). This is the result of a reduction in the number of DZ twins born (as estimated by Weinbergs method). In 1938 7 out of 10 twin births were DZ (approximate DZ:MZ ratio - 2:1), but in 1983 this has fallen to 6 out of 10 twin births (OPCS birth statistics 1984). Therefore the estimated MZ:DZ ratio for a given twin sample must also take account of the birth cohorts used. If only like-sexed twins are gathered the MZ:DZ ratio should be approximately 1:1. Opinion is divided over the importance of this principle, and it has been suggested that a skewed sample in favour of MZ twins is irrelevant, providing there is not an over-representation of concordant pairs (Bundey 1991). This may be correct if the factor to be studied is not connected with the reason for the over-sampling of MZ twins, but in disease-related twin studies the subjects are usually aware of the reason for the study. Difficulty in gathering enough pairs is an inevitable feature of traditional twin study methodology. If the disease is completely random then the proportion of affected people who are also twins will be approximately the same as the twin ratio within the general population (1/100). However, if the disease is uncommon (for example prevalence < 100/100,000 people), only a limited twin sample will be available. Many researchers are undeterred by this problem, and most of the published twin studies relating to Parkinson's disease, Alzheimer's disease and multiple sclerosis are impaired because of small sample numbers. Much of the criticism surrounding twin studies arises because of this.

(i) TRADITIONAL METHODS OF SAMPLE COLLECTION

Volunteer-Based Twin Samples: This method involves advertising the study and inviting subjects to come forward. The impetus to volunteer depends on the individual twin pairs. Straight away this biases the sample, because of the unknown and unmeasurable intrinsic differences between the twins who volunteer for medical research studies and those who abstain. In two reviews of volunteer-based twin studies investigating a variety of traits, Lykken et al (1978 & 1987) found a consistent bias across the different samples which they called the "rule of two-thirds". This states that studies of adult same-sex twins, which rely upon volunteers, will consist of about two-thirds female and two-thirds monozygotic pairs, thus producing a ratio of 4MZF:2DZF:2MZM:1DZM. Instead of the expected equal ratio of both sexes and twin types ie. 1MZF:1DZF:1MZM:1DZM.

If the extrinsic incentives to volunteer are the same for all twins, then intrinsic factors must be less important to DZ twins. There are some known attributes in which volunteer subjects differ from the non-volunteers (Rosenthal & Rosnow 1975). Females are more willing to be

experimental subjects unless the research promises to be stressful. Volunteer subjects also tend to be more intelligent, better educated and have a higher social class status than non-volunteers. Therefore, the interests and values which are conducive to volunteering for research studies, must be commoner among these sections of society. Personal interest in the topic under investigation will enhance the likelihood of collaboration (Rosenthal & Rosnow 1975). It follows that MZ twins come forward for research about twins, because as a group they are more interested in twinship as a phenomenon than DZ twins (Lykken et al 1978). The effect of these biases is that the males and DZ pairs must be less representative of their respective populations than the female and MZ pairs, so that volunteer DZ samples are more homogenous than the comparable MZ sample. This is important because estimates of heritability for continuous traits, using twin data, are based upon comparisons of computed intraclass correlations for the MZ and DZ twin samples. This in turn assumes that the total variances are the same for both groups and requires the same amount of heterogeneity. Restriction in the range of total variance for the DZ twins will result in underestimation of the intraclass correlations computed to represent within-pair similarities, causing overestimation of the heritability of the trait concerned (Lykken et al 1978).

It is extremely plausible that similar forces affect the volunteering of twins for physical disease related studies. As MZ twins are more attracted to twin investigations, it is inferable that concordant MZ pairs for a study disease will have greater reason to volunteer. This will also tend to inflate the genetic component, as a disproportionate number of the concordant pairs are likely to be MZ. In addition, other elements influence the willingness of unaffected co-twins to participate in disease related studies. The vulnerability or threat they perceive from the specific disease will affect there response, along with any anxiety regarding possible invasive investigations.

Illustration of the biased samples obtained by volunteer-based twin studies into Parkinson's disease (PD) and multiple sclerosis (MS) is given in tables 4 and 5. Predictably, in both cases the twin type distribution is highly skewed, with the MZ twins representing approximately 43% to 69% of the sample, and the concordance rate for MZ and DZ twins being particularly high. The sex ratio cannot be assessed as inadequate data is given. In the one MS study where the proportion of each sex is presented, there are nearly twice the number of females as would be expected (Williams et al 1980). In the PD studies the bias towards males is unexplainable.

Population-Based Studies: A twin register recording details of every twin birth in a geographical area, is the ideal source for a population-based twin study. Only the Scandinavian countries have established such registers, and details can be cross-linked with hospital and national insurance records when identifying twins diagnosed with a certain disorder. For all other countries, the twin sample can only be gathered by national trawl of

REFERENCE	NUMB. OF TWIN PAIRS	SEX RATIO M:F expec 1:1.7	MONOZYGOTIC TWIN PAIRS			DIZYGOTIC TWIN PAIRS		
			Total No.	No. Concordant (%)	Sex Ratio M:F	Total No.	No. Concordant (%)	Sex Ratio M:F
VOLUNTEER-BASED STUDIES								
Mackay & Myrianthop. 1966	68	1:3.9	39	9 (23.1)	1:4.6	29	6 (20.7)	1:3.1
Cendrowski 1968	107	U/K	51	15 (29.4)	U/K	56	5 (8.9)	U/K
Williams et al 1980	24	1:3.8	12	6 (50.0)	1:11	12	2 (16.7)	1:2
Currier & Eldridge 1982	51	U/K	22	8 (36.6)	U/K	29	3 (10.3)	U/K
TOTAL	**250**	**1:3.9**	**124**	**38 (30.6)**	**1:5.4**	**126**	**16 (12.7)**	**1:2.7**
POPULATION-BASED STUDIES								
Bobowick et al 1978 (male twin pairs only)	9	N/A	5	1 (20.0)	N/A	4	0 (0.0)	N/A
Heltberg & Holm 1982 (like-sexed twins only)	47	1:1.6	19	4 (21.1)	U/K	28	1 (3.6)	U/K
Ebers et al 1986	70	1:1.8	27	7 (25.9)	1:2.4	43	1 (2.3)	1:1.5
Kinnunen et al 1987 (like-sexed twins only)	21	1:1.6	11	1 (9.1)	1:0.8	10	0 (0.0)	1:4
Mumford et al 1992	105	U/K	44	11 (25.0)	U/K	61	2 (3.3)	U/K
Sadovnick et al 1993	42	U/K	19	5 (26.3)	1:3.8	23	0 (0.0)	U/K
Alpérovitch et al 1992	54	1:2.2	17	1 (5.9)	U/K	37	1 (2.7)	U/K
TOTAL	**294**	**1:1.7**	**142**	**30 (21.1)**	**1:2.2**	**206**	**5 (2.4)**	**1:1.8**

TABLE 4: Multiple Sclerosis Twin Studies

REFERENCE	NO. OF TWIN PAIRS	SEX RATIO M:F expec 1.1:1	MONOZYGOTIC TWIN PAIRS			DIZYGOTIC TWIN PAIRS		
			Total No.	No. Concordant (%)	Sex Ratio M:F	Total No.	No. Concordant (%)	Sex Ratio M:F
VOLUNTEER-BASED STUDIES								
Marsden 1987	22	U/K	11	1 (9.1)	U/K	11	1 (9.1)	U/K
Ward et al 1983 (typical P.D.)	60	U/K	42	1 (2.4)	1.6:1	18	0 (0.0)	U/K
(+ atypical & possible P.D.)	65	U/K	47	4 (8.5)	1.6:1	18	1 (5.5)	U/K
Vieregge et al 1992	21	1.3:1	9	3 (33.3)	2:1	12	3 (25.0)	1:1
TOTAL (typical P.D.)	**103**	**U/K**	**61**	**5 (8.2)**	**1.7:1**	**41**	**4 (9.7)**	**U/K**
(+ atypical & possible P.D.)	**108**	**U/K**	**66**	**8 (12.1)**	**1.7:1**	**41**	**5 (12.2)**	**U/K**
POPULATION-BASED STUDIES								
Marttila et al 1988 (like-sexed twins only)	32	1.2:1	18	0 (0.0)	U/K	14	1 (7.1)	U/K
TOTAL	**32**	**1.2:1**	**18**	**0 (0.0)**	**U/K**	**14**	**1 (7.1)**	**U/K**

Key: U/K = unknown
N/A = not applicable

TABLE 5: Parkinson's Disease Twin Studies

all cases with the disorder via hospital, community and voluntary organisation records. Then enquiry with every case whether they are a twin. Obviously, this method is reliant on comprehensive and accurate information, and also the availability and accessibility of such records. Depending in general, on the size of the geographical area to be covered, this is a difficult, and time-consuming exercise. It also requires the cooperation of medical and administrative staff at many levels, which is probably a near impossible task. Identification of the twins is still dependent on the twins revealing themselves when questioned, and the attitudes of the pair to the disease.

To circumvent some of the practical and monetary problems, many "population-based" samples use very restricted groupings. For example, the Maudsley Hospital London twin register which is compiled from all patient referrals to the hospital (Karlinsky et al 1992), and the twin register based on US Veterans Administration records (Bobowick et al 1978). Any population-based study gathered purely from hospital records cannot be taken to represent the diseased population in the community it serves. This is because 1) hospital clinics attract more severely affected and "interesting" patients, especially those who contradict present expectations; 2) not all patients diagnosed with a disorder will be referred to a hospital specialist, especially elderly people. Over-representation of concordant pairs is a possible bias in such twin studies, because of associated ascertainment. Concordant pairs attract more attention and thus a mildly affected co-twin is more likely to be identified on hospital records (Allen et al 1967).

The difficulties associated with population-based twin studies are reflected in the sample distributions of the studies presented in tables 4 & 5. The MS study based on a twin register comprising male twin pairs who were both recruited into the US military services (Bobowick et al 1978), has an equal MZ:DZ twin ratio, but represents a highly selected population and cannot be taken to reflect the total USA male population. Only half the expected number of twins with subsequent MS were detected. Perhaps this reflects the fact that the young males picked for military service were at the peak risk age for MS, and any sufferers would have been rejected, but some would still have been alive when the study was carried out.

Two MS and one PD study are based on population study registers. The zygosity and sex ratio of the two Finnish studies into MS (Kinnunen et al 1987) and PD (Marttila et al 1988) are as expected, but unfortunately the numbers are very small. The Danish MS twin study has a skewed zygosity (MZ:DZ = 1:1.5) distribution, but this could have been caused by adding twins from a MS register (Heltberg & Holm 1982).

The Canadian study is more informative because the sample is larger (Ebers et al 1986). This was gathered from the population attending MS centres established in each province for the centralisation of medical care. Unfortunately, the authors fail to make clear that the

population attending the centres during the study period was far from complete (Hawkes personal communication), so neither was the twin sample. This is illustrated by the exclusion of twin pairs who volunteered for the study, but were excluded presumably because they had not been found among the MS centre populations. Whether the zygosity and sex ratio are as expected is not commentated upon and cannot be assessed. There is possibly slight excess of MZ twins, ie. 27 when 21-24 would be expected, assuming the MZ:DZ ratio is approximately 30:70. Overall the sex ratio appears representative, assuming the Canadian MS Male:Female ratio is 1:1.7, but the MZ group has too many females.

The way large samples help to even out any discrepancies or distribution bias is nicely illustrated by comparing Ebers et al's original study, and a follow-up investigation using only two of the MS centres (Sadovnick et al 1993). In the latter study, both the zygosity and the sex ratio are very skewed in favour of MZ twins and females, and near to the Lykken's "two thirds rule" for voluntary studies. It is possible that this distribution has occurred because of the publicity generated around the first study, which resulted in MZ and female MS sufferers requesting referral to the centres.

The largest sample of twins collected for the investigation of MS has recently been constructed in the United Kingdom (UK) (Mumford et al 1992). The sample has been collected by asking neurologists to find out who among their clinic patients are twins, and then informing the researchers. Obviously, this system will only access the proportion of patients with MS who attended a neurology clinic during the study period, and is totally dependent on the interest and cooperation of the individual neurologists. In fact only 10% of the available twins with MS were identified, with a predictable excess of MZ twins in whom concordance is probably overestimated.

Contradictory results have been obtained by the French Research Group, with concordance being independent of zygosity (Alpérovitch et al 1992). Unfortunately, their sample is not representative, as it was obtained from a volunteer register of MS patients (higher proportion of females), but the twin selection was unbiased (expected MZ:DZ ratio).

Proponents of these methods would argue that because the concordance rate in both types of twins is similar, then their methods are adequate for overcoming recruitment bias. It is possible they all have similar inherent bias, because this assumption has now been challenged (Alpérovitch et al 1992), and the one true population-based MS twin study has a considerably lower MZ and DZ concordance rate (Kinnunen et al 1987). As a whole, the evidence provided by the population-based twin studies into MS and PD can only be taken as provisional because of small sample size, or lack of vigour in the adherence to epidemiological principles.

<u>Individual Case Studies:</u> MZ twins reared apart (MZA) are of great value. Clearly, in such circumstances concordance for a disease in a pair implies a major genetic contribution, but such twins are rare. Detailed case studies of MZ pairs may provide aetiological clues, and some commentators declare this to be the only worthwhile use of twins in medical research (Bundey 1991). Case-control studies using both types of twins for researching environmental factors can be helpful, providing a representative sample is taken so the intrapair or intraclass frequency of a factor (eg smoking) is meaningful. A PD twin sample was used for this purpose, and the findings suggested smoking was protective against PD (Bharucha et al 1986). However, the twin sample was highly selective, so this finding may be misleading.

In MND, all but one twin study have been case-studies as follows: Three individual twin pairs have been described, two concordant DZ pairs (Dumon et al 1971, Estrin 1977), and one discordant MZ pair (Jokelainen et al 1978). All previous descriptions of MND in twins are valueless (Dumon et al 1971). One of the DZ concordant pairs were offspring of consanguineous parents and could represent a recessive trait (Dumon et al 1971). Concordance in the other DZ pair was theorised to be due to an intrauterine assault, because of inadequate evidence for inheritance or similar adult environmental toxifications (Estrin 1977). The discordant MZ twins were used as a case-control pair for environmental factors (Jokelainen et al 1978). A larger case-control study using discordant like-sexed twins has been published (Currier & Conwill 1989). This found a higher occurrence of influenza and greater physical activity in the affected twins compared to the co-twins, but the sample were volunteers. MND twin studies so far have shed little light on its aetiology.

(ii) TEMPORAL SPREAD

An important element is the length of time a discordant pair must be followed before they can be categorically declared discordant. It has been shown that in PD there can be a temporal spread of 14 years in disease onset between a concordant pair, though in others expression of the disease appeared in both twins around the same time (Vieregge et al 1992). In MS a difference of 4 to 22 years was recorded between disease onset in 6 concordant pairs (Williams et al 1980). The only guide available to the possible temporal spread of MND is evidence from familial studies, which have found great diversity in the age of disease onset between family members. For example, in one generation of a family pedigree, onset of MND occurred between the ages of 39 to 63 (Li et al 1988). Obviously, the effect of large temporal spread will be underestimation of any genetic contribution, unless pairs can be studied for long periods of time.

In diseases occuring mainly in old age like MND and PD, potential concordant pairs will be lost due to death of the co-twin from another cause, before such an extended time period has elapsed. Technology now allows researchers to attempt to circumvent this problem by scanning for subclinical pathological changes in the co-twins CNS. For example, MRI scanning for MS (Ebers et al 1986, Sadovnick et al 1993, Mumford et al 1992),and PET scanning for AD and PD (Burn et al 1992). The results of such investigations can be very misleading, unless a truly representative or complete sample of co-twins is used. This avenue of disease detection also creates great ethical problems, because of the dilemma associated with scanning a clinically well person for an incurable terminal disease, which may never express itself, barring some pathological changes, before the person dies.

(iii) HERITABILITY ESTIMATION

The degree of influence exerted by genetic factors in specific diseases is given by the comparison of MZ and DZ concordance rates. There are two distinct definitions of the concordance rate, which have caused much misunderstanding (Allen et al 1967).

The more obvious definition is the **pairwise concordance rate**, which is the proportion of twin pairs affected by the disease where both members have the condition within the total twin sample. The **pairwise concordance rate** is calculated using the formula C / (C + D), where C = the number of concordant pairs, D = number of discordant pairs.

The other definition is termed the **proband concordance rate**, which identifies the proportion of affected individuals among the co-twins of previously defined index cases. **Probands** are all those twin individuals affected by the disease who were ascertained without dependence on their co-twin. If both members of a twin pair are affected and identified independently, then effectively the pair is counted twice. The **proband concordance rate** is given by (C + C')/ (C + C' + D), where C' = the number of concordant pairs where both members were ascertained independently. It produces an estimation of the relative risk for a co-twin to be affected by the same disease.

The proband concordance rate can be used to make inferences about the disease in broader populations, and is regarded as a definitive expression. It can be compared across studies, even if each has different ascertainment probabilities, and be used to accurately estimate the population casewise rate which forcasts individual risk (McGue 1992). The pairwise concordance rate is of limited value (Allen et al 1967), because it only represents the conditions within that particular sample, and reflects the thoroughness of twin ascertainment. It cannot be compared across different studies or populations (McGue 1992).

Under certain circumstances the pairwise concordance rate can be useful (Hrubec & Allen 1975); 1) As a lower limit of the proband rate, especially when there is doubt concerning the independence of doubly ascertained pairs; and 2) in the comparison of a samples MZ and DZ concordance rates, because it is amenable to the recognised statistical tests.

An important aspect of the proband concordance rate is that it can be used to provide estimates of correlation ('r') among MZ or DZ twins in liability to a trait or condition (Smith 1974). Smith 1974 demonstrated that the MZ and DZ correlations can then be interpreted genetically by the expression $G = 2(r_{MZ} - r_{DZ})$. This eliminates nongenetic familial effects on twins, and estimates the coefficient of genetic determination for the trait. The expression 'G' is based on Falconer's 1965 "threshold model" for **multifactorial inheritance**, which measures the correlation between relatives for a disease, using incidence rates for the general population and relatives of affected individuals. The model assumes an underlying continuous liability to a disease, consisting of many environmental and genetic factors, and thus normally distributed. Manifestation of the disease occurs if an individuals liability exceeds a critical threshold level. Revision of the model was made by Smith and presented as a graph for convenient usage (Smith 1970). Unfortunately, there are limits to this model, which also apply to the 'G' expression. The estimation of heritability is only meaningful if a single major gene does not contribute to the cause of the study disorder, and likewise, that there is no genetic heterogeneity causing the disorder (Emery 1986).

Sadly, calculation of heritability estimations appear to be ignored, or dismissed as unhelpful by the authors of many twin studies. Certainly no figures are presented in any of the published twin studies into MS, PD or AD. This may partly be due to the complicated mathematics involved in calculating the coefficient of genetic determination ('G'); or because it was thought inappropriate, considering the limitations of the data involved. Instead, only the difference between the MZ and DZ pairwise concordance rates have been presented as an indication of the role of genetic factors in a disease's aetiology (Ebers et al 1986, Sadovnick et al 1993).

SECTION 2

THE STUDY METHODOLOGY

2.1 IDENTIFICATION OF THE RESEARCH PROBLEM

Perhaps the most puzzling aspect of MND is that 5% - 10% of patients have a dominantly inherited disorder, which is clinically indistinguishable from the apparently sporadic syndrome affecting the rest.

Very little literature was found that attempted to address this paradox. The majority of epidemiological studies concentrated on identifying possible environmental factors relevant to sporadic MND. Some familial pedigrees, collected during FMND studies, had no known cases in previous generations (Mulder et al 1986, Li et al 1988, Williams et al 1988), and were classified familial because of more than one affected sibling or cousin. These could represent low-penetrance FMND (Williams et al 1988), a new genetic mutation, or even a chance occurrence. Alternatively, it could be the result of the affected generation having a genetic predisposition to a particular environmental assault, and therefore arguably developed sporadic MND. It seemed pertinent therefore, to design an investigation into the possible contribution of genetics in sporadic MND, compared with environmental influences. A twin study was deemed the most appropriate method for tackling this question (Hrubec & Robinette 1984).

Operational Definitions: MND has already been operationally defined in the previous chapter, along with interpretations of sporadic and familial MND. Genetics refers to the DNA coding that determines all heritable characteristics of a person. As a science it is concerned with how heritable differences originate and relate to an individuals genetic programme, and the method by which traits are transmitted through a lineage (Singer 1985). Environmental factors are any extrinsic phenomena that may influence and interact with a person, causing temporary or permanent alterations to physiological and psychological mechanisms.

Stating the Research Question: No hypothesis was developed for this particular study, because that involves making suppositions about the relationship between variables acting within a specific situation (Williamson 1987). This is not appropriate when the purpose of the study is only to explore and describe the relationship between variables in an empirical situation, without making any prior assumptions. As the aim of this study was to help clarify the significance of the many theories on MND aetiology, a neutral stance was taken regarding them all. Therefore, a set of aims and objectives were identified to indicate the purpose and direction of the study.

AIM: To determine the relative importance of genetic and environmental factors in the aetiology of MND.

by: 1) Obtaining an unbiased sample of twins where at least one member
of each pair suffered from MND.

2) Establishing which twins have a family history of autosomal dominant inherited MND, classified as FMND.

3) Identifying all concordant pairs for MND, and establishing whether the difference between the MZ and DZ rates implies a definite genetic influence.

4) Estimating inheritance of liability for MND (Smith 1974) using the concordance rates found for MZ and DZ twins.

5) Identifying any environmental factors which have a significant intrapair difference in the exposure level experienced by discordant twins.

6) Determining any common environmental factors among the affected twins, compared with the co-twins, which may have a role in MND aetiology. Likewise, any unifying environmental exposures among the co-twins, which may have a protective role.

7) Attempting to establish which of the many environmental influences that have been implicated in MND aetiology in the past are worth pursuing in a more detailed study.

2.2 STUDY DESIGN

Many twin samples are only collected to produce concordance rates for MZ and DZ twins. These are then compared to establish whether the rates are significantly different, thus producing an estimation of the genetic contribution. However, the findings are not utilised further. The value of twins as matched-pairs for comparing exposure to environmental factors is usually ignored, despite the recognition of the value of twin studies in identifying environmental aetiological factors (Allen 1965, Harvald & Hauge 1956, Macdonald 1993).

A population-based twin study was chosen as the most appropriate approach for estimating the genetic contribution to sporadic MND. This design facilitates the collection of a complete and unbiased sample of twins which will be representative of the diseased population, provided the general principles of such studies are followed (see section on twin studies). It was decided that the twins should also be used as matched-pairs for a case-control study of

environmental factors that the literature has suggested are possibly relevant to MND. This would maximise the potential information available from a carefully constructed twin sample.

The importance of the case-control design is clearly presented by Schlesselman (1982). It is particularly suitable for the study of diseases like MND which are rare and possibly have long latencies and allows the investigation of multiple potential causes. Also it requires fewer subjects than the cohort design to overcome two potential problems when investigating a population sample. These are known as Type I error - claiming that exposure is associated with disease, when it is not (rejecting a true null hypothesis); and Type II error - claiming that exposure is not associated with disease, when it is (supporting a false null hypothesis) (Altman 1991). Estimating the minimum sample size required to avoid these errors is an important consideration in the planning stage of any case-control study. However, frequently in practice the size of a sample is constrained by other considerations.

In this study the number of available subjects determined its maximum size. In such circumstances, when the sample size is known, an estimation of the power of the study to detect an acceptable alteration in disease risk, which is of biological or public health importance can be calculated for different environmental exposures (Schlesselman 1982). However, the broad descriptive nature of this particular study was not amenable to such manipulation because many different environmental exposures were investigated, and it was impractical to determine the study power for detecting the desired relative risk (Odds Ratio) for every individual factor. It could be argued that this study was too encompassing, but it was decided that the effort involved in identifying and interviewing the co-twins justified maximising the information gathered. As most of the MND research has produced statistically weak results because of small numbers, and covered a hugh range of possible aetiological factors, further clarification is needed using the largest population sample available. It was decided that to meet this aim of the study, a loss of statistical power was acceptable.

As with all research methods, there are disadvantages to the case-study design, which must be recognised in relation to the study's findings. The main problems applicable to this study were: 1) reliance on memory recall for information on past exposures and activities; 2) inability or difficulty in validating information; and 3) incomplete control of extraneous variables (Schlesselman 1982).

The Suitability of Twins as Matched-Pairs: All case-control studies have the problem of deciding how best to select an unbiased sample of controls, and whether they should be matched. Match sampling involves pairing each case with one or more controls on the basis of specified variables. By doing this the effects of such variables are eliminated from the comparison, and thus allows the control of confounding variables, for example, age will have

considerable bearing on the risk of developing a degenerative disease. Using twin pairs instantly overcomes this problem, but produces possible complications.

It can be argued that twins are "overmatched" because of the likelihood of similarities in environmental exposures studied. This was noticed by Ward et al 1983 among their discordant twins for Parkinson's disease. The result of using "too similar" controls is that true relationships may be obscured because heterogeneity in exposures is lacking. This is probably true for the early experiences of twin pairs, but as they get older their lifestyles and exposures will become more diverse. Even though MND may have a long latency, there is no evidence at present to indicate childhood exposures are particularly important, except possibly to the poliovirus. Recall of later experiences will be better and more detailed, which will help to overcome this potential problem of overmatching. The most important reason for using twins as matched pairs is that genetic susceptibility to the effects of extrinsic factors is controlled, and thus the genetic effects as a confounding variable are controlled.

2.3   METHODOLOGICAL CONSIDERATIONS

The major problem at the outset of this study was the small size of the potential twin sample available, if a traditional population-based method was employed. There were two reasons for this: 1) the rarity of MND, with a prevalence rate of approximately 4-7/100,000; (this is obtained from the annual incidence rate of between 1.5 - 2.2/100,000 multiplied by the mean life expectancy of 2.5 - 3 years (tables 1 and 3). 2) the proportion of twins within the general population. The ratio of multiple births to singletons was only 1:80 live births before 1940, when the majority of recent MND sufferers were born. The ratio of living twin pairs is reduced by the frequency of infant death, which was greater among twins. This ranged from 151 per 1,000 live births in 1901 to 60 per 1,000 live births in 1941 (Nissel 1987), giving a survival ratio of approximately 1 twin pair per 100 people. Therefore, it can be seen that for a population of 48.5 million (England and Wales), there are between 1900 and 3400 people living with MND at any time, of which around a hundredth part will be twins (19 -34), a very small number. Difficulties identifying and tracing the twins would reduce this figure further and create bias.

To overcome this fundamental problem, an alternative and novel methodology was devised, termed the DEATH DISCORDANT TWIN PAIR METHOD. Basically, this involved identifying twins among the people who have already died from MND, and tracing their co-twins. Any living co-twins could then be approached about participating in the study, and death certificate details obtained for the dead co-twins. Information pertaining to both twins would be sought from the co-twin.

Initial negotiations with the Office of Population Censuses and Surveys for England and Wales (OPCS) were concerned with the availability and access to the necessary documentation, the logistics of conducting such a study, and use of OPCS medical research services for tracing the co-twins.

Data Collection: Interviewing the subjects was chosen as the most appropriate method for collecting the data. This is a time consuming exercise, but often yields better results because of personal contact, especially when dealing with sensitive topics.

Interviewing allows expansion and exploring of an area from the initial question, enabling more detailed information to be gathered. Obviously this is not possible with self-administrated questionnaires, which need to be very precise and carefully worded, to avoid confusion and alienation which may produce unusable answers (Oppenheim 1966).

It is important to ensure that information is gathered from all subjects under similar conditions. This is to reduce the unknown effects of the environment, and interaction with the interviewer, on the subject's responses. Often, very little can be done to control the physical environment. Visiting the subjects at home however, provided a comparable place which was the least threatening and hopefully most congenial for the subjects. In an effort to control the effect of the researcher on the subjects responses, the interviews followed a set questionnaire and the researcher attempted to behave similarly with all the subjects.

Validity of Proxy Data: Since the MND cases had all died, it was necessary to use proxy data to obtain the required information, but obviously the co-twin data was given directly. This would mean that the quality of the data would inevitably be unbalanced. Usually data is obtained directly from both cases and controls, or alternatively, if the case has died, relatives of both can be used instead to avoid differential recall. Little methodological work has been undertaken to validate proxy data against direct information. Linet et al 1989 examined the recall of past medical history by cases, as compared with that of a relative surrogate, and validated each opinion against hospital records. Generally, the surrogate recall was inferior, although this depended on the disease. This has also been shown for smoking data. Therefore, the general effect of proxy data collection would appear to be the underestimation of actual events (a type II error), rather than overestimating any exposure (a type I error). In most case-control studies the opposite effects can occur since cases have a tendency for selectively recalling events they feel are important to their disease, which supports their own theory as to a cause (Sackett 1979).

To improve the quality of data gathered for the case twin, it was decided that where possible, any living spouses could be approached through the co-twins after their interview. This

would provide two independent sources of information and thus increase the validity of the data collected for the case twins.

2.4   ETHICAL CONSIDERATIONS

A major responsibility of the researcher is to ensure the rights of any people affected by the study, especially the subjects, are protected. This requires an understanding of ethical principles, which can vary between different societies, and change over time. Medical questionnaires often involve probing deeply into a person's private experiences, and such intrusion raises issues concerning the right to privacy and protection from exploitation (Stolley & Schlesselman 1982). The principle mechanism used for ensuring that an individuals' rights are respected, is informed consent (Williamson 1987). This means the research subjects voluntarily decide to participate, following adequate information about the nature and purpose of the study, and any possible risks or benefits. Subjects must also consent, knowing they can withdraw at any time, without fear of coercion or retaliation. Complying with the concept of confidentiality is also important. Informed consent was obtained from all participants in this study, following both written and verbal information. This was given prior to the researcher visiting the subjects in their homes, and before commencing the interviews. Confidentiality was protected by allocating code numbers to all twin pairs.

The methodology of this study created an ethical dilemma, because of causing possible distress to the co-twins, if the initial letter sent to them about the study mentioned MND. Alternatively, repressing information regarding the prime purpose of the study could also be deemed unethical. The decision to withhold the true purpose of the study was made by the ethical committee that vets all OPCS based research studies. At a later stage this decision was revoked, providing extra precautions were taken to prevent unnecessary anguish, because of the negative effect it was having on the number of co-twins willing to participate in the study.

2.5   THE DEATH DISCORDANT TWIN PAIR METHODOLOGY

Abridged copies of all death certificates stating MND as an underlying or contributory cause of death (ICD 335.2) were obtained from OPCS for the eleven year period 1979-1989, and the details transferred onto a computer database. Rearranging the people into birth order, and accessing the appropriate Birth Indexes, an entry for all those born in England and Wales was located. This enabled the identification of those who were born with a living twin. Before September 1911, the only indication of a possible twin pair within the index, was the registration area of the birth, along with a volume and page number. This meant common

surnames from large populous areas often produced several possible co-twins for one person who had died from MND. To solve this problem, agreement was reached with OPCS personnel that they would check each possible twin pairing in the full birth register to confirm authenticity. Fortunately, after September 1911 the mother's maiden name was added to the birth index, thus greatly reducing the number of questionable twin pairings.

The next stage was to trace the co-twin. This was achieved by accessing the National Health Service Central Register (NHS-CR), which holds the Family Practitioner Register. From the information carried on all registered citizens since instigation of the NHS, the present status of the co-twins was identified, and if living, with which General Practitioner (GP) the co-twin was registered. A letter was then forwarded, through the Family Health Services Authority, to the relevant GP explaining the purpose and nature of the study and asking permission to approach the co-twins about the study. If access was approved, the co-twin was contacted by letter, and if a positive reply was received, further information and arrangements were made by telephone. All the co-twins were interviewed in their homes (Figure 1).

Results of the Tracing Process: A total of 10,872 death certificates were classified under the ICD rubric 335.2 (MND) during 1979-1989. After removing those born outside England and Wales, or incorrectly classified from the information given on cause of death, 9,780 people remained. Misclassified certificates were those that gave a diagnosis of cerebrovascular accident or metastatic cancer, along with bulbar palsy or MND. Searching the birth indexes from 1900 onwards revealed 131 twin pairs, among 9371 cases. Earlier births were not used because of the unlikelihood that the co-twins were still living.

Tracing at NHS-CR located 66 co-twins of whom 23 died as adults ($\geq$ 18 yrs) and 1 in infancy ($\leq$ 2 yrs), 2 had emigrated, and 40 were living. Of the remaining 65 co-twins, 62 were returned as no-trace cases, and 3 were registered with the armed forces.

Only 50% of the identified twins were traced at the NHS-CR during the initial sweep, with a high percentage of those undiscovered being women. Steps were therefore taken to minimize twin loss by searching Death and Marriage Register Indexes to identify possible entries that could relate to the untraced co-twins. This earmarked co-twins who died before 1948 when the NHS-CR was first instigated, and females who married before that time and who were only registered with the NHS-CR under their married name. A search was made of the death indexes for the 65 untraced co-twins, and if necessary any remaining women were traced in the marriage indexes. This produced 28 co-twins who died in infancy, 19 married females, 2 who died as young adults, and 16 remained untraceable. The 19 married co-twin details were re-submitted to NHS-CR, which resulted in 12 being traced alive, 5 had died and 2 emigrated.
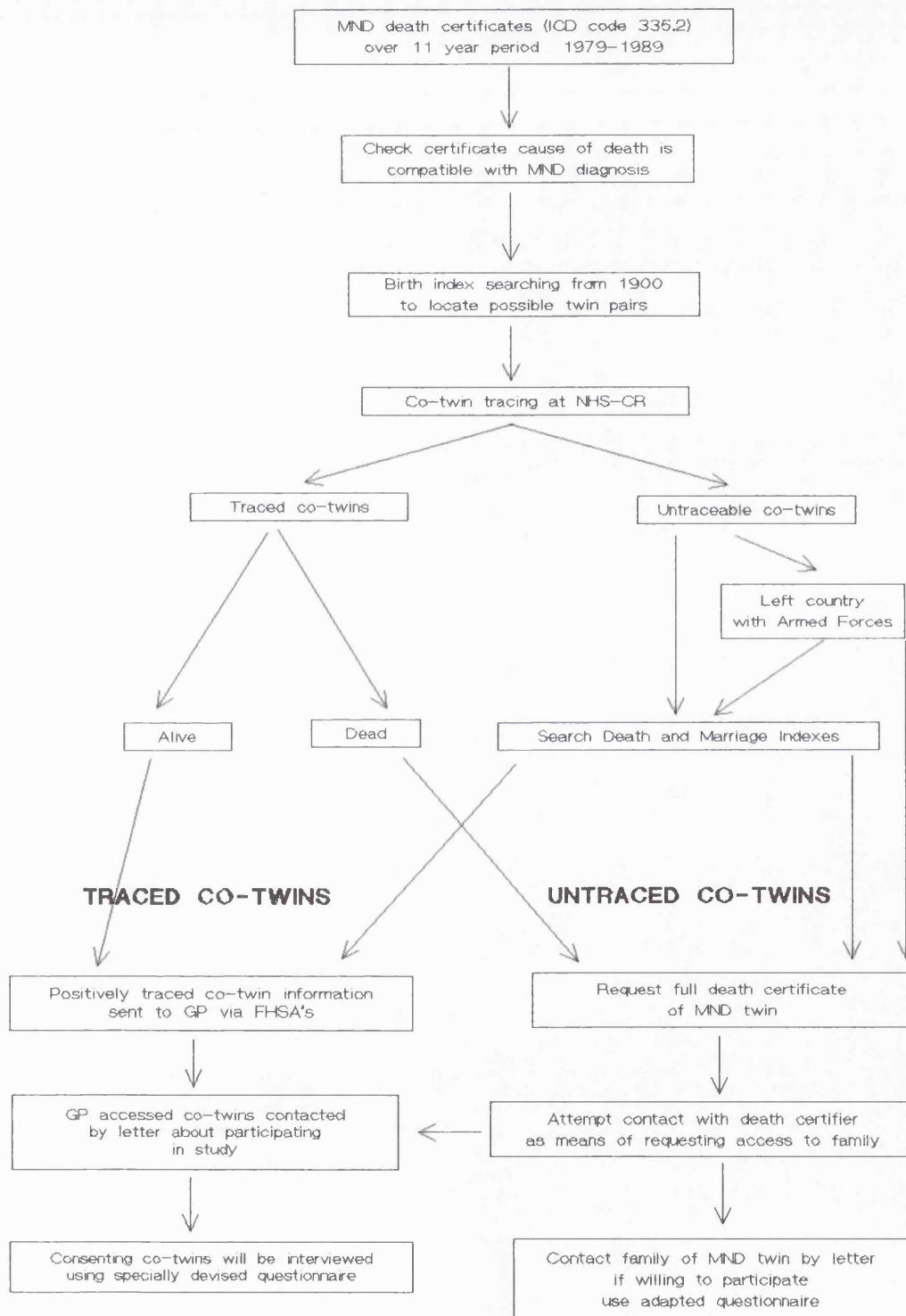
Fig. 1   Flowchart Illustrating the Co-twin Tracing Process

In another strategy OPCS agreed to release complete copies of the death certificates for the MND twins when their co-twin was found to have died, or could not be traced by the methods described. From information on the death certificates, attempts were made to contact the hospital or doctor involved, to seek access to the family of the twin who had died from MND. During this procedure 5 further co-twins were traced (3 living, 1 emigrated and 1 died abroad), and another 3 were discovered to have been incorrectly diagnosed at the time of death. The final result of the co-twin tracing process produced: 55 living co-twins, 31 adult deaths, 29 infant deaths, 5 emigrated, 8 untraceable co-twins, and 3 incorrectly diagnosed index twins (Figure 2).

This second strategy enabled the inclusion of twins where both had died as adults or the co-twin had emigrated, using relatives as the source of information. Obviously, information obtained in this way was of varying quality, but at least it provided some general information about the twins lifestyles, and of most value their zygosity if like-sexed.

Validation of MND Diagnosis in Case Twins: The authenticity of the MND diagnosis was obviously a problem when identifying case twins for this study. Attempts to validate the diagnosis were made by requesting hospital and general practice records using information given on the death certificate. Records were only available on a limited number of patients, because many hospitals destroy the medical records of deceased patients after 8 years, and FHSA's after 3 years. In the cases where such information was available, a consultant neurologist (CHH) studied the patients' history and test results to verify the death certificate diagnosis. All interviewees were questioned about the index twins illness and diagnosis, including a description of its symptoms and progression. This provided some evidence for the validity of the MND diagnosis.

Detection of MND in Living Co-twins: This was achieved by observation and careful questioning of the co-twin regarding the common signs and symptoms of early MND, and contact with their GP. If the co-twin gave a history of any neurological or muscular symptoms that could vaguely be suggestive of MND, this was verified with the co-twin's GP, and pursued further by a neurologist as necessary.

Zygosity Verification: This was achieved using a refined version of the questionnaire devised by Magnus et al 1983 which focuses on the similarities between twin pairs. Obviously, other methods comparing blood samples were impossible.

The reliability and validity of this questionnaire was clearly demonstrated by Magnus et al 1983. The questions selected from those used in the original study, were chosen because they were the most efficient in discriminating between the twin types during their survey. The
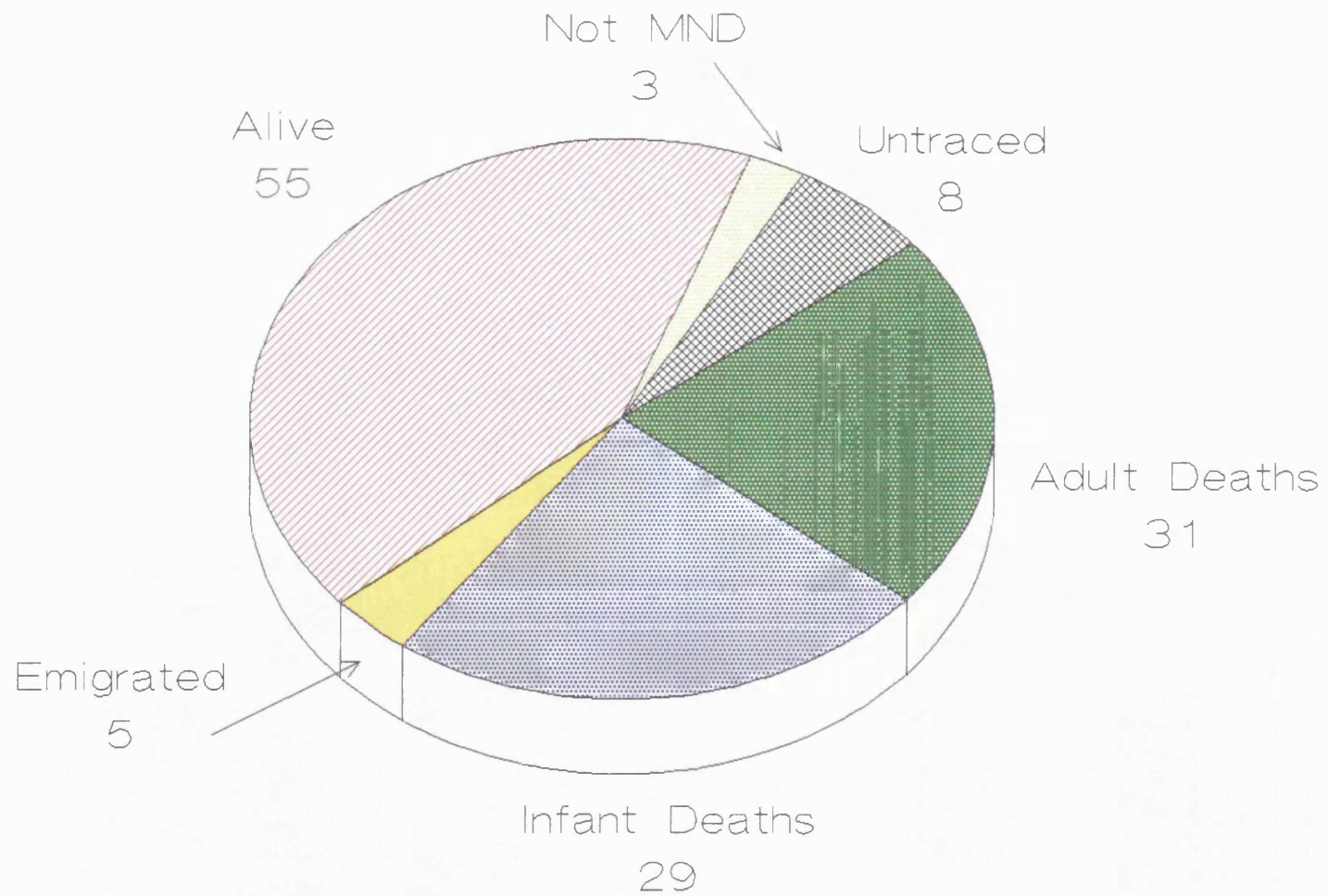
Fig.2   Results of Co-twin Tracing Process

others were rejected as they would be of little benefit and greatly lengthen this section of the interview. Photographs of the twins were requested, especially as children, to help confirm zygosity. This is a technique used routinely by twin researchers at the Maudsley Hospital. A copy of the zygosity questionnaire used is given in the appendix.

(i) DEVELOPMENT OF THE STUDY QUESTIONNAIRE

The first stage was to decide which causative factors could be included in this study. Many were thought important, and as all previous case-control studies of MND have produced weak associations with conflicting elements. It was decided that a large range of different factors should be included. Exposure to various chemicals and metals are mainly associated with the workplace, or area of residence, so a full history of these features was inserted in the questionnaire. This type of information is also better recalled than detail of precise exposures, which a person may never have even considered. Therefore, a rough estimation of chemical and heavy metal exposure was derived by adding together all the situations where exposure had been definite or possible, after the interview. A source list was complied for both types of exposure, which was used as a guide when conducting the interviews.

Considering the extent and complexity of the elements to be covered by the interviews, the questionnaire was designed to investigate the twins lifestyles, thus enabling an estimation of exposure to various specific factors later. It was divided into the following sections:-

Residential History; including classification by the interviewee of every location into rural, semi-rural, or urban.

Employment History; including any known chemical/metal usage.

Hobbies and Leisure Activities; including degree of participation.

Chemical Exposure; to weedkillers, insecticides/fungicides, fertilisers, commercial crop spraying, wood preservatives /treatments, glues and adhesives, paints, inks and dyes, petrochemicals, cosmetics and hair solutions, dry cleaning agents and solvents.

Heavy Metal Exposure; to lead, mercury, arsenic, thallium, aluminium, copper, selenium, zinc, cobalt, chromium, silver, tin, molybdenum.

Diet; broad outline of dietary habits in later adult life only including beverages; any particular customs.

Alcohol Consumption; over lifetime.

Smoking Habits; over lifetime.

Usage of Prescribed and Freely Available Drugs; including illegal designer drugs.

Medical History; particularly neurological conditions, trauma and surgery, infectious diseases, immunisations and allergies.

Family Medical History; particularly neurological conditions.

Sexual Habits; number of sexual partners, use of condoms, any homosexual experiences (men).

Advice was sought from several quarters regarding the format of the questionnaire, and the best approach to adopt when eliciting certain types of information. The design and content of other questionnaires from epidemiological studies were also viewed. It was suggested that because the subjects were being asked to remember events over a long time, that an individualised diary of main life events would be useful. This was constructed at the beginning of each interview, and provided the researcher with personalised prompts by which to stimulate the subjects memory to events and their timing. The use of an autobiographical framework has been shown to improve the recall of personal happenings (Bradburn et al 1987, Jobe & Mingay 1989).

A pilot study was conducted for testing the reliability and validity of the questionnaire, along with the interview technique adopted by the researcher. To preserve all the identified co-twins for the main study, a group of 7 siblings and spouses of local deceased MND patients were approached and their cooperation sought. As a consequence of the pilot study, the format of the questionnaire was slightly modified in certain sections. The length of time required to carry out the interviews was variable, being around 2 hours. However, this was very dependent on the reactions and responses of the interviewees, as many wished to talk about MND generally and how it had effected their lives. (See appendix for full questionnaire).

Conversion of the Data for Analysis: Where possible official classification systems were used for handling the data, such as Postal Codes, the 'Standard Occupational Classification 1990', and the 'International Classification of Disease (9th edition)'. For other data, such as chemical exposure an ordinal scoring system was devised, because the material collected was not accurate enough to treat as continuous data. Smoking data was converted into grammes of tobacco (Doll & Hill 1954) and alcohol data into the recognised official alcohol units.

2.6 DATA ANALYSIS AND STATISTICAL METHODS

Standard statistical procedures were applied to the analysis of the data obtained, and the p-value to indicate statistical significance was taken at $p \leq 0.05$. The analysis of the data was divided into three different sections:-

1) Overview of the twin sample: This was important to establish whether the twin sample was representative, and so the following tests were applied where appropriate: "the two sample t test"; and the hypothesis test for two independent groups (z) so that when $z \geq 1.96$, $p \leq 0.05$.

2) Determination of the genetic contribution to MND: The calculations involved in this process have already been presented in the section titled "twin studies".

3) Results from the environmental questionnaire: Analysis of this data was carried out using statistical methods for matched pairs. Odds Ratio (OR), ie. ratio: number of pairs where only case exposed / number of pairs where only control exposed, along with 95% confidence intervals (CI) were calculated using the methods given by Schlesselman 1982 and Altman 1991 for proportional data. Statistical significance of the OR's was found using McNemar's test including a continuity correction, which is important when the numbers involved are small. The influence of different environmental factors on the estimated risk of being afflicted with MND were also analysed by conditional logistic regression using the statistical package 'GLIM' (Generalised Linear Interactive Modelling). This system is specifically designed for analysing discordant data with categorical rather than continuous variables. Conditional logistic regression models allow the analysis of individual and joint effects of several variables to disease risk to be carried out. Particularly is this so when the cases and controls are matched, and where there may be only a small number of individuals in each variable subset.

Initially a tentative selection was made of those environmental factors which appeared to be possible risk factors for MND, or alternatively protective against it. The factors which produced interesting findings were then analysed more closely individually, and only those which exhibited statistically significant differences between the cases and controls were selected for inclusion in the conditional logistic regression model. When fitting a logistic regression model, only information from the case/control pairs with a known value for every variable included in the model would be used, subsequently excluding any pairs with missing data. The model was constructed firstly using the data obtained for all the death discordant twin pairs, and then selecting different subsets of the sample.

In view of the small sample size the unlike-sexed twin pairs were included in the analysis of the environmental factors. Though these pairs were not strictly matched as the same-sexed

twins, this decision was justified because the group contained equal numbers of male and female cases and controls, which allows the balancing of exposures that could be sex related.

SECTION 3

THE STUDY RESULTS

3.1 OVERVIEW OF SAMPLE

A MND population of 9371, as obtained, would be expected to yield approximately 117 twin pairs, assuming the twinning rate between 1900 - 1950 was 1 twin birth per 80 maternities. An excess of 14 pairs was found, suggesting the twinning rate during the earlier years was greater than the first published rate in 1938, and more in the region of 1:70 live births. This implies that the twin sample identified is representative and probably complete for this MND population. Supporting evidence is given by the twin proportions, and the sex and age distributions of the sample (figure 3) compared to the study population (all dead MND sufferers born in England and Wales from 01/01/1900 onwards).

Age Distribution: The age distribution at death of the index twin sample was comparable with that of the study population (see table 6 & figure 3). The small age differences between these two groups was not statistically significant (t = 0.445; p > 0.05).

	Mean Age at Death (+/- SD) (in years)	Age Range (in years)
Complete MND Twin Sample	64.5 (10.1)	33 - 85
MND Death Certificate Population	66.4 (10.4)	13 - 89
Male MND Twin Sample	64.5 (10.2)	33 - 84
Male MND Death Certificate Pop.	65.4 (10.8)	17 - 89
Female MND Twin Sample	64.6 (10.0)	37 - 85
Female MND Death Certificate Pop	67.5 (9.8)	13 - 89

Table 6: Comparison of the Mean Age and Age Range at Death Between the Index Twins and MND Death Certificate Population (born since 01/01/1900).

Twin Proportions: The complete twin sample included 86 (67.2%) like-sexed twins and 42 (32.8%) unlike-sexed twins. The exact proportions of these at birth, during the period when most of the study twins were born, is unknown, as no such statistics were kept. However, the study twin distribution is very similar to that given with the first twin birth statistics for
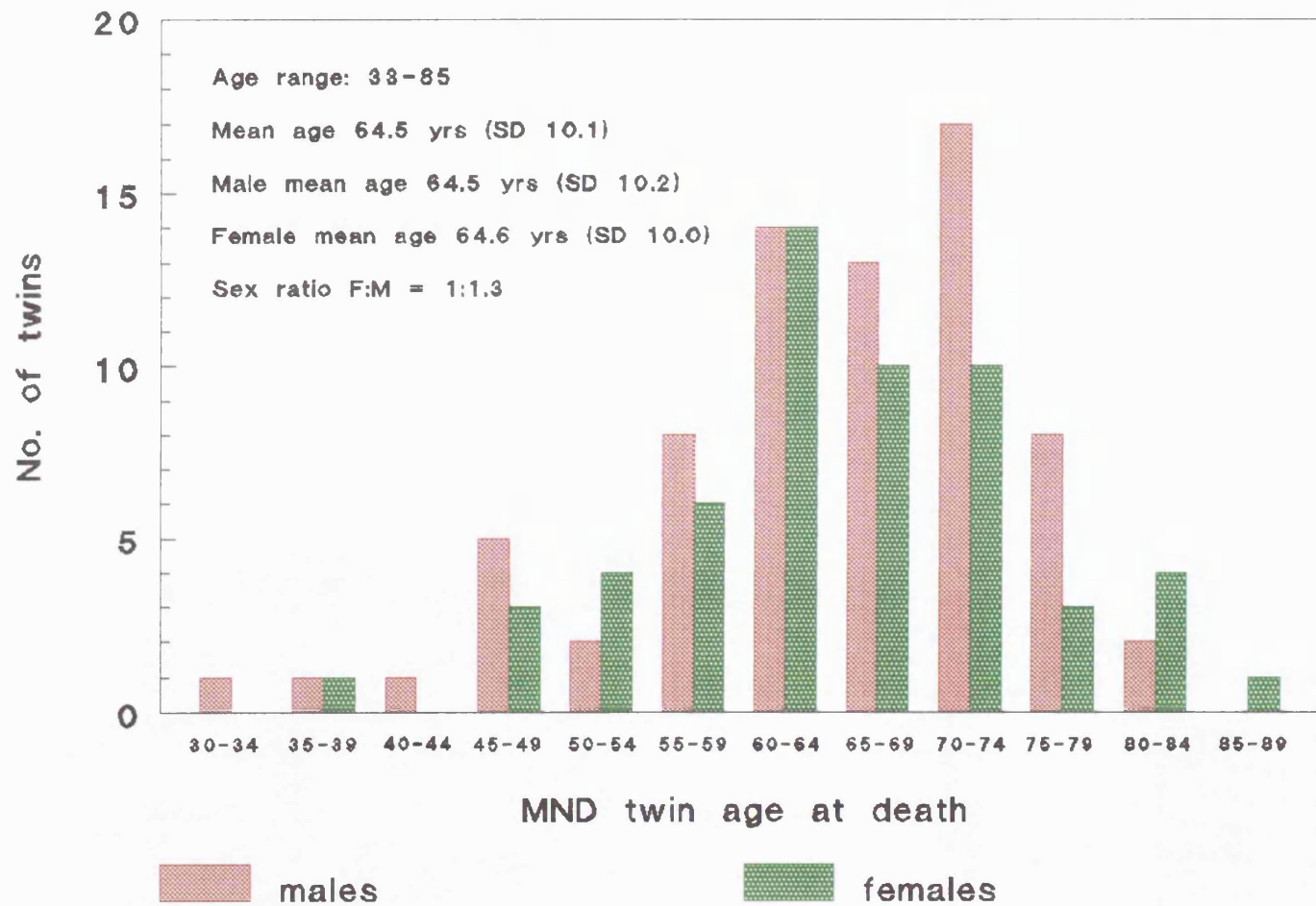
Fig.3   Age & Sex Distribution of MND Twin Sample

England and Wales, which recorded an average of **64.4%** like-sexed twins and **35.6%** unlike-sexed twins between 1938 - 1940.

Sex Distribution: Within the complete twin sample the sex distribution of the index twins consisted of **72** males and **56** females, giving a M:F ratio of 1.3:1. This was not significantly different from the sex ratio of the study population M:F = 1.2:1 (z = 0.384; p = 0.7) (figure 3).

(i) TWIN SAMPLE STATUS

Through contact with the relatives of the case twins, three of the emigrated co-twins were found to be alive, but the remaining two were classified as untraceable. This produced the following co-twin status: **58** living, **31** adult deaths, **29** infant deaths, and **10** untraceable (table 7).

Unfortunately, the twin distribution between the groupings was uneven (table 8). The unlike-sexed twins were over-represented among the infant deaths (**48.3%**) and untraceable co-twin pairs (**50.0%**), compared with the complete twin sample (**32.8%**). These differences however, were not statistically significant (z = 1.57; p = 0.12, and z = 1.1; p = 0.27 respectively). The reasons for this skewing can only be surmised. The sex distribution of the co-twins among the infant death pairs is similar to the whole sample, but females are over represented among the untraceable pairs (M:F 1:1.5), though this difference is not statistically significant (z = 0.90; p = 0.37). One reason for this bias among the untraceable co-twin pairs, was the inability to identify marriage registrations for females with common names, with the amount of information available. The other untraced co-twins may have emigrated. Following a systematic search of the public records, the untraceable co-twin figure was only **7.8%** of the total number of identified twin pairs.

The mean age at death of the index twins whose co-twins died as infants or were untraceable was **67.6 (SD +/- 10.7)**, age range **33 - 85** years. The greater mean age reflects the higher risk of infant death at the time when the older twins were born, and likelihood that the co-twin would have married or emigrated before the instigation of the NHS-CR. However, because these factors equally apply to the whole population, the surviving twin pairs must be representative of the remaining population.

Twin Sample Portion	No. of Twin Pairs	Like - sexed pairs			Unlike - sexed pairs		
		No. & Proportion	No. of males	No. of females	No. & Proportion	No with female Co-twin	No with male Co-twin
Complete Twin Sample	128	86 (67.2%)	50	36	42 (32.8%)	22	20
Living Co-twin Portion	58	45 (77.6%)	23	22	13 (22.4%)	8	5
Adult Death Co-twin Portion	31	21 (67.7%)	15	6	10 (32.3%)	3	7
Infant Death Co-twin Portion	29	15 (51.7%)	10	5	14 (48.3%)	8	6
Untraceable Co-twin Portion	10	5 (50.0%)	2	3	5 (50.0%)	3	2

Table 7: Twin Type and Sex Distribution of the Complete Twin Sample and Among the Four Different Groupings Differentiated by Co-twin Status.

73

(ii) PROBAND RATE FOR MND

The co-twins of two previously defined index cases, who also died from MND, were identified independently of their twin during death certificate processing. This increased the complete sample to **130** probands and co-twins, leaving **91**, when the probands with untraceable or infant death co-twins were removed.

The 91 probands and co-twins consisted of **68 (74.7%)** like-sexed probands and **23 (25.3%)** unlike-sexed probands. The sex distribution among the probands was **50** males and **41** females, giving a ratio of 1.2:1, and the mean age at death was **63.4 (SD +/- 9.6)**, age range **37 - 85** years.

(iii) PARTICIPATING TWIN PAIRS

Probands with Living Co-twins: Forty seven of the fifty eight living co-twins agreed to be interviewed. Of the remaining eleven, 2 lived abroad, access to 2 was denied by the GP because of ill-health (1 x senile dementia; 1 x psychotic depression), and 7 refused to participate. However, of these 11, relatives of 6 probands supplied some information about the twin pairs concerned, including probable zygosity. The remaining 5 pairs consisted of 2 unlike-sexed pairs; and the zygosity of the others was obtained from the co-twin, or their medical records, via their GP. These GP's also confirmed whether the co-twins were presently suffering from MND.

Probands with Adult Death Co-twins: Positive contact was made with the relatives of **19** probands where both twins had died. Attempts to contact the others were unsuccessful. The participating relatives were again able to recall some information regarding the life-styles of both twins, including probable zygosity.

Probands with Infant Death Co-twins: Attempts were also made to contact all the relatives of the probands within this group. The main reason was to establish if any of these probands came from families with FMND. If possible a sibling was recruited as a control for these probands, because apart from sharing the pre-natal environment, siblings are genetically equivalent to dizygotic twins, and if close in age, will have shared the same environment as children. This was done to enable a broader cross section of probands to be sampled and allow comparison with those whose co-twin lived through adulthood.

Six siblings were willing to be interviewed as controls. The data obtained from these pairings was treated separately at all times, but added to the twin pair data when appropriate. If

exposure to a particular environmental factor had produced interesting results, the sibling data was added to see whether the relationship was enhanced or weakened. This extra data proved a useful means of validating the findings as the sample size was relatively small.

Recruitment of Living Probands and Co-twins: During this study, details of 7 living probands and 1 who died in 1990 with a living co-twin, were sent by neurologists or the Motor Neurone Disease Association. Interviews were carried out with these twins, but they were not included in the genetic determination calculations as they were a non-population sample. The data obtained from the environmental questionnaire was treated in the same way as the sibling data.

3.2   RESULTS OF ZYGOSITY VERIFICATION

Applying Weinberg's rule to the complete twin sample for an approximation of the expected MZ and DZ twin proportions produces the following:

$$\text{The proportion of MZ pairs} = \frac{86 - 42}{128} = 34.4\% \quad (44 \text{ pairs})$$

$$\text{The proportion of DZ pairs} = \frac{2 \times 42}{128} = 65.6\% \quad (84 \text{ pairs})$$

Out of the 88 like-sexed probands identified, obviously the zygosity of the like-sexed twins among the untraceable (5) and infant death (15) co-twin pairs, and the adult death pairs for who no information was available (10), was not discernible. Among the remaining 58 like-sexed probands and co-twins, zygosity questionnaire data was available for 55, and analysis identified 25 MZ and 30 DZ probands and co-twins. The twins were classified MZ if they scored near maximum points on the questionnaire, along with a positive response to the 'like two peas in a pod' question.   All other scores were classified DZ.   According to the information obtained from the GP's of the non-participating living co-twin pairs this increased the proportions to 26 MZ and 32 DZ probands and co-twins. An equivalent group of unlike-sexed probands and co-twins was included in the genetic calculations (19 pairs), giving a total of 77 probands and co-twins (figure 4).

Unknown zygosity
adult death
co-twins   10

Monozygotic
living co-twins
18

Dizygotic unlike-sexed
adult death
co-twins   10

Dizygotic like-sexed
adult death
co-twins   5

Total No.
Probands
= 91

Monozygotic adult
death co-twins
8

Dizygotic Like-Sexed
living co-twins
27

Dizygotic unlike-sexed
living co-twins
13
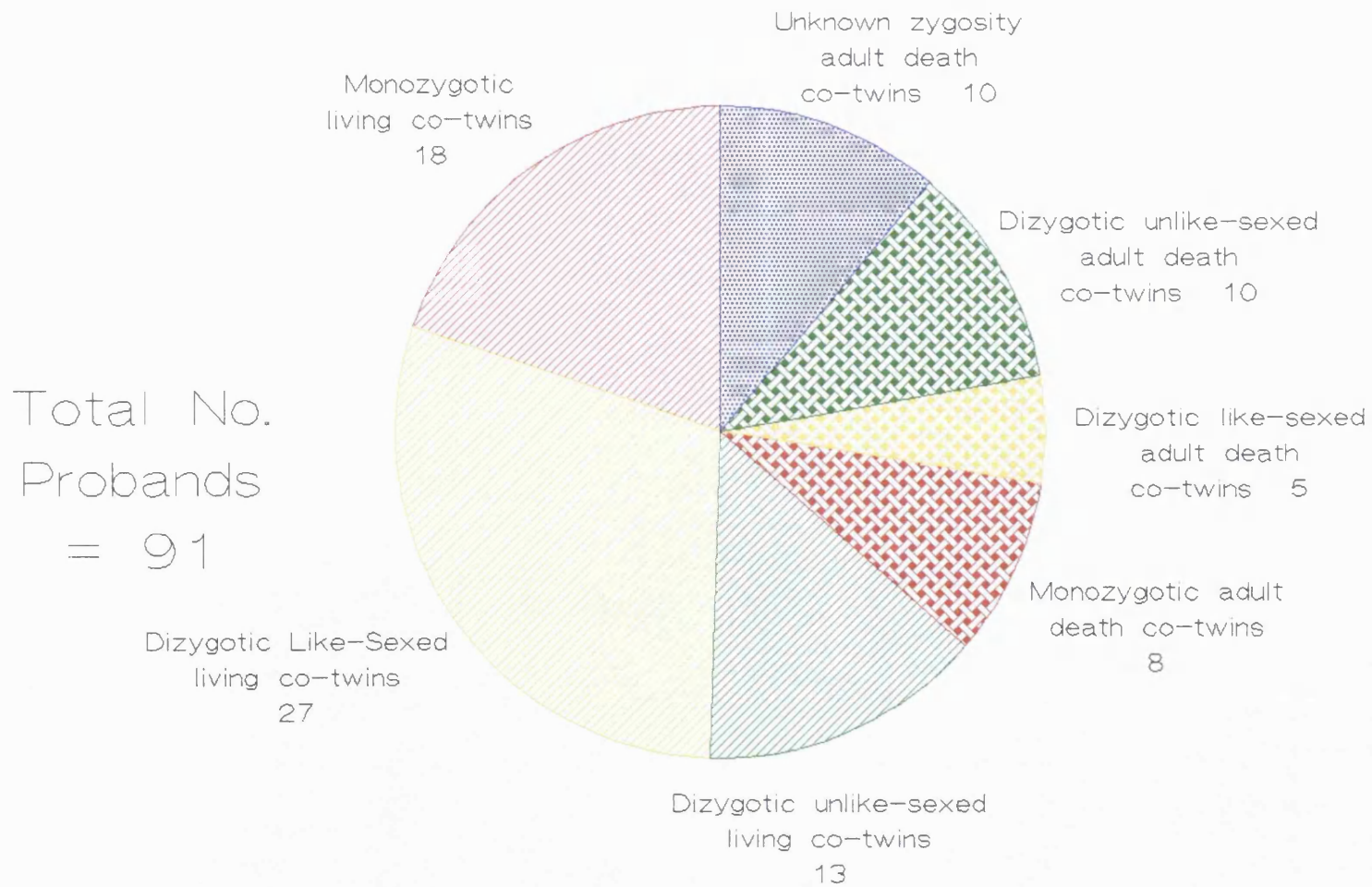
Fig. 4   Distribution of Proband Zygosity

3.3 DETERMINATION OF THE GENETIC CONTRIBUTION TO MND

At the time of the co-twin interviews, none presented with any symptoms that after further investigation were considered to be a feature of MND. In addition, none of the co-twins who died from other disorders prior to the study period, had undisclosed MND according to the relatives interviewed. Therefore among this twin sample, 4 MZ probands from 2 pairs were identified as concordant for MND, but 0 DZ probands and co-twins were found to be concordant.

Before a concordance rate for MND among MZ twins was calculated, two other factors had to be considered: 1) Whether any of the concordant probands had a family history of FMND; and 2) how many of the MZ pairs, where the co-twin had also died, should be considered discordant for MND.

Proportion of Twins from FMND Pedigrees: Among the seventy two probands, where a family medical history was taken, 5 probands (3 MZ and 2 DZ) from 4 families were identified with a positive history of FMND. In addition there was no known family history of FMND reported for 10 probands whose co-twin died as an infant. Therefore, the proportion of probands found to have a positive history of FMND was approximately 6.0%. In two families, a parent had died from MND; in one family a parent, and probably a sibling had suffered from MND; and in the fourth family, a maternal aunt had probable MND, and the daughter of the proband had diagnosed MND.

Only two probands from 1 MZ pair, among the four families identified, were concordant for MND. The other MZ co-twin had remained discordant, since the onset of his twin's illness for 6.5 years when interviewed, and will be followed up in the future. One like-sexed DZ co-twin had been discordant for 7.5 years, and one unlike-sexed co-twin died from acute myeloid leukaemia 5 years after the onset of the proband's illness.

Discordant Adult Death Co-twin Pairs: When considering discordance for MND among probands and their co-twins, temporal spread of the disease may be an important factor. No figures are presently available, though the variation in age of FMND sufferers, would indicate that MND has a long latency, and possible large temporal spread. Therefore following epidemiological advice, it was decided that when the co-twin died before the proband (3 MZ pairs and 11 DZ pairs), these should not be considered discordant for the disease when calculating the concordance rate.

Increased Risk for Co-twins: A very simple and crude estimation of the increased risk for the co-twins of MND affected twins can be obtained using the raw data. Obviously, an

unknown proportion of this risk will be the result of genetic factors, and shared environmental exposures.

The risk of developing MND among the general population of England and Wales is approximately 2:100,000 (see table 1), assuming MND is a random sporadic disease. However, among the non-FMND twin sample, who reached the defined risk period, this chance was 2:65 over 11 years; a risk **140** times greater than that for the general population. Among the MZ twins alone, the chance is 2:21 over 11 years; a risk **433** times greater than that for the general population. Therefore, this suggests that there is probably a significant genetic influence in the aetiology of MND.

MZ Proband Concordance Rate: A crude MZ proband concordance rate for this twin sample was calculated using the basic MZ proband numbers obtained [**15.4% (Standard Error –** **SE: 7.1%)**]. This rate increased to **17.4% (SE: 7.9%)**, when the MZ probands with a co-twin dying **before** themselves were excluded from the calculation. When estimating the genetic contribution to an apparently sporadic disease, any probands with a family history that strongly suggests dominant inheritance should be excluded (Emery 1986; Alberman and Macdonald personal communications). Two MZ probands met this criteria, which yielded an amended MZ proband concordance rate of **10.0% (SE: 6.5%)**. In turn this produced the following estimate for the MZ "correlation of liability" (Smith 1974) 'r' = **0.717 (SE: 0.130)**. These figures are based on <u>one</u> pair, but confidence that this figure truly reflects the genetic influence in MND is supported by the pair being female, therefore their disorder cannot have been Kennedy's Syndrome, and that there was no suspicion of any other affected family members. The twins died from a rapidly progressing MND within 20 months of each other aged 57 and 59 years respectively.

DZ Proband Concordance Rate: It was not possible to calculate a proband concordance rate for the DZ twins as no concordant pairs were found. This is probably an artifact of small numbers, which has arisen despite collecting <u>all</u> the twin pairs from the MND population identified, because MND is relatively rare. Therefore, unless MND was highly genetic, it would be very unlikely that a concordant DZ pair would be found. This situation has occurred in previous twin studies of rare diseases (Rutter et al 1990). Two logical alternatives can be applied to provide estimates of the DZ concordance rate so heritability calculations can be made. These are:

  1) Using the sibling rate for the disease calculated from all the siblings who lived to adulthood (> 20 years) among the twins families.

2) Calculating the DZ proband concordance rate for the twin sample obtained, assuming the next DZ pair identified would be concordant for the disease.

These two solutions can be used to provide a minimum and maximum boundary for the DZ concordance rate. Among the sibling data collected for the study twins, only 1 in 399 had probable MND, giving a minimum estimate for DZ concordance of **0.25% (SE: 0.25%)**. If it is assumed that the next DZ pair identified would be concordant for MND, a maximum estimate for DZ concordance would be **2.22% (SE: 2.20%)**. Using these figures to calculate the limits of DZ "correlation of liability", the true figure must fall between **'r'= 0.291 (SE 0.004)** (when utilising the minimum DZ concordance rate), and **0.525 (SE 0.101)** (when utilising the maximum rate).

Genetic Contribution to Sporadic MND: Unfortunately, without a "correlation of liability" estimation for the DZ twins, calculating the heritability of MND would be prohibited. However, by substituting in the maximum and minimum DZ "correlation of liability" estimates above, the range in which the true coefficient of genetic determination ('G') can be calculated. Using $'G' = 2(r_{MZ} - r_{DZ})$ (Smith 1974), 'G' ranges between **0.383**, when substituting the maximum DZ 'r', to **0.852**, when substituting the minimum DZ 'r'. Therefore the true coefficient of genetic determination for MND must lie somewhere between these two extremes.

A calculation commonly given to support the findings of twin studies is to compare the MZ and DZ pairwise concordance rates. In this case such a comparison is rather meaningless because of the small numbers of twins and 0 values involved, due to MND rarity, and not surprisingly the difference between the MZ and DZ pairwise concordance rate is not statistically significant ($z = 0.41$; $p = 0.68$). Neither is it when the two given alternative DZ rates are substituted. An alternative method of displaying the difference between MZ and DZ pairwise concordance rates as a ratio (Gottesman & Shields 1982) is also impossible with a DZ rate of 0/44. However, when using the two alternative DZ rates above, meaningful figures are obtained. If the maximum DZ concordance rate is substituted the MZ:DZ ratio = 2.25, and taking the minimum DZ rate the MZ:DZ ratio = 19.95.

3.4 RESULTS OBTAINED FROM THE ENVIRONMENTAL QUESTIONNAIRE DATA

Preliminary analysis of the case-control twin data revealed that because of small numbers, the most informative results from the environmental factor data, were those pertaining to the complete death discordant twin sample. The monozygotic twin data alone was found to be insufficient for identifying any reliable relationships, except for the variables which had a strong association for all the twin data.

Several possible risk factors for MND were identified when the environmental variables were analysed individually. There were also factors which appeared to be associated with protection from MND. The affects of adding the extra data from the sibling and living MND pairs on the results for these variables is given in brackets [...] (table 8).

Occupation: The broadest classification used that reflects occupation is 'social class'(classes I - IV). Comparing the intrapair social class distribution, the difference between the twins was not statistically significant when all 6 classes were considered at once. This was despite an individual Odds Ratios (OR) for class II = 0.50 (95% confidence interval (CI): 0.19 - 1.26), [OR = 0.48 (CI: 0.19 - 1.13)], and class III non-manual OR = 5.0 (CI: 0.88 - 66.240, [OR = 4.0 (CI: 0.93 - 25.74)]. In view of the professional / non-professional demarkation between the twin pairs, the social class distribution was divided into these two groups. Non-professional social class as a risk factor for MND yielded an OR = 2.0 (CI: 0.71 - 3.63), [OR = 2.25 (CI: 0.86 - 6.30)]. The difference is not statistically significant for the death discordant twins alone, but is nearing significance when the extra data is included (z = 1.76, p = 0.078).

All the occupations pursued during the working life of both cases and controls were compared, using the "Standard Occupational Classification" 1990. This classification firstly divides occupations into nine main divisions which are then further subdivided. The first subdivision covers jobs of a similar type; eg. all the occupations involved in education are classified under rubric 23 (representing division 2, subdivision 3). A further subdivision is available, but the relatively small sample size of this study, prevented use of this more detailed classification. The cases and controls were fairly evenly distributed among the nine broad occupation categories. However, some variation was detectable at the second level of classification.

Two occupational groupings produced OR's that could be of clinical value, though the numbers were too small for statistical significance. These occupational groups were "metal machining/fitting and instrument making trades" (rubric 51), with an OR = 2.5 (CI: 0.34 - 37.46), [OR = 3.0 (CI: 0.45 - 43.60)]; and "vehicle trades" (rubric 54) with an OR = 2.5 (CI: 0.34 - 37.46), [OR = 2.5 (CI: 0.34 - 37.46)].

Leisure Activities: Only data concerning three types of leisure activities were identified as risk factors for MND - car / vehicle maintenance, DIY, and participation in a group of crafts which involve a variety of chemicals.

Car maintenance was the most prominent risk factor yielding an OR = 7.0 (CI: 1.33 - 89.90), [OR = 5.33 (CI: 1.31 - 33.07)], which is statistically significant (z = 2.75, p = 0.006) for both sets of data.

DIY yielded an OR = 2.17 (CI: 0.70 - 7.48), [OR = 2.0 (C.I: 0.74 - 5.71)], which is not statistically significant, but maybe of clinical value. The difference in the frequency with which the case and controls carried out DIY type activities, supports this supposition. The cases were reported to undertake DIY type activities significantly more frequently than controls ($0.05 > p > 0.02$) when using both data sets. The increase in risk for MND with greater regularity of DIY activity yielded an OR = 1.57 (CI: 1.02 - 2.42), [OR = 1.47 - CI: 1.02 - 2.13)].

The crafts included in the group using any type of chemicals were upholstery, picture framing, photography, painting, model making, wine / beer making. This variable yielded an OR = 4.33 (CI: 1.02 - 27.58), [OR = 2.00 (CI: 0.69 - 6.25)]. The OR for the death discordant twin data is statistically significant ($z = 2.25$, $p = 0.024$), but not when the extra data is added. If wine / beer making is removed from the craft category, as the chemicals involved are of a different nature to those used in the other crafts, the adjusted figures yield an OR = 4.0 (CI: 0.66 - 54.92), [OR = 2.25 (CI: 0.55 - 11.22)]. Even though these O.R.'s are not statistically significant, they may represent clinical value.

Chemical Groups: Chemical exposure was analysed from two angles. During the interviews the twins chemical exposure was estimated using both occupational and non-occupational contact. Later, work orientated chemical exposure was estimated from the occupational history of the twin pairs using a database constructed by the Medical Research Council Epidemiological Unit at Southampton. This database provides information related to the likelihood and regularity of exposure to different chemical groups in a broad spectrum of specific occupations.

Paints: This was the only chemical group that was estimated to be used by more case twins than controls during their work. Taking the 'definite' and 'probable' classifications for the likelihood of exposure from the Southampton database for positive usage of paint, this yielded an OR = 3.75 (CI: 1.05 - 17.12, [OR = 3.75 (CI: 1.05 - 17.12)], which is statistically significant ($z = 2.29$, $p = 0.022$). When total paint exposure was considered, the twin pairs appeared more evenly matched. The measurement scale utilised was rather crude, but indicated that case twins were more regularly exposed to paint in total compared to their controls.

Wood Preservatives / Finishes: The number of pairs where one twin had more than just occasional exposure to this chemical group in any situation was very small, and therefore statistical analysis was not appropriate. However, it is interesting that among the pairs with one regular user of such chemicals (> once/week), the case twin was the more likely candidate.

Name of Variable	Odds Ratio (O.R.)	95% Confidence Interval Lower - Upper Limit	Level of Statistical Significance
Car / Vehicle Maintenance	7.00 5.33	1.33 - 89.90 1.31 - 33.07	0.006 0.006
Crafts Using Chemicals	4.33 2.00	1.02 - 27.58 0.69 - 6.25	0.024 N.S.
Paints Used in Job	3.75 3.75	1.05 - 17.12 1.05 - 17.12	0.022 0.022
Metal Machining / Fitting	2.50 3.00	0.34 - 37.46 0.45 - 43.60	N.S. N.S.
Vehicle Trades	2.50 2.50	0.34 - 37.46 0.34 - 37.46	N.S. N.S.
Non- Professional Soc. Clas.	2.00 2.25	0.71 - 3.63 0.86 - 6.30	N.S. N.S.
Petro- chemical Length/Time	1.49 1.69	0.78 - 2.82 0.97 - 2.94	N.S. N.S.
D.I.Y. Regularity	1.36 1.47	0.90 - 2.05 1.02 - 2.13	< 0.05 < 0.05

Table 8: **Positive Environmental Factors in Order of their Odds Ratio (O.R.) Value, with 95% Confidence Intervals and Statistical Significance.**

(The upper figures relate to the data obtained from the death certificate twin sample, and the lower figures are those obtained when the extra sibling and living twin data is included.)

<u>Petro - Chemicals:</u> This was a difficult chemical group to analyse because it is ubiquitous, and exposure extremely variable. Very few twins were considered to have 'no' exposure, though many reported only indirect contact. Therefore, during analysis only direct exposure was taken as positive contact and the estimations made of the degree of exposure were crude. The twins were equally proportioned among those with reported direct contact and 'no' contact. Degree of exposure was then analysed using two variables - regularity and length of time. When regularity was analysed by the GLIM programme, the results were inconsistent and did not prove amenable to standard statistical evaluation. Length of time however, did reveal a near significant trend for cases to more frequently experience long-term petrochemical exposure (> 15 years) compared to the controls. These patterns were seen using both the death discordant twin data and when the extra data was included.

<u>Medical History:</u> Experience of any disease by the cases did not increase the risk of MND. There were however, three disease groupings, as defined by the International Classification of Disease 9th edition, which were reported more frequently for the control twins compared to the case twins (ie 'protective' factors).

<u>Heart Disease:</u> OR = 0.20 (CI: 0.015 - 1.14), [OR = 0.29 (CI: 0.062 - 1.04)].
The death discordant twin data OR was statistically significant (z = 2.07, p = 0.039), as was the OR for the complete data set (z = 2.12, p = 0.034).

<u>Malignant Neoplasm:</u> OR = 0.125 (CI: nearing 0 - 1.21), [OR = 0.30 (CI: 0.045 - 1.36)]. Only the OR for the death discordant twin data was statistically significant (z = 2.06, p = 0.039).

<u>Endocrine Disorders:</u> OR = 0.143 (CI: nearing 0 - 1.44), [OR = 0.286 (CI: 0.02 - 1.82)]. These are not statistically significant, but may be of clinical value.

Two other factors related to medical history were of interest, The controls reported a greater number of surgical operations than the cases, OR = 0.79 (CI: 0.59 - 1.03), [OR = 0.78 (CI: 0.62 - 1.02)], which was just short of being significantly different. More controls also reported sustaining a 'head injury', (any injury to the face or skull), yielding an OR = 0.35 (CI: 0.11 - 1.04), [OR = 0.33 (CI: 0.12 - 0.83)], which was statistically significant for both the death discordant twin data (z = 2.09, p = 0.037) and the when the extra data was included (z = 2.65, p = 0.008).

Name of Variable	Odds Ratio (O.R.)	95% Confidence Interval Lower - Upper Limit	Level of Statistical Significance
No. Surgical Operation	0.79 0.78	0.59 - 1.03 0.62 - 1.02	0.06>P>0.05 0.06>P>0.05
Head Injury	0.35 0.33	0.11 - 1.04 0.12 - 0.83	0.037 0.008
Heart Disease	0.20 0.29	0.015 - 1.14 0.062 - 1.04	0.039 0.034
Endocrine Disorder	0.143 0.286	near 0 - 1.44 0.02 - 1.82	N.S. N.S.
Malignant Neoplasm	0.125 0.30	near 0 - 1.21 0.045 - 1.36	0.039 N.S.

Table 9: Negative Environmental Factors in Order of their Odds Ratio (O.R.) Value, with 95% Confidence Intervals and Statistical Significance.

(The upper figures relate to the data obtained from the death certificate twin sample, and the lower figures are those obtained when the extra sibling and living twin data is included.)

(i)   CONDITIONAL LOGISTIC REGRESSION MODELLING

'Head injury' was excluded from the conditional logistic regression model, because of the large number of twin pairs with missing data for this variable, and the likelihood that the great variation in reported 'head injury' was biased by the co-twins recall of their own injuries, but not their twins. Surrogate recall must be particularly problematic for this type of injury, where it is often a minor occurrence and sometimes leaves no visible sign. This left eight variables to be involved in construction of the conditional logistic regression model - professional/non-professional social class; car/vehicle maintenance; DIY regularity; occupational paint use; degree of petrochemical exposure; heart disease; and malignant neoplasm.

Data from 12 death discordant pairs which contributed to the individual variable analysis above, was not included in the conditional logistic regression model.

Fitting all eight variables to the model produced a highly significant value for the combined eight OR's $(0.01 > p > 0.001)$. This remained constant when different fractions of the data was used, for example all the death discordant twin pairs, the death discordant twins pairs where the co-twin was interviewed, and when the extra data was included. However, following manipulation of the conditional logistic regression model by fitting different combinations of the 8 chosen variables, only two were found that consistently contributed to the model at a statistically significant level. All the others were therefore removed from the final model. The two model variables were 'occupational paint exposure'; and carrying out 'car/vehicle maintenance' on a regular basis. Within the conditional logistic regression model selected, occupational paint exposure yielded an OR = 1.68 (CI: 0.96 - 2.94), [OR = 1.70 (CI: 0.97 - 2.96)]; and car/vehicle maintenance yielded an OR = 9.68 (CI: 1.23 - 74.47), [OR = 5.85 - CI: 1.31 - 26.12)]. The combined risk of these two variables with regard to MND was statistically significant $(p < 0.001)$ for the death discordant data and when the extra data was included.

In addition to these two variables there were two others, that when added to the logistic regression model together, contributed an increased risk of MND which was just short of statistical significance. These were the 'DIY regularity' and 'non-professional social class' variables.

(ii)   ENVIRONMENTAL FACTORS WITH NO APPARENT INFLUENCE ON MND

The factors which the case and control twins had very similar reported experiences of or exposures to were as follows:-

Birth order of the twins;

Degree of participation in sporting or physical activities after leaving school;

Contact with domestic animals, mainly cats and dogs;

Direct use, or indirect contact with the following chemicals, either in the workplace or at home: agricultural or garden chemicals; glues and adhesives; inks and dyes; cosmetics and hair solutions; industrial and domestic solvents;

Exposure to all the heavy metals listed previously;

Broad pattern of diet - intake of main food groups and frequency, including common beverages. Any food fads or long term diet restrictions.

Intake of alcohol over lifetime;

Use of illegal drugs, vitamins and herbal concoctions;

Use of tobacco over lifetime, including length of time and amount smoked;

Known bouts of infectious diseases, particularly viral, including poliomyelitis and personal contact with infected people;

Lifetime experience of immunisations and vaccinations to various diseases;

Known allergies - trigger and response.

Number of serious injuries (ie required hospital treatment) - fractures, abrasions, joint sprains;

Number of blood transfusions;

Past experience of mental illness, or periods of psychological distress causing anxiety or depression.

Experience of any other neurological diseases.

Residential Area: Many of the twin pairs lived within the same geographical area for most of the case twin's life, though a few pairs resided in more diverse locations. For most of the male twins, joining the armed forces during the Second World War, or for National Service, accounted for a large portion of the variation in residential location to their twin. In view of the relatively small sample size, the interviewees were asked to classify each of the areas lived into 'urban', 'semi-rural' and 'rural' for comparison. Again there was little difference between the length of time case and control twins spent in each type of location once they lived apart. The sample was too small for detecting any significant geographical variation from the population distribution across England and Wales.

S E C T I O N   I V

DISCUSSION AND CONCLUSIONS

The study's findings will be discussed under the following aspects:-

Methodological issues and twin studies.

The death discordant twin method.

Results of the environmental questionnaire.

Results of the genetic analysis.

Conclusions.

4.1 METHODOLOGICAL ISSUES AND TWIN STUDIES

Potentially, twin studies can be a useful research tool for investigating the aetiology of diseases, both genetic and environmental factors. There has been recently some debate as to the true value and interpretation of using twins to investigate the genetic influence in particular diseases. Phillips 1993 argued that twin studies which draw conclusions inferring genetic origins for diseases may be misleading. The premise is that the adverse prenatal environment of MZ twins, relative to DZ twins, will result in increased MZ concordance and fallacious overestimates of genetic aetiology will ensue. Many others however disagree (Leslie & Pyke 1993, Duffy 1993), and advocate the value of twins to medical research. For example, Macdonald (1993) points out that since MZ twins have greater within-pair variability in their prenatal environment, then this will lead to discordance and make MZ twin less alike. Methodological difficulties associated with conducting twin studies have justifiably resulted in criticism of their value (Bundy 1991). Many twin samples gathered are incomplete, skewed, and unrepresentative of the populations from which they were obtained, producing results which are meaningless and misleading. This has particularly been the case with twin studies into neurological diseases, as discussed in detail in the literature review. There are several factors which are important for a successful twin study with reliable results, such as the correct zygosity ratio, and ensuring the twin sample truly represents a defined population. The aim of the death discordant twin method was to satisfy these conditions when investigating a rare disease.

4.2 THE TWIN SAMPLE OBTAINED USING THE DEATH DISCORDANT
TWIN METHOD

The new methodology designed for this study has been demonstrated with success, creating the largest population-based sample of twins for the investigation of MND ever gathered worldwide. Many of the pitfalls associated with traditional twin studies, have been overcome, especially that of biased subject selection, which occurs when using some form of public appeal (Lykken et al 1978 & 1987), or when not all the twins within a population are

identified (Mumford et al 1992, Alpérovitch et al 1992). The twin sample collected for this study is probably complete and unbiased, and therefore representative of the study population. Corroboration is given by:

1) A total number of 131 index twins was identified among the 1979-89 MND death certificate population (born since 01/01/1900 = 9371 people), giving a ratio of approximately 1:70. The twins form a greater proportion of the study population than the first published birth incidence for twins in England and Wales (1938-1940), which was approximately 1:80 live births (OPCS Birth Statistics 1984). In view of the steady reduction of twin births in England and Wales since 1940, as the number and distribution of pregnancies among women have changed, it would be plausible that the twin ratio during the early part of the century was greater than the first published rate, therefore implying the MND twin sample is probably complete for the study population:

2) the proportion of like-sexed (67.2%) to unlike-sexed (32.8%) twins among the complete twin sample, is comparable with the first published ratio (1938-1940), 64.4% and 35.6% respectively:

3) the sex ratio M:F = 1.3:1, and age distribution of the twin probands at death (mean 64.5 years, SD +/- 10.1 years), were very similar to that found in the utilised MND death certificate population (M:F ratio = 1.2:1, mean 66.5 years, SD +/- 10.4 years):

4) the study ratio of DZ:MZ twins (2:1) found among those whose zygosity was established (77 probands), is the same as the estimated ratio in the general population (Emery 1986). Such a ratio has rarely been achieved in previous neurological twin studies, where MZ pairs are generally over-represented as demonstrated in the literature reviews (tables 4 & 5):

In most population-based twin studies, index twins are selected from hospital/medical records (Ebers et al 1986, Mumford et al 1992), and therefore are probably not representative of the diseased population as a whole. With this study method there is no selection bias, because the diagnosis of MND in the index twins will have been obtained via all the same routes as the study population. This method also allows the generation of a relatively large database of twins when a rare disease is being studied, with some information being gathered on 77 probands. In the three chronic neurological diseases often compared with MND, multiple sclerosis, Parkinson's disease, and Alzheimer's disease, only Mumford et al 1992 have obtained a larger number of subjects in a population-based twin study into multiple sclerosis, which has a prevalence rate 20 times larger than MND.

As with all research methodologies, the death discordant twin method has weaknesses. Attempting to overcome such difficulties had to be weighed against the available resources, and practical solutions needed to be found.

1) Accuracy of Death Certification: Reliance on the diagnosis certified at the time of death was one of the main problems with the death discordant twin method.   Therefore, the authenticity of the MND diagnosis in the probands could not be fully guaranteed.   However, in retrospective studies in Britain and U.S.A, 72% - 91% of known MND sufferers death certificates were found to quote the correct diagnosis (O'Malley et al 1987, Buckley et al 1983, Juergens et al 1980, Hoffman & Brody 1970).   To help confirm the legitimacy of the probands' MND diagnoses, the co-twins and/or relatives were asked to state the diagnosis given for the probands' illness when they were alive, and give a description of its symptoms and progression. Using information gained from the death certificates, medical records were also obtained where possible, though this proved more difficult with the increasing number of years since the proband's death.   All the available records were studied by a consultant neurologist (CHH) for authenticity of the MND diagnosis, and this led to the removal of three probands because of incorrect certification at death.

2) Dependence on Proxy Information: Another important weakness with this methodology was that information relating to the proband could only be obtained second-hand from their co-twin or relative.   Obviously, it would have been more satisfactory to have been able to interview the probands themselves.   However, as clearly demonstrated earlier, the rarity of MND would have involved using only a very small biased twin sample, whose information would have limited value. Certainly, the determination of the heritability of sporadic MND, which was one aim of this study, would have been impossible without this approach. Retrospective information gathering from diseased patients can also be problematic, because patients tend to emphasise the aspects of their lives which they think are important to the causation of their illness (Sackett 1979).   The possible result of such behaviour, is the erroneous identification of factors associated with a disease, when in fact they are not (a Type I error).   This was an important error to avoid, as there are so many different environmental exposures that have been associated with MND in the past, and the other purpose of this study was to pinpoint the most probable causes. When using proxy information the error more likely to arise is the overlooking of factors which in fact are associated with MND (Type II error), because the surrogate may not be able to provide detailed information about aspects of the probands lifestyle. In an attempt to reduce this problem the spouse or other close relative of the proband was interviewed if possible. The interviewing method had the above weakness but this was accepted in return for the opportunity to estimate the genetic contribution to sporadic MND using a large unbiased twin sample.

3) Loss of Twin Pairs from the Sample: The number of living co-twins traced (45.3%) was less than expected for three reasons that were only quantified by doing a formal search of the co-twins. These were:-

a) The rate of infant mortality between 1900-1950 resulted in many co-twins being dead (22.7%), with a disproportionate number being unlike-sexed twins.

b) With the age range for MND development being 45-75 years for most sufferers, some co-twins succumbed to other diseases before or shortly after the index twin (24.2%);

c) The advanced age of several female co-twins meant that many married before instigation of the NHS-CR in the late 1940's. Hence, it was nearly impossible to identify the correct marriage entries for women with fairly common surnames, given the limited amount of information available (5.5%).

The loss of twin pairs to the study because of co-twin infant death will not have biased the twin sample because this is a population phenomenon, affecting all born twins in England and Wales during the same time period. Unfortunately, only the total infant mortality rate was published during the birth years of the MND twins, which ranged from 15% in 1901 to 6% in 1940 (Nissel 1987). Obviously, mortality among twins was greater than that for singletons, but there is no evidence to suggest that development of MND in one twin was linked to increased infant mortality of their co-twin, above that of any twins born at the same time. The reasons for the greater loss of unlike-sexed pairs because of infant mortality can only be surmised, though the relatively small number of twins involved could account for this skewing. The remaining sample of twins (99) were still representative of their population, as any collection of adult twins within a defined population.

Early co-twin death in adulthood would remove unknown numbers of twin pairs from any conventional twin study. The effect is to produce a twin sample with a slightly lower average age than that of the population they represent. However, the methodology of this study allowed the inclusion of such pairs, and therefore produced a more diverse twin sample, though when the co-twin died prior to the proband, the pair had to be excluded from the concordance rate calculations. The first (a) and third (c) reason for twin loss when collecting a death discordant twin sample will diminish with time because of reducing infant mortality and increasing numbers of women who married post 1948, and thus identifiable.

4) Twin Zygosity: Determination of the twins zygosity was essential to the accurate calculation of concordance rates and thus heritability of sporadic MND. Classification of zygosity using immunological testing on blood was obviously not possible, instead a validated

zygosity questionnaire was used (Magnus et al 1983), supported by childhood photographs. This method produced no sets of answers, when taken as a whole, that were not clearly classifiable as MZ or DZ. Magnus et al 1983 had not attempted to classify twin zygosity using their questionnaire through a first degree relative, so validity of its use for determining the zygosity of the pairs when both twins had died is unknown. Nonetheless, there is no reason to believe that its accuracy should be vastly lower than when only one twin supplies the information.

5) Miscellaneous Sources of Possible Biases: During the selection process of the co-twins, there were several junctures where bias could have inadvertently been introduced into the sample, though none were systematic. These included human error during searching the birth, marriage and death indexes, the reaction of GP's to the study, and whether the co-twin agreed to be interviewed or refused. It has not been feasible to validate the fallibility of the index searching, but no co-twins were lost through GP indifference, and only 9 living co-twins were too ill or refused to be interviewed. A final potential source of bias could have arisen if the interviewer had preconceived ideas about the aetiology of MND, and thus unwittingly focused the interviews on supporting elements. In an attempt to prevent this a tightly structured questionnaire was followed during each interview.

4.3   ENVIRONMENTAL RISK FACTORS FOR MND

The results obtained from analysis of the environmental questionnaire strongly imply that exposure to petro-chemicals, paints and associated industrial chemicals greatly increase the risk of developing MND. Most of the evidence for this conclusion comes from the large Odds Ratio's (OR) obtained individually for activities that involve exposure to such chemicals. 'Car/vehicle maintenance' and 'occupational paint usage' produced highly statistical significant OR's individually and within the conditional logistic regression model. 'DIY regularity' and 'non-professional social class' individually produced OR's that were significant, or nearly significant at the accepted maximum 'P' value ($p < 0.05$) respectively, though they only produced a combined effect in the logistic regression model. For the other 4 variables ('crafts using chemicals', 'metal machining/fitting trades', 'vehicle trades', and 'length of exposure to petro-chemicals'), the OR's were large, but only two were nearing statistical significance individually, and all contributed very little within the conditional logistic regression model.

At face value the relationships between these environmental factors and MND appear fairly weak, because unfortunately the 95% Confidence Intervals (CI) of all the variable OR's are extremely wide, and in many cases includes 1.0, thus implying lack of precision. The main reason for this problem was small numbers, which is an unavoidable problem with any

investigation of a rare disease such as MND. In matched pair analysis, only the difference between the discordant pair data is considered, and for most variables investigated in this study, this involved only a small portion of the total sample. Therefore, only the two strongest variables ('car/vehicle maintenance' and 'occupation paint usage') had a lower CI value above 1.0, though all eight variables had OR's that could be of clinical value. Support for this is given by the fact that most of these OR's were statistically significant or nearly so, despite having CI's that suggested a spurious finding. The nature of the exposure in most cases was chronic, rather than acute, which as discussed earlier makes causative relationships very difficult to identify, and by definition any statistical associations weak.

From a statistical viewpoint, it is undesirable to investigate a large number of variables, when a comparatively small number of subjects are involved, because any statistical evaluation will be very weak. Also it can be argued that any positive relationship found between a variable and the study subject purely occurred by chance. Both these aspects can be applied to the case-control section of this study, and therefore a major criticism as the methodology failed to adequately address them. Unfortunately, the diversity of factors that have been suggested to have a causative relationship with MND and rarity of the disease, meant it was impossible to address these issues without altering the aims of the study. Most epidemiological surveys into rare diseases must be affected by the same statistical weaknesses, and this is certainly true among all the case-control studies into MND, and must be an accepted requisite when carrying out epidemiological research into rare diseases. If not, valuable information that is gathered under such conditions could be lost or ignored.

Interest in the possible role of noxious chemicals in the aetiology of MND has increased in recent times, though the evidence presented so far has been weak, causing much scepticism. Several studies of occupational data and MND have suggested a positive relationship between MND and solvent exposure (Hawkes et al 1989, Gunnarsson et al 1991, Chio et al 1991, Deapen & Henderson 1986), though the occupations involved have varied, and other chemicals would have been used as well. Unfortunately, solvent exposure alone did not appear to be a risk factor for MND among the twin sample. Solvents as a group are so widely used in both industry and for domestic purposes, that it is not surprising to find no direct relationship between solvents and MND. They are however important components used in conjunction with both petro-chemicals and paints, and may therefore have a positive relationship with MND under certain conditions. There have also been case reports of a MND type syndromes developing after acute exposure to specific chemicals such as ant pesticide (Pall et al 1987). The male preponderance seen among MND sufferers (The Scottish MND Research Group 1992, Yoshida et al 1986, Tysnes & Aarli 1991) supports the likelihood that industrial chemical exposure has a significant causative role, as men are more likely to carry out activities using noxious chemicals. However, the results of this study, suggest that direct contact with petro-

chemicals or paints may not be necessary if these chemicals are aetiologically important to MND. More subtle or hidden exposure could be sufficient, as many index twins did not report any direct contact, but may well have experienced significant degrees of exposure within working or local environments, of which they were unaware.

Gathering reliable data on chemical exposure over a person's lifetime is extremely difficult, especially identifying individual chemicals and quantifying the degree of exposure. People change their jobs, or the chemicals involved alter over time, as do the employee protection measures. Therefore, any retrospective epidemiological study can only hope to produce nonspecific findings, that suggest trends and highlight areas for more detailed study. Only prospective longitudinal studies can hope to produce detailed data, though for a disease like MND the problems associated with this type of study would make it prohibitive.

The possible association between exposure to petro-chemicals and paints with an increased risk of MND found in this study raises interesting questions relating to the cause of MND. Firstly, both petro-chemicals and paints consist of complex mixtures of individual chemicals, so is it specific shared derivatives within these two groups which are important, which are not found in the other chemical groupings investigated? Alternatively, could it be the combined effects of several chemical constituents in both groups, which may also differ from one another, that produces a neurotoxic cocktail? Secondly, how could exposure to such chemicals result in the pathology seen in autopsy material from MND patients? As discussed earlier several theories have been proposed to explain how degeneration of the motor neurones may be instigated. It is possible that the protective metabolic processes that detoxify petro-chemical and paint constituents which enter the body could become defective (Williams 1991) resulting in a slow accumulation of raw chemical or toxic metabolic byproducts such as free radicals (Mitchell et al 1987). Recently it has been discovered that there is tight genetic linkage between a marker for the FMND gene and one on chromosome 21q that encodes a cytosolic CU/ZN-binding superoxide dismutase (SOD1), involved in detoxifying a common free radical (Rosen et al 1993). Rosen et al 1993 hypothesised that alterations in the activity of SOD1 could result in the progressive accumulation of neurotoxic free radicals, resulting in motor neurone degeneration. Alternatively, it may be possible that unadulterated or partly metabolised constituents of petro-chemicals and paints could act as neuroexcitotoxins in vulnerable people, either directly or through changes to the homeostasis of the amino acid neurotransmitters (Plaitakis & Caroscio 1985, 1987, Rothstein et al 1990, Perry et al 1987).

As MND is a neurodegenerative disease which exhibits increasing incidence with age (Yoshida et al 1986, Lilienfeld et al 1989, Hawkes et al 1992), any environmental influence on its development is more likely to be of a chronic nature, possibly over long time periods. Alternatively, the ageing nervous system may become more sensitive (Calne et al 1986),

particularly the motor neurones, to environmental assaults, so smaller amounts of chemicals entering the body over time inflict increasing damage. Therefore, the time scale for MND to become overt would be shorter.

4.4 NEGATIVE ENVIRONMENTAL RISK FACTORS

The findings of this study do not support the positive role of many environmental factors in the development of MND, suggested by the authors of previous epidemiological studies.

Viruses: The possibility of viruses playing a role in the aetiology of MND was investigated from several different angles. There was no indication from the study's findings of a viral cause for MND following vaccination using attenuated viral material. For most of the twins their experience of immunisation was influenced greatly by whether or not they had been members of the armed forces around the time of the Second World War, but generally both twin members were equally affected. Most twin pairs had received tetanus and small pox vaccine, but very few voluntarily travelled outside Europe during their lives. Recall of receiving polio vaccine at any time was very poor, so analysis was not possible. The possibility of viral contamination following the receipt of blood transfusions also proved negative.

Prolonged contact with live and dead animals (Hanisch et al 1976, Tarras et al 1985) and consequential viral infections that could possibly lead to MND, was also unsupported by this twin study, because similar numbers of MND twins and co-twins lived with domestic pets, and handled animal carcases as adults. Other case-control studies have also found no excess of animal contact among MND patients (Mulder et al 1983, Norris & Padia 1987).

The hypothesis put forward by Martyn et al 1988 regarding latent poliovirus infection and MND in later life is not supported by the experience of poliomyelitis reported for the twin pairs. The similarity of the twins experience could partly be the result of living together as children and sharing similar environments, during the time period when poliomyelitis was a common infectious disease. As many viral infections are much more commonly contracted in childhood the twins had similar histories of these illnesses too. Therefore, these may be factors for which the twins environment was too similar for revealing any differences. However, the likelihood of adverse effects being revealed 40 - 50 years after viral contamination of the motor neurones seems improbable, unless there is a secondary assult on already weakened motor neurones (Martyn et al 1988). Also at present there is no supporting microbiological or immunological evidence (Bharucha et al 1983, Weiner et al 1980, Kurent et al 1979).

Even though the findings of this study and many others do not support a viral hypothesis for the causation of MND, there have been reports of Creutzfeldt-Jakob disease developing in a tiny proportion of patients who received cadaveric growth hormone (Ellis et al 1993), following incubation periods ranging from 8 to 28 years (Preece 1991). This disease is caused by infectious agents called prions, which may have been transmitted through the hormone material. If an infectious agent is responsible for causing MND, then the characteristics of prions make them a possible candidate, though so far there is no pathological evidence.

Heavy Metals: The negative relationship found in this study between heavy metals and MND is not surprising, as evidence for any connection between these two is rather questionable (Section 1.6 (ii)). Several different metals produce motor neuropathy following acute poisoning episodes (Raffle et al 1987) that are reversible (Campbell et al 1970, Adams et al 1983). Retrospective study of heavy metal exposures is difficult to investigate, as many different metals are hidden elements of both domestic and industrial items commonly used. Therefore only overt handling of metallic products is generally recalled by the interviewee. This was found to be the case during this study, though hidden sources of metals were also explored by the interviewer. Often exposure from such sources could only be recorded as possible.

Identifying variation in lead compound exposure is particularly difficult to determine, especially for older people. This is because lead was extensively used in the domestic environment, for example in water piping and paint, so everyone at sometime would have experienced chronic low dose exposure. This was the situation found among the twins. Both mercury and aluminium also have uses that affect large proportions of people; for example, mercury alloys have been one of the main constituents of teeth amalgams (Nichols 1984), and aluminium has been widely used in the construction of affordable cooking utensils. Therefore, determining any significant difference in exposure levels between the twins for these two metals was also not possible. Even studies that have compared the heavy metal concentrations within blood and tissue between patients and controls (Currier & Haerer 1968, Mitchell et al 1984, Kurlander & Patten 1979), have produced conflicting results, and the reliability of the analytical techniques used have been questioned (Tandan & Bradley 1985(b)). Considering these aspects, chronic poisoning by any heavy metals is unlikely to be a significant factor in the development of MND for the majority of people.

Physical Strain and Trauma: As MND is a degenerative disease, and anterior horn cells are lost during the normal ageing process (McComes et al 1973), accelerated ageing caused by physical strain and trauma leading to MND is an attractive hypothesis. Unfortunately, the data obtained during this twin study does not support this theory, as will now be discussed.

There is often within-pair variability in the birth weight of twins (Phillips 1993) suggesting one twin has developed physically at the expense of the other, and possibly leaving the smaller twin at greater risk of disease later in life (Barker 1992). Obviously it was not possible to find out the birth weight of the twins during this study, but records of birth order were available, which may provide a possible indicator of differing physical attributes. Among the entire twin sample (128 pairs) the index twins were equally distributed between being the first and second born twin. This variable does not support the premise that pre-natal physical status is an important factor in the development of MND in adulthood.

In contrast to several previous case-control studies into MND (Felmus et al 1976, Kurtzke & Beebe 1980, Provinciali & Giovagnoli 1990, Gunnarsson & Palm 1984), the index twins were not reported to participate in strenuous sporting activities to any greater degree than the co-twins. Likewise, they were not found to be employed in occupations requiring hard manual labour any more frequently. Related to physical strain is trauma, both accidental and planned. Index cases have been reported to have experienced an excess of "Mechanical injuries" and severe electrical shocks before the onset of MND (Kurtzke & Beebe 1980, Gawel et al 1983, Gallagher & Sanders 1987, Deapen & Henderson 1986). Unfortunately, these findings were not replicated during this study, and the converse found with the co-twins reporting more frequent surgical operations and 'head injuries'. The statistically significant Odds Ratio (OR) for 'head injury' to be a 'protective' factor [$z = 2.09$, $p = 0.037$; ($z = 2.65$, $p = 0.008$)] is most probably the result of recall bias on behalf of the co-twins remembering their own injuries, but not their twins' injuries. This type of bias is not surprising, since many injuries to the head are of a minor nature which are easily forgotten, especially when they happened to another person. The greater occurrence of surgical operations among the co-twins compared to their index twin could also be the result of recall bias, but this seems less likely with a more dramatic event. Therefore, the index twins did not appear to experience increased neuromuscular 'wear and tear' leading to accelerated ageing of the motor neurone pathways compared to their co-twins. However, it is possible that this conclusion is misleading since if 'head injuries' were underestimated for the index twins, the same could be true for other physical injuries. The implication being that this study may have erroneously missed an increased risk of MND following some forms of physical trauma, though there is no indication that the negative finding for excessive strain and 'wear' could be a misinterpretation.

Ingestion of Foods and Drugs: This was felt to be an important area to investigate in view of the potentially neurotoxic chemicals found in many food items such as pulses and nuts (Spencer et al 1986(a), 1986(b), Nunn - personal communication) and the role diet has on health and protection from disease. The broad categorical data obtained regarding diet of the twins revealed no interesting differences between the pairs. This was probably an area which should have been removed from the questionnaire, because of all the problems and

shortcomings associated with investigating dietary habits retrospectively (Bingham 1987, Marr 1971). The two greatest problems in this particular study were:-

1) This was the area in which the co-twins felt the least confident in answering on behalf of their twin, meaning many pairs had only one set of data instead of two.

2) Even though data was gathered on the dietary habits of the twins during the index twins later life, it is questionable whether this was the most appropriate time period to study.

Eating habits have changed the most radically during the last 10 - 20 years, with increasing variety and interest in different and foreign food stuffs, and earlier eating patterns may be more important. A study carried out in 1982 however, suggested that food habits may remain fairly stable during adult life (Møller Jensen et al 1984). Only a tiny number of twins were reported to have very restricted or strange dietary habits, which individually may have been relevant to their disease, but collectively meant nothing.

Alcohol, tobacco and 'street' drugs all have powerful effects on the nervous system. The data from this study confirmed the general belief that alcohol consumption is irrelevant to MND, but hinted at the possibility that tobacco consumption may be neuro-protective for MND. A similar concept has been suggested for Parkinson's disease and Alzheimer's disease (Ward et al 1983, Brenner et al 1993). The evidence was contradictory, because more co-twins reported having ever smoked on a regular basis, but the index twins tended to be the heavier smoker within a pair. This finding could have purely been the result of co-twin bias in reporting tobacco consumption, which they may have under-estimated for themselves, as smoking is generally perceived as a 'bad' habit. Interestingly however, a case-control study into Alzheimer's disease, found the greatest risk reduction was among those who smoked, but only to a small degree (Brenner et al 1993), and suggests a dose-dependent protective response. No members of any twin pairs were reported to have indulged in 'street' drugs, though both members of one discordant female monozygotic pair had fallen victim to benzodiazepine addiction. It would be unlikely that 'street' drug abuse would contribute to the disease process of this population of MND sufferers, as the fashion and availability of such drugs is a more recent phenomenon.

Association between MND and other Physical/Psychological Disorders: An increased risk of MND was not found to be associated with any previous medical disorders as highlighted in earlier studies, particularly neoplasms (Brain et al 1965, Chio et al 1991), thyroid disease (Appel et al 1986, Chio et al 1991, Armon et al 1991), and gastro-intestinal problems (Chio et al 1991, den Hartog Jager 1987). The findings of this study detected a converse

relationship, with heart disease, neoplasms and endocrine disorders being far more frequent among the co-twins, and thus being negative risk factors for MND. Whether these findings really represent a protective role against MND when other disease processes are present is debatable. It could just be an inevitable finding, as these diseases are the most common ones found in older age groups leading to mortality, which the twin sample represents. The index twins could have died before such disorders could develop or be diagnosed. Alternatively, perhaps this data indicates that there are some common physiological processes occurring during ageing, that lead to disorder of the most vulnerable organ/system first. Interestingly, the proportions of co-twins reportedly affected by heart disease and neoplasms were however smaller than the those found among the total population deaths in England and Wales (Mortality statistics for 1985) for these diseases.

Among the 19 co-twins who gave a history of heart disease, only 4 died prior to the index twin, and could arguably have contracted MND if they had lived to a greater age. On the other hand, 5 additional co-twins have developed heart problems since the index twins' death. A similar pattern was seen among the co-twins who developed malignant neoplasms. Three died before the index twin, but a further 3 have been diagnosed since the study interviews, and have subsequently died recently.

Obviously, it is impossible to assess if there is any increase in the incidence of neurological disorders among family members of MND patients, when the twins share identical relatives. There was however only 1 twin pair, who had a parent who was reported to have suffered from Parkinson's disease, and 12 twin pairs who had a parent who developed some degree of "forgetfulness and confusion". Unfortunately, it was not possible to determine which of these had Alzheimer's disease, as this categorisation was partly dependent on the co-twins opinion, rather than a definitive diagnosis. No excess of previous neurological or psychological problems among the index twins, compared to the co-twins was detected, which has been the conclusion of other case-control studies (Armon et al 1991, Leone et al 1987). This suggests that the association of sporadic MND with other neurological diseases is most probably a chance occurrence. The more recognised link between MND and dementia in a small proportion of patients (Myrinathopoulos & Smith 1962, Editorial Lancet May 26 1990) has possibly a stronger relationship in view of the frequency of dementia among Chamorro Indians suffering from Western Pacific MND, but is also concomitant with the increasing numbers of very old people (> 75 years) being diagnosed as having MND, and their increased risk of dementia.

Immunological Responses and MND. Only a very crude assessment was made of the subjects immunological functioning during this study. This was achieved by investigating any history of allergic responses, but there was no difference in the number, or types of known allergies

between the index and co-twins. Even though this is a very superficial way of assessing immunological mechanisms, it does indicate that the capacity of the twins to produce inappropriate immunological responses is very similar, and does not support the immunological hypothesis (Drachman & Kunel 1989, Antel et al 1990).

MND and Metabolic Disorders. Abnormal carbohydrate metabolism does not appear to be associated with MND among the twin sample, based upon a diagnosis of diabetes mellitus, though these have been linked earlier (Saffer et al 1977, Reyes et al 1984). None of the index twins were known to suffer from type I or type II (maturity onset) diabetes, though five co-twins developed type II diabetes in later life, suggesting again a possible lower risk of MND in the presence of another chronic progressive disorder.

Likewise there were no indications among the twin sample to suggest that calcium metabolism is disrupted in MND patients. Contrary to some previous case-control studies (Felmus et al 1976, Kurtzke & Beebe 1980, Provinciali & Giovagnoli 1990), the index twins were not reported to have sustained more frequent, or larger numbers of skeletal fractures than the co-twins. This finding is supported by the results of other investigations (Deapen & Henderson 1986, Pierce-Ruhland & Patten 1981, Armon et al 1991), increasing the evidence against chronic calcium deficiency leading to sporadic MND, though this is one of the more favoured hypotheses regarding Western Pacific MND (Gajdusek 1990, Yase 1988).

Geographical Location and MND. There was no evidence of geographical clustering among the index twins based on residential post codes throughout life, though the sample was probably too small to detect this phenomenon in a rare disease. Similarly, the index twins were not found to have resided any longer in either urban or rural settings compared to the co-twins. This implies that agricultural practices, such as crop spraying with various chemicals, is not associated with an increased risk of MND.

4.5 THE GENETIC ELEMENT OF MND

The monozygotic (MZ) proband concordance rate of 10.0% (0.10) obtained for this sample of monozygotic twins is quite small, but to be expected in a disease like MND which has a tiny population frequency equalling 0.005% (5 per 100,000). It has been stressed that this does not mean heritability is negligible (Smith 1970). In rare diseases the concordance rate for monozygotic twins will be low, unless genetic factors have a major role in determining disease manifestation. An indication of the importance of genetic factors in the development of sporadic MND is given by the "correlation of liability" (Smith 1974) calculated for the MZ twins 'r' = 0.717 (SE: 0.130). This figure implies that the shared environmental and genetic

factors affecting both the twins together have a fairly large contribution in determining whether an individual person develops MND. Unfortunately, the "correlation of liability" estimation is less reliable when the disease frequency is extremely small. Therefore, the calculated figure for the MZ twins is probably an overestimation of the degree to which shared factors determine the manifestation of MND, though it clearly demonstrates a significant contribution. The relatively small MZ twin sample means the standard error quoted for the MZ "correlation of liability" is large, which also indicates imprecision, but does not negate the overall interpretation. The MZ proband concordance rate being 433 times greater than that expected for the general population supports this conclusion.

Under the same circumstances, it is not surprising that no concordant dizygotic (DZ) twins were found among the 44 DZ pairs. A much larger twin sample would have been required for a concordant pair to have been revealed. The two alternative methods used to produce substitute estimations for the DZ proband concordance rate and "correlation of liability" provided an indication of the true DZ figures. Unfortunately, the differences in the two sets of substitution calculations created a very wide range in which the DZ proband concordance rate and "correlation of liability" must fall. The extremes of this range therefore represent rather opposing interpretations of the controlling elements in the manifestation of MND.

If the true DZ figures are nearing the minimum extreme for the proband concordance rate 0.25% (0.0025) and "correlation of liability" ['r'= 0.291 (SE 0.004)], then this implies that the combined affects of shared genetic and environmental factors only have a (small) influence on the development of MND in DZ twins. If these figures for DZ twins, in conjunction with the equivalent MZ estimations, are used to calculate the coefficient of genetic determination for MND ['G'= $2(r_{MZ} - r_{DZ})$ (Smith 1974)], then a large value for 'G' is obtained ('G' = 0.852). Assuming both types of twins are equally influenced by their shared environment, then the difference in disease manifestation must be due to the genetic component of the "correlation of liability". In this case suggesting genetic factors have a very important role in MND.

Conversely, if the maximum estimations of the DZ proband concordance rate 2.22% (0.0222)] and "correlation of liability" ['r'= 0.525 (SE 0.101)] are closer to the true DZ figures, then a different conclusion is drawn. The coefficient of genetic determination in this case has a much lower value ('G' = 0.383), because the MZ and DZ "correlations of liability" are closer. This indicates a greater role for shared environmental factors in the manifestation of MND, and therefore less genetic influence.

The true coefficient of genetic determination for MND probably lies somewhere around the middle of these two extremes (0.852 > 'G' > 0.383), indicating a moderate, but significant contribution of genetic factors in the manifestation of MND. Unfortunately, it has not been

possible to demonstrate conclusively the degree to which genetic factors are influential. This has occurred because of the rarity of the disease resulting in small numbers of twins, and low concordance rates, and the weakness of the available analysis techniques when being applied under such circumstances.

The epidemiological pattern of MND with a large age range, increasing occurrence with age, and possible large temporal spread means that these are important considerations in relation to the concordance rate and corresponding "correlation of liability" for the MZ and DZ twins. The present figures could very well increase with time if any of the living unaffected co-twins succumb in the future. Therefore, this study provides a minimum estimation of the true figures, and only time will reveal the need for any alterations.

(i)   FAMILIAL MOTOR NEURONE DISEASE

The percentage of probands who had a family history of MND (6.0%), which followed an autosomal dominant inheritance pattern, was very similar to that found in previous studies (5% - 10%)(Li et al 1988, Tandan & Bradley 1985). Perhaps one of the most interesting occurrences is that among the two families with MZ probands, only one pair have both succumbed to the disease so far. The other pair had remain discordant for MND up to interview over 6.5 years, and are still discordant after a further two years. Three possible explanations for this are: 1) the discordant MZ pair were not in fact monozygotic twins despite evidence that they were, but unfortunately this can not be proven. 2) FMND is not the result of a single gene defect, but multiple genetic factors, that are not all shared by monozygotic twins, because some occur during cell division after the gamete has divided. 3) Different intra-uterine influences affecting the manifestation of the inherited factors, and protecting one twin from the disease. The published findings of large FMND linkage studies, support a multiple gene defect in FMND (Siddique et al 1991), because so far only a strong link has been achieved between a FMND marker and a gene that encodes a cytosolic CU/ZN-binding superoxide dismutase (SOD1) found on chromosome 21q, among a small proportion of affected families (Rosen et al 1993). For the other families no such link can be established, and instead alternative much weaker loci of interest have been revealed and are presently under scrutiny (Siddique et al 1989).

4.6   CONCLUSION

The study provides:- 1) Validation of a new methodology that facilitates the collection of a large twin sample in rare diseases, that is truly representative of the chosen population.

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2) Collection of the largest twin sample reported worldwide for the investigation of MND.

3) Evidence to implicate industrial chemicals, particularly the constituents of paints and petrochemicals, in the aetiology of MND.

4) Evidence that there is a fairly strong genetic influence in the aetiology of sporadic MND, leading to a predisposition for MND which is triggered by one or many exogenous factors.

The findings of this study may indicate that the aetiology of sporadic MND is heterogeneous, and that a combination of both multiple environmental and genetic factors are probably required before the disease is manifested. The characteristics of MND mean any epidemiological study can only produce tentative findings, which hopefully will be valuable in guiding subsequent investigations. Perhaps such investigations should concentrate on hypotheses that accommodate both genetic and environmental facets together. The environmental factors found in this study to exhibit an increased risk for MND, collectively suggest that industrial chemical exposure, particularly petro-chemical and paint products are the most likely agents to consider as aetiological elements in MND. This could only be proven by much more detailed epidemiological and physiological investigation. It is also disappointing that the degree of genetic influence in sporadic MND was not demonstrated more conclusively, but by following the twins via the NHS-CR for any future MND concordance, the genetic element will possibly become clearer.

The value of the new methodology developed for this study remains doubtful, particularly for the assessment of environmental risk factors. It may be more relevant to other diseases with a prevalence rate which exceeds 20 per 100,000. The searching time and delays would be reduced if only people born after 1910 were used, or even better after 1925, because the birth indexes contain better information, or most women would have been registered at NHS-CR under their maiden name. Obviously, its success in relation to other diseases would be dependent on the validity of death certificate diagnosis for that disease. It has been shown for example, that for a disease like Alzheimer's disease this is not a suitable approach to take (Martyn & Pippard 1988) because people do not usually die from the direct effects of the illness. However, where the disease is more distinct and death certificate diagnosis reliable, such as multiple sclerosis (Kurtzke & Lux 1985), or myocardial infarction, this twin method could be a valuable way of collecting an unbiased population sample. Previously this has not been achieved in any twin studies into progressive neurological disorders.

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S E C T I O N 6

APPENDIX

TWIN STUDY QUESTIONNAIRE

IDENTIFICATION CODE: _____

DATE OF INTERVIEW: _____

[Source of information if twin is DEAD]_____

[Relationship to twin] _____

GENERAL PERSONAL DETAILS

1) Please could we start with some basic personal details:

Surname _____   Maiden Name _____

First Names _____   Gender _____

Address _____

_____ Telephone Number _____

Marital Status _____   Age _____   Date of Birth _____

Husbands Occupation _____
    (Females only)

Twins name _____

Gender _____   Marital Status _____   Date died _____

Spouse living **YES // NO**   Possible interview _____

Address _____

_____ Telephone Number _____

Twin's Husbands Occupation _____
    (Females only)

ZYGOSITY

2) Were you and your twin the same sex?   **YES // NO** (Go to question 3)

2.1) During childhood were you and your twin considered to be alike as **"two peas in a pod"** or only to have the likeness of two brothers or sisters?

    "Two peas in a pod" _____   As brothers/sisters _____   Don't Know _____

2.2) Did any of the following people have difficulty in telling you and your twin apart when you were children?

	Frequently	Occasionally	Never	Don't Know
a] Teachers	_____	_____	_____	_____
b] Strangers	_____	_____	_____	_____

2.3) In your judgement, to what degree were you and your twin similar in the following features. (Tick appropriate column)

	The Same	Almost The Same	Different	Don't Know
a] Hair Colour	_____	_____	_____	_____
b] Eye Colour	_____	_____	_____	_____
c] Voice	_____	_____	_____	_____
d] Dexterity	_____	_____	_____	_____
e] Muscular Strength	_____	_____	_____	_____

2.4) Do you believe that you and your twin were identical or non-identical?

Identical _____ Non-identical _____ Don't Know _____

2.5) Which, if any of the following, are your reasons for giving that answer.

a] _____ You both looked alike

b] _____ You didn't look alike

c] _____ Your parents were told that you were identical

d] _____ Your parents were told that you were non-identical

e] _____ There was only one placenta

f] _____ There were two placentas

g] _____ You both had the same blood group

h] _____ You both had different blood groups

i] _____ Other reasons. Specify

_____

3) Please would you list all your brothers and sisters in the order they were born:

Birth Order . Gender . Age . If Died - Age Died
1
2
3

4	
5	
6	
7	
8	

BRIEF LIFE HISTORY OF BOTH TWINS

Some of the questions I will be asking you during this interview will involve you trying to remember events that may have happened many years ago. To try and help you recall these things, I am going to first ask you some brief questions that focus on main life events, so I can use your answers to stimulate your memory later on.

4.0) At what age did you first go to school? _____yrs old   What year was this? _____

4.1) Was first school close by to where you lived?   **YES // NO**

    (IF NOT) - Where was your school situated?

_____

4.2) Did your twin go to the same school?   **YES // NO // DON'T KNOW**

    (IF NOT) - Where was your twin at this time?

_____

5.0) Did you change schools   **YES // NO // DON'T KNOW**

    (IF YES) - Can you tell me how old you were then, the year and where your new schools were located?

Age	. Year	. Close to Home	. If not-Where	. B
a]				
b]				
c]				

5.1) Did you board at any of your schools? **YES // NO** (IF YES - Mark above)

5.2) Did your twin also attend the same schools? **YES // NO // DON'T KNOW**

    (IF NOT) - Where was your twin at this time?

_____

6.0) At what age did you leave school? _____ yrs old

6.1) After leaving school did you go straight into a job or looking for work; or instead move onto some form of further education?

WORK _____ (go to question 6.3)

FURTHER EDUCATION _____ (go to question 7)

6.2) Did you ever go to college/ university full-time later in life? **YES // NO**
(**IF YES**) Where did you go to college or university?

_____

6.3) When was this? _____ to _____

6.4) What did you study? _____

6.5) At what age did your twin leave school? _____ yrs old

6.6) Did your twin go to college/university after leaving school? **YES // NO // DON'T KNOW**

(**IF NO**) - Did he/she attend full-time further education later? **YES // NO**

(**IF YES**) - Where did your twin attend college/university?

_____

6.7) When was this? _____ to _____

6.8) What did he/she study? _____

7.0) When did you start your first job after leaving school or college?

_____

7.1) During your working life did you ever have to change your job through redundency?

YES // NO

How many times? _____

When was this? _____

7.2) Have you ever experienced a period of unemployment? **YES // NO**

(If yes) How many times? _____

When were you unemployed and for how long?

_____

_____

7.3) When did your twin start his/her first job after leaving school or college?

_____

7.4) During your twin's working life did he/she ever have to change his/her job through redundancy?

YES // NO

How many times? _____

When was this? _____

7.5) Did your twin ever experience a period of unemployment?   **YES // NO**

(If yes) How many times? _____

When was he/she unemployed and for how long?

_____

_____

8.0) Are you, or have you in the past, been married? **YES // NO** (If no - go to question 8.3)

Have you been married more than once? **YES // NO** (If yes - go to question 8.2)

8.1) When were you married? _____   How old were you? _____

Are you married at present? **YES // NO**

(If no) Is that because of widowhood / divorce / separation / other

When did this happen? _____

8.2) How many times have you been married? _____

Please will you tell me the when you got married each time and how old you were?

Year:- 1st Marriage _____ Age _____: 2nd Marriage _____ Age_____

Year:- 3rd Marriage _____ Age _____: 4th Marriage _____ Age_____

Are you married at present? **YES // NO**

(If no) Is that because of widowhood / divorce / separation / other

When did this happen? _____

Did your previous marriage(s) end because of widowhood or divorce?

1st marriage _____ When did this happen _____

2nd marriage _____ When did this happen _____

3rd marriage _____ When did this happen _____

8.3) Did your twin ever get married? YES // NO / DON'T KNOW
(Lived with someone on a long-term or permanent basis)

Was he/she married more than once?   YES // NO / DON'T KNOW

8.4) When did he/she get married? _____   How old was he/she? _____

Was your twin married when he/she died ?   YES // NO / DON'T KNOW

(If no) Was that because of widowhood / divorce / separation / other
When did this happen? _____

8.5) How many times was your twin married? _____

Please will you tell me the when your twin got married each time and how old he/she
was?

Year:- 1st Marriage _____ Age _____: 2nd Marriage _____ Age_____

Year:- 3rd Marriage _____ Age _____: 4th Marriage _____ Age_____

Was your twin married when he/she died?   YES // NO

(If no) Was that because of widowhood / divorce / separation / other

When did this happen? _____

Did your twin's previous marriage(s) end because of widowhood or divorced?

1st marriage _____   When did this happen _____

2nd marriage _____   When did this happen _____

3rd marriage _____   When did this happen _____

9.0) Do you have any children?   YES // NO (go to question 9.2)

(If yes) How many do you have? _____

Please would you list them in the order they were born giving their year of birth?

1st child _____ 2nd child _____

3rd child _____ 4th child _____

5th child _____ 6th child _____

7th child _____ 8th child _____

9.1) For each of your children will you tell me a) age left school/year; b) if he/she went to
college / university; c) what their 1st full-time job was; d) if / when he/she got married //
left home.

1st _____

2nd _____

3rd_____

4th_____

5th _____

6th_____

10.0) Did your twin have any children?   YES // **NO** (go to question 11)

(If yes) How many did your twin have? _____

Please would you list them in the order they were born giving their year of birth?

1st child _____ 2nd child _____

3rd child _____ 4th child _____

5th child _____ _____ 6th child _____

7th child _____ 8th child _____

10.1) For each of your twin's children will you tell me a) age left school; b) if they went to college / university; d) what their 1st full-time job was; e) if / when they got married // left home.

1st _____

2nd _____

3rd_____

4th_____

5th _____

6th _____

(For all subjects who have reached retirement age >55)

12.0) Are you still working now, or have you retired from your regular employment?

Still Working _____   Retired _____

(If retired) When did you retire? _____

(For all twins who reached retirement age >55)

12.1) Did your twin retire from their regular employment YES // **NO / DON'T KNOW**

(If yes) When did he/she retire? _____

ENVIRONMENTAL EXPOSURES

RESIDENTIAL LOCATIONS:

13.0) Using the brief history of life events we have just constructed as a guide, please would you now think about the places you have lived within Britain for longer than 3 months, starting with the place you were born.

Place of Residence	. Age .	Dates Residence	. Total Time .	Classification.	Perceptions of
	. .	From . To	. Residence .	of Location .	Detrimental Fac.

a]_____

b]_____

c]_____

d]_____

e]_____

f]_____

g]_____

RESIDENTIAL LOCATIONS:

Place of Residence	Age	Dates Residence From To	Total Time Residence	Classification of Location	Perceptions of Detrimental Fac.
h]					
i]					
j]					
k]					
l]					
m]					
n]					

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RESIDENTIAL LOCATIONS: (TWIN)

13.2) Please could you now list all the additional places where your twin lived but not with you?

Place of Residence	Age	Dates Residence From	To	Total Time Residence	Classification of Location	Perceptions of Detrimental Fac.
a]___						___
b]___						___
c]___						___
d]___						___
e]___						___
f]___						___
g]___						___

RESIDENTIAL LOCATIONS:

Place of Residence	Age	Dates Residence From	To	Total Time Residence	Classification of Location	Perceptions of Detrimental Fac.

h]____

i]____

j]____

k]____

l]____

m]____

n]____

13.1) How often did you and your twin visit one another, once you no longer lived together?

Lived at a)_____ = _____

Lived at b)_____ = _____

Lived at c)_____ = _____

Lived at d)_____ = _____

Lived at e)_____ = _____

Lived at f)_____ = _____

FOREIGN TRAVEL:

14.0) Have you ever travelled abroad for any reason?   YES // NO (go to question 14.3)

14.1) Were you ever taken ill while visiting a foreign country? YES // NO

    (IF YES) – What was the illness / symptoms and in which country?

_____

_____

14.2) Have you ever been ill shortly after returning from a foreign country?   YES // NO

    (IF YES) – What was the illness / symptoms and following which country?

_____

_____

14.3) Did your twin ever travel abroad for any reason? YES // NO (go to question 15)

14.4) Was he/she ever taken ill while visiting a foreign country?   YES // NO / D.K

    (IF YES) – What was the illness / symptoms and in which country?

_____

_____

14.5) Was your twin ever ill shortly after returning from a foreign country?

                                      YES // NO / D.K.

    (IF YES) – What was the illness / symptoms and following which country?

_____

_____

OCCUPATION / VOLUNTARY WORK OUTSIDE THE HOME: - FOR INTERVIEWEE

15.0) This next question is concerned with your working activities **outside** the home, including both paid employment and voluntary work. Please could you think carefully about each one starting with the first job you did after leaving school or college.

Job Title	.	Main Tasks and Activities	. Type of firm or . Establishment	. Year Started . or Age	. L.Time Spent . Job	. Location of . Work	. Perception Detrimental . factors

a]_____

b]_____

c]_____

d]_____

e]_____

OCCUPATION / VOLUNTARY WORK OUTSIDE THE HOME: FOR INTERVIEWE

Job Title	.	Main Tasks and Activities	. Type of firm or Establishment	. Year Started or Age	. L.Time Spent Job	. Location of Work	. Perception Detrimental factors
f]							
g]							
h]							
i]							
j]							
k]							
l]							

OCCUPATION / VOLUNTARY WORK OUTSIDE THE HOME: - FOR TWIN

15.0) This next question is concerned with your working activities **outside** the home, including both paid employment and voluntary work. Please could you think carefully about each one starting with the first job you did after leaving school or college.

Job Title	.	Main Tasks and Activities	. Type of firm or Establishment	. Year Started . or Age	. L.Time Spent . Job	. Location of . Work	. Perception Detrimental . factors
a]___							
b]___							
c]___							
d]___							
e]___							

OCCUPATION / VOLUNTARY WORK OUTSIDE THE HOME: FOR TWIN

Job Title	.	Main Tasks and Activities	. Type of firm or Establishment	. Year Started or Age	. L.Time Spent Job	. Location of Work	. Perception Detrimental factors
f]____							
g]____							
h]____							
i]____							
j]____							
k]____							
l]____							

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DOMESTIC ACTIVITIES, HOBBIES & LEISURE ACTIVITIES: - FOR INTERVIEWEE

16.0) Now I would like you to think along similar lines in relation to the domextic activities you perform, any working activity you did/do from home, and your hobbies and leisure activities. Please include such activities that you have done in the past, but don't do now.

Brief description of Hobby, Leisure or Domestic Activity	. Approx.Age. or Year . Started	Location of . Activity	. Regularity of . Activity	. No. Years . Pursued . Activity	. Perception Detrimental factors
a]___					___
b]___					___
c]___					___
d]___					___
e]___					___

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DOMESTIC ACTIVITIES, HOBBIES & LEISURE ACTIVITIES CHART: - FOR INTERVIEWEE

Brief description of Hobby, Leisure or Domestic Activity	. Approx.Age. or Year . Started	Location of . Activity	. Regularity of . Activity	. No. Years . Pursued . Activity	. Perception Detrimental factors
f]					
g]					
h]					
i]					
j]					
k]					
l]					

DOMESTIC ACTIVITIES, HOBBIES & LEISURE ACTIVITIES: - FOR TWIN

16.0) Now I would like you to think along similar lines in relation to the domextic activities you perform, any working activity you did/do from home, and your hobbies and leisure activities. Please include such activities that you have done in the past, but don't do now.

Brief description of Hobby, Leisure or Domestic Activity	Approx.Age. or Year . Started	Location of . Activity	Regularity of . Activity	No. Years . Pursued . Activity	Perception Detrimental factors
a]					
b]					
c]					
d]					
e]					

DOMESTIC ACTIVITIES, HOBBIES & LEISURE ACTIVITIES CHART: - FOR TWIN

Brief description of Hobby, Leisure or Domestic Activity	. Approx.Age. or Year . Started	Location of . Activity	. Regularity of . Activity	. No. Years . Pursued . Activity	Perception Detrimental factors
f]___					
g]___					
h]___					
i]___					
j]___					
k]___					
l]___					

17) CHEMICAL EXPOSURE: - FOR INTERVIEWEE

Group of Chemical Substances	. Ever Used . /Indirect . Contact	. Regularity of Use	.Time .Span	. Location . of Use	. Specific Names . of Substances	. Rating .

a] WEEDKILLERS (FUNGICIDES)
eg:Tumble Weed; May & Baker;ICI
Verdone 2; Cuprinol Fungi.sp;
Sandtex; Polycell Mould Cleaner

b] INSECTICIDES (PESTICIDES)
eg:Safers Organic Insecticide;
Vapona flykiller; Nippon antki;
Murphy Slug Pellets; Jeyes Fl.

c] FERTILISERS
eg:Fisons Lawn Fertilizer + W.K
Evergreen Extra; Maxicrop;
Liquid Growmore; Arthur Bowers

d] CROP SPRAYING (other spraying)

e] PRESERVATIVES FOR WOOD ETC.
eg:Cuprinol Wood Pres. Stains
Sadolin; Creosote; Linseed Oil
Aquaseal & Bitumen Ext. Paint

f] GLUES / ADHESIVES
eg:Copydex; Unibond; Evo Stick
Bostik; Unibond/Polyfilla/
Tetrion Tile Adhes.(fungi)

Group of Chemical Substances	. Ever Used. .Indirectly.	Regularity of Use	.Time .Span	. Location . . of Use .	Specific Names of Substances	. Rating .

g] PAINTS (specify base:
lead/cellulose/emulsion/oil)
International Ext; Red oxide &
Aluminium Primers- Hammerite
Crown; Dulux; Polyur.Var.+Seal

h] PRINTING / DYING FABRIC/
FABRIC FINISHERS/LEATHER
eg:commercial dyes & fabric
finishers; tanning;

i] PETRO-CHEMICALS
eg:synthetic fibres/plastics
production; paraffin; petrol
various solvents.

j] COSMETICS / HAIR DYES/RESTORER
eg:Avon; Boots-No.7 & 17; Maxi
Clarol; Nice& Easy; Polycolour
Harmony; Asian Makeup- Cohl

k] DRY CLEANING AGENTS
eg: Perchlorethylene

l] SOLVENTS (cleaning sols.
bases-other substances)
eg:Household Cleaners; Bases
for glues/spray fert.+paint
(glue/solvent "sniffers)

17) CHEMICAL EXPOSURE: - FOR TWIN

Group of Chemical Substances	Ever Used /Indirect Contact	Regularity of Use	Time Span	Location of Use	Specific Names of Substances	Rating
a] WEEDKILLERS (FUNGICIDES) eg:Tumble Weed; May & Baker;ICI Verdone 2; Cuprinol Fungi.sp; Sandtex; Polycell Mould Cleaner						
b] INSECTICIDES (PESTICIDES) eg:Safers Organic Insecticide; Vapona flykiller; Nippon antki; Murphy Slug Pellets; Jeyes Fl.						
c] FERTILISERS eg:Fisons Lawn Fertilizer + W.K Evergreen Extra; Maxicrop; Liquid Growmore; Arthur Bowers						
d] CROP SPRAYING (other spraying)						
e] PRESERVATIVES FOR WOOD ETC. eg:Cuprinol Wood Pres. Stains Sadolin; Creosote; Linseed Oil Aquaseal & Bitumen Ext. Paint						
f] GLUES / ADHESIVES eg:Copydex; Unibond; Evo Stick Bostik; Unibond/Polyfilla/ Tetrion Tile Adhes.(fungi)						

Group of Chemical Substances	. Ever Used .Indirectly.	Regularity of Use	.Time .Span	. Location . of Use	. Specific Names . of Substances	. Rating .

g] PAINTS (specify base:
lead/cellulose/emulsion/oil)
International Ext; Red oxide &
Aluminium Primers- Hammerite
Crown; Dulux; Polyur.Var.+Seal

**h] PRINTING / DYING FABRIC/
FABRIC FINISHERS/LEATHER**
eg:commercial dyes & fabric
finishers; tanning;

i] PETRO-CHEMICALS
eg:synthetic fibres/plastics
production; paraffin; petrol
various solvents.

j] COSMETICS / HAIR DYES/RESTORER
eg:Avon; Boots-No.7 & 17; Maxi
Clarol; Nice& Easy; Polycolour
Harmony; Asian Makeup- Cohl

k] DRY CLEANING AGENTS
eg: Perchlorethylene

**l] SOLVENTS (cleaning sols.
bases-other substances)**
eg:Household Cleaners; Bases
for glues/spray fert.+paint
(glue/solvent "sniffers)

18) HEAVY METAL EXPOSURE: - FOR INTERVIEWEE

Other elements found commonly within our environment are HEAVY METALS. They occur naturally and are used in the manufacture of many different commodities. Please could you tell me if you are aware of coming into contact with any heavy metals during both your working life and at home. (If not) In that case

Heavy Metal	. Level . . Contact .	Specific Sources of Exposure	. Regularity . . of Contact .	Time . Span .	Nature of . Contact	Rating

LEAD:

MERCURY:

ARSENIC:

THALLIUM:

Heavy Metal	Level Contact	Specific Sources of Exposure	Regularity of Contact	Time Span	Nature of Contact	Rating
ALUMINIUM:						
COPPER:						
MANGANESE:						
ZINC:						
SELENIUM:						
COBALT:						
CHROMIUM:						
SILVER:						
TIN:						
OTHER METALS:						

18) HEAVY METAL EXPOSURE: - FOR TWIN

Heavy Metal	. Level . Contact .	Specific Sources of Exposure	. Regularity . of Contact .	Time . Span .	Nature of Contact	. Rating

LEAD:

MERCURY:

ARSENIC:

ALUMINIUM:

Heavy Metal	. Level . Contact .	Specific Sources of Exposure	. Regularity . of Contact .	Time . Span .	Nature of Contact	. Rating

THALLIUM:

COPPER:

MANGANESE:

ZINC:

SELENIUM:

COBALT:

CHROMIUM:

SILVER:

TIN:

OTHER METALS:

FOOD CONSUMPTION - FOR INTERVIEWEE

19.0) Do you eat any of the following types of food, and please could you tell me how often you eat them (indicate by entering the number times eaten a day)

Food Group	.Ever.1x .Eat	1x .Day	.4-6 .xwk	.2-3 .xwk	.1x .Wek	.1x .2wk.	1x Mth.	.4-6 xyr	.2-3. .xyr.	=<1 xyr
NUTS:										
CHEESE:										
EGGS:										
MILK:										
OTHER DAIRY PRODUCE:										
PULSES:										
RICE TYPE CEREALS:										
COOKED VEGETABLES:										
RAW VEGETABLES:										
FRUIT:										
SHELLFISH:										
WHITE FISH:										
OILY FISH:										
BEEF:										
LAMB:										
PORK:										
POULTRY/ GAME:										
ANY TYPE OFFAL:										
ANIMAL FATS:										
VEGETABLE FATS:										

19.1) Please could you now tell me the specific types of food you regularly eat within each food group.

NUTS: (Peanuts)_____

_____

CHEESE: (Blue & Soft)_____

_____

_____

MILK: _____

OTHER DAIRY PRODUCE: (cream; yoghurt; ice cream)

_____

PULSES: _____

_____

_____

COOKED VEGETABLES: (Brassicas)_____

_____

Root veg._____

_____

RAW VEGETABLES: _____

_____

_____

RICE TYPE CEREALS: _____

_____

FRUIT: _____

_____

_____

SHELLFISH: _____

_____

WHITE FISH: _____

_____

_____

OILY FISH: _____

_____

SMOKED FISH: _____

POULTRY/GAME: _____

_____

_____

ANY FORM OF OFFAL: (include sausages, pate etc;[Brain]-brawn)

_____

_____

ANIIMAL FATS: _____

_____

VEGETABLE FATS: _____

_____

19.2) Please would you tell me whether you follow a particiular type/special diet for any reason?

(For example: Vegetarian; Weight Reducing; Diabetic; Low Fat; Low/High Protein; Low Salt; Diet to lower Blood Cholesterol; High/Low Residue; Milk/Gluten Free)

Please specify - (source of information & when):_____

_____

19.3) Has your diet changed much over the years?   **YES // NO**

(IF YES) - How? _____

_____

FOOD CONSUMPTION - FOR TWIN

19.0) Did your twin eat any of the following types of food, and please could you tell me how often he/she ate them (indicate by entering the number times eaten a day)

Food Group	.Ever. Eat	1x .Day	.4-6 .xwk	.2-3 .xwk	.1x .Wek	.1x .2wk.	. 1x Mth.	.4-6 .xyr	.2-3. .xyr.	=<1 xyr
NUTS:										
CHEESE:										
EGGS:										
MILK:										
OTHER DAIRY PRODUCE:										
PULSES:										
RICE TYPE CEREALS:										
COOKED VEGETABLES:										
RAW VEGETABLES:										
FRUIT:										
SHELLFISH:										
WHITE FISH:										
OILY FISH:										
BEEF:										
LAMB:										
PORK:										
POULTRY/ GAME:										
ANY TYPE OFFAL:										
ANIMAL FATS:										
VEGETABLE FATS:										

19.1) Please could you now tell me the specific types of food your twin regularly eat within each food group.

NUTS: (Peanuts) _____

_____

CHEESE: (Blue & Soft) _____

_____

_____

MILK: _____

OTHER DAIRY PRODUCE: (cream; yoghurt; ice cream)

_____

PULSES: _____

_____

_____

COOKED VEGETABLES: (Brassicas) _____

_____

Root veg. _____

_____

RAW VEGETABLES: _____

_____

_____

RICE TYPE CEREALS: _____

_____

FRUIT: _____

_____

_____

SHELLFISH: _____

_____

WHITE FISH: _____

_____

_____

OILY FISH: _____

_____

SMOKED FISH: _____

POULTRY/GAME: _____

_____

_____

ANY FORM OF OFFAL: (include sausages, pate etc;[Brain]-brawn)

_____

_____

ANIIMAL FATS: _____

_____

VEGETABLE FATS: _____

_____

19.2) Please would you tell me whether your twin followed a particiular type/special diet for any reason?
(For example: Vegetarian; Weight Reducing; Diabetic; Low Fat; Low/High Protein; Low Salt; Diet to lower Blood Cholesterol; High/Low Residue; Milk/Gluten Free)

Please specify - (source of information & when):_____

_____

19.3) Did your twins diet change much over the years?   YES // NO

(IF YES) - How? _____

_____

BEVERAGE CONSUMPTION - INTERVIEWEE

20.0) People also tend to have very individualised drinking habits involving the consumption of both non-alcoholic and alcoholic beverages. Please could you tell me how frequently you take the following drinks and how much you have each time.(cups/mugs/glass/bottle/can/carton-per day/week)

Frequency	. Tea .	Herb. Tea .	Instant. Coffee .	Ground. Coffee.	Tap . Water.	Min . Water.	Fruit . Squash .	Carbon-. ated .	Fruit. Juice.	Milk.	Alcohol
a] EVERY DAY											
b] MOST DAYS											
c] 2-3 TIMES A WEEK											
d] ONCE A WEEK											
e] FORTNIGHTLY											
f] MONTHLY											
g] SPECIAL OCCASIONS ONLY / RARELY											
h] MEDICAL PURPOSES											
i] NEVER											

ALCOHOL CONSUMPTION:-

20.1) How often have you had a drink of (......) during the last 12 months?

(For each positive response ask:-)

a] How much (......) have you usually drunk on any one occasion during the last 12 months?

Alcoholic Beverage	Most. Days.	3-4. per. Wek.	1-2. per. Wek.	1-2. per. Mth.	1-2. per. 6Mth.	1-2. per. Yr	Not. at . All.	Ask Q.a]	
SHANDY									_____pts
									_pts
BEER, LAGER, STOUT, CIDER									_____pts
									pts
SPIRITS / LIQUEURS (eg. gin, whisky, rum,brandy, vodka, advocaat)									_____sings
									sings
SHERRY/ MARTINI/ PORT/ VERMOUTH (eg. dubonnet cinzano)									_____glas
									glas
WINE / CHAMPAGNE (incl. babycham)									_glas
									glas

OTHERS- Specify

_____

_____

20.2) Do you regularly drink homemade ......?

a] BEER:_____   b] WINE:_____   c] SOFT DRINKS/MIXERS:_____

20.3) Are your drinking habits today very different to what they were at an earlier time in your life?

                     YES // NO

        (If yes) When was this? _____
               (repeat above)

BEVERAGE CONSUMPTION   - FOR TWIN

20.0) People also tend to have very individualised drinking habits involving the consumption of both non-alcoholic and alcoholic beverages. Please could you tell me how frequently your twin took the following drinks and how much they had each time.(cups/mugs/glass/bottle/can/carton-per day/week)

Frequency	Tea	Herb. Tea	Instant. Coffee	Ground. Coffee	Tap. Water	Min. Water	Fruit Squash	Carbon-. ated	Fruit. Juice	Milk.	Alcohol
a] EVERY DAY											
b] MOST DAYS											
c] 2-3 TIMES A WEEK											
d] ONCE A WEEK											
e] FORTNIGHTLY											
f] MONTHLY											
g] SPECIAL OCCASIONS ONLY / RARELY											
h] MEDICAL PURPOSES											
i] NEVER											

TWINS ALCOHOL CONSUMPTION:-

20.4) Would you rate your twins alcohol consumption to have been generally less than / similar / greater than your own.

<div align="center">LESS THAN / SIMILAR / GREATER</div>

20.5) How often did your twin usually drink (......) during the later years of their life?

(For each positive response ask:-)

a] How much (......) did he/she usually consume on any one occasion?

Alcoholic Beverage	Most. Days.	3-4. per. Wek.	1-2. per. Wek.	1-2. per. Mth.	1-2. per. 6Mth.	1-2. per. Yr.	Not. at. All.	Ask Q.a]	
SHANDY								_____pts	
								_pts	
BEER, LAGER, STOUT, CIDER								pts	
								pts	
SPIRITS / LIQUEURS (eg. gin, whisky, rum,brandy, vodka, advocaat)								_____sings	
								sings	
SHERRY/ MARTINI/ PORT/ VERMOUTH (eg. dubonnet cinzano)								_____glas	
								glas	
WINE / CHAMPAGNE (incl. babycham)								glas	
								glas	

OTHERS- Specify

_____

_____

20.6) Did your twin regularly drink homemade ......?

a] BEER:_____ b] WINE:_____ c] SOFT DRINKS/MIXERS:_____

20.7) Were your twins drinking habits much different during an earlier period in his life?

<div align="center">**YES / NO**</div>

(If yes) When was this?_____
(repeat above)

SMOKING HABITS

The following questions are about your smoking habits.

21.0) Have you ever smoked a cigarette, a cigar , or a pipe?   YES // NO (go quest 21.7)

How old were you when you started to smoke regularly? _____ yrs old

21.1) Do you smoke cigarettes at all nowadays?   YES // NO (go to question 21.2)

About how many cigarettes **a day** do you usually smoke at weekends? _____

About how many cigarettes **a day** do you usually smoke during the week? _____

21.2) Have you ever smoked cigarettes regularly? YES // NO (go to question 21.3)

About how many cigarettes did you used to smoke in a day? _____

When did you stop smoking cigarettes regularly? _____

21.3) Do you smoke cigars of any type at all nowadays? YES // NO (go to question 21.4)

About how many cigars do you smoke in **a day?** _____

21.4) Have you ever smoked cigars regularly? YES // NO (go to question 21.5)

About how many cigars did you used to smoke in a day? _____

When did you stop smoking cigars regularly? _____

21.5) Do you smoke a pipe at all nowadays? YES // NO (go to question 21.6)

About how many ounces of tobacco do you smoke in a week? _____

21.6) Have you ever smoked a pipe regularly? YES // NO (go to question 21.8)

About how many ounces of tobacco did you used to smoke in a week? _____

When did you stop smoking a pipe regularly? _____

21.7) Do you live with a smoker or work closely with people smoking? YES // NO

About how many cigarettes / cigars / ounces of tobacco do they smoke a day // week

---

TWINS SMOKING HABITS:-
The following questions are about your twins smoking habits.

21.8) Did your twin smoke cigarettes, cigars, or a pipe? YES //
NO / DON'T KNOW (go to question 21.14)

How old was he/she when he/she started smoking regularly? _____yrs old

21.9) Did he/she smoke cigarettes? YES // NO / DON'T KNOW (go to question 21.10)

About how many cigarettes **a day** did he/she usually smoke at weekends? _____

About how many cigarettes **a day** did he/she usually smoke during the week? _____

21.10) Did he/she smoke cigars? YES // NO / DON'T KNOW (go to question 21.11)

About how many cigars did he/she smoke **a day?** _____

21.11) Did he/she smoke a pipe? YES // NO / DON'T KNOW (go to question 21.12)

About how many ounces of tobacco did he/she smoke in **a week?** _____

21.12) Had your twin ever smoked in the past? YES // NO / DON'T KNOW (go quest 22)

When did he/she stop smoking? _____

How old was he/she when he/she started smoking regularly? _____yrs old

21.13) About how many of the following did he/she smoke before he/she stopped?

Cigarettes per **day?** _____

Cigars per **day?** _____

Ounces of tobacco per **week?** _____

21.14) Did your twin live or work closely with people smoking? YES / NO / DON'T KNOW

(If yes) About how many cigarettes/ cigars/ ounces of tobacco did they smoke a day/week

_____

DRUG CONSUMPTION

The following questions are related to any prescribed or over-the-counter drugs / medications / remedies / tonics that you take on a regular basis, or have used for long periods of time in the past.

22.0) Are you presently on any drugs prescribed by your doctor? YES / NO (go quest 22.1)

Please could you list them and tell me how long you have been taking them:

DRUG NAME   DURATION

a] _____

b] _____

c] _____

d] _____

e] _____

f] _____

g] _____

22.1) Have you been on any long-term prescribed medication in the past, that you have not already mentioned.

YES / NO (go to question 22.2)

DRUG NAME   YEAR   DURATION

a] _____

b] _____

c] _____

d] _____

22.2) Do you regularly take drugs / medications / remedies / tonics purchased from the chemist?

YES / NO (go to question 22.3)

DRUG NAME   DURATION

a] _____

b] _____

c] _____

d] _____

22.3) Have you taken such medications in the past for a long period of time.   YES / NO

DRUG NAME   YEAR   DURATION

a] _____

b] _____

c] _____

d] _____

22.4) Do you take vitamin or herbal tablets?   YES / NO (go to question 22.5)

DRUG NAME   DURATION

a] _____

b] _____

c] _____

d] _____

22.5) Have you ever regularly taken vitamin or herbal tablets?   YES / NO (go quest 22.6)

	DRUG NAME	YEAR	DURATION
a]			
b]			
c]			
d]			

TWINS DRUG CONSUMPTION:-

The following questions are related to any prescribed or over-the-counter drugs / medications/ remedies / tonics that your twin took on a regular basis, or have used for long periods of time.

22.7) Was your twin ever on long-term medication prescribed by his/her doctor? **YES //**
or **NO / DON'T KNOW** (go to question 22.8)

Please could you list them, including the length of time he/she took them for and when:

	DRUG NAME	YEAR	DURATION
a]			
b]			
c]			
d]			
e]			
f]			
g]			

22.8) Did your twin ever regularly take drugs / medications / remedies / tonics purchased from the chemist?

YES //   NO / DON'T KNOW (go to question 22.9)

	DRUG NAME	YEAR	DURATION
a]			
b]			
c]			
d]			

22.9) Did your twin ever taken vitamin or herbal tablets?   **YES //   NO / DON'T KNOW**
(go to question 23.0)

	DRUG NAME	YEAR	DURATION
a]			
b]			
c]			

PERSONAL MEDICAL HISTORY

These questions are related to your general health, present health problems, and past illnesses and injuries.

23.0) Are you **presently** suffering from any troublesome health problems or medically diagnosed condition.

DURATION

a] _____

b] _____

c] _____

d] _____

e] _____

f] _____

g] _____

h] _____

23.1) Have you ever had any of the following illnesses or injuries, particularly as a child?

a] _____ Mumps: age _____   b] _____ Whooping Cough: age _____

c] _____ Measles: age _____   d] _____ German Measles (Rubella): age _____

e] _____ Chicken Pox: age _____   f] _____ Rheumatic Fever: age _____

g] _____ Broken Bones: no/times _____ ages _____ places _____

h] _____ Head Injuries/concussion: no/times _____ ages _____

j] _____ Abrasions requiring hospital treatment: no/times _____ age _____

places_____

Please specify any other illness or injury you suffered that is not listed above

k] _____: age

l] _____: age

m] _____: age

23.2) In relation to the answers you have just given, can you recall experiencing any immediate complications or long-term consequences resulting from the illnesses and injuries you had?

(please describe briefly, stating the preceding illness/injury)

a] _____

b] _____

23.3) As we are particularly interested in neurological problems, please would you tell me whether you have suffered from any of the following disorders:-

a] Poliomyelitis or anything that was vaguely similar?   **YES / NO**

(If yes) Age at onset: _____ Symptoms:-_____

_____

(If no) Have you been in contact with people who developed polio? **YES / NO**

b] Physical Tremor of any muscle group such as in the arm or hand?   **YES / NO**

c] Nervous Problems?   **YES / NO**

Specify:_____

d] Any other Neurological Disorder?   **YES / NO**

Specify:_____

Symptoms of
above:_____

_____

23.4) Have you ever required hospital treatment as an in-patient?   **YES or**
NO (go to question 23.5)

Please would you list those occasions, stating why you were admitted, in what year, and for how long.

	Reason for In-patient Hospital Treatment	Age / Year
a]		
b]		
c]		
d]		
e]		
f]		

23.5) Please would you tell me how many times you have received the following items:-

	Never	Once	Twice	3-5 Times	>5 Times
A Blood Transfusion (or Equivalent):					
Severe Electric Shock or Lightning Strike:					
A General Anaesthetic:					

(If get a positive reply:)
      Did you suffer from any immediate complications or long-term consequences after receiving .....? (depends on their answers to above)

a] Blood Transfusion:_____
      (or equivalent)

_____

b] Severe Electric Shock:_____
      (or Lightning Strike)

_____

c] General Anaesthetic:_____

_____

23.6) Many medical and surgical treatments are carried out in out-patient clinics <u>ONLY</u>. Have you ever been an out-patient?
               YES or NO (go to question 23.7)

Please give below details of the out-patient treatments you consider important.

	Reason for Out-patient Visit	Age/Year
a]	_____	
b]	_____	
c]	_____	
d]	_____	
e]	_____	

23.7) Most of the illnesses, injuries or health problems we experience throughout our lives are never treated at a hospital, and sometimes not even seen by our G.P. Can you remember any such illness, injury or health problem that you have suffered in the past, and have not already mentioned. (Include any flu-like illnesses, but not common colds)

```
Illness, Injury or Health Problem   . Year/age . Duration
```

a] _____

b] _____

c] _____

d] _____

e] _____

f] _____

23.8) Have you ever been given an immunisation against any particular disease / infection?

YES // NO / DON'T KNOW (go to question 23.9)

(IF YES) Can you remember if you were immunised against any of the following specific disease or infection?

a] _____ Measles: b] _____ German Measles: c] _____ Mumps:

d] _____ Whooping Cough: f] _____ Tetanus: g] _____ Small Pox:

h] _____ Polio (Sugar Lump):   i] _____ Polio (Injection):   h] _____ Cholera:

i] _____ Yellow Fever: j] _____ Typhoid: k] _____ Hepatitis:

l] _____ Other Condition: (Please name):_____

Did you ever experience an **adverse** reaction following any vaccination?

YES // NO /or DON'T KNOW (go to question 26.9)

Can you remember which ones and the reaction?

_____

_____

23.9) Do you feel you have ever suffered from any allergies?

YES / /   NO /or DON'T KNOW (go to question 23.10)

Do you feel you are allergic to any of the following **groups of substances**, or have been in the past?

a] FOODS: (ie. fruits, meats, shell fish, dairy products) .... _____

b] ANIMALS, BIRDS or INSECTS: (ie. feathers, stings, fur) .... _____

c] PLANTS AND PLANT EXTRACTS: (ie. hay, grass, oils) .... _____

d] AIRBORNE SUBSTANCES: (ie. dust pollen) .... _____

e] DRUGS or MEDICAL TREATMENTS: (ie. penicillin, aspirin, anaesthetics) _____

f] CHEMICAL SUBSTANCES: (ie. bleach, washing powder, paint, fertiliser) . _____

g] NON-CHEMICAL MANUFACTURED MATERIALS OR GOODS .... _____

Can you name any **specific** substances that you feel you are presently allergic to, or have been in the past?

a] _____   b] _____

c] _____   d] _____

e] _____   f] _____

What do you feel are/were the major symptoms of each of your allergies?

Allergy a] – Symptoms _____

Allergy b] – Symptoms _____

Allergy c] – Symptoms _____

Allergy d] – Symptoms _____

Allergy e] – Symptoms _____

Allergy f] – Symptoms _____

TWINS PERSONAL MEDICAL HISTORY

These questions are related to any health problems, illnesses or injuries your twin may have experienced during their life.

23.11) Did your twin ever have any of the following illnesses or injuries, particularly as a child?

a] _____ Mumps: age _____   b] _____ Whooping Cough: age _____

c] _____ Measles: age _____   d] _____ German Measles (Rubella): age _____

e] _____ Chicken Pox: age _____   f] _____ Rheumatic Fever: age _____

g] _____ Broken Bones: no/times _____ ages _____ places _____

h] _____ Head Injuries/concussion: no/times _____ ages _____

j] _____ Abrasions requiring hospital treatment: no/times _____ age _____

  places _____

Please specify any other illness or injury your twin suffered that is not listed above

k] _____: age

l] _____: age

m] _____: age

23.12) In relation to the answers you have just given, can you recall your twin experiencing any immediate complications or long-term consequences resulting from the illnesses and injuries he/she had? (please describe briefly, stating the preceding illness/injury)

a] _____

b] _____

23.13) Can you remember your twin ever having **poliomyelitis** or anything vaguely similar
                                                        YES / NO / DON'T KNOW

(If yes) Age at onset: _____ Symptoms:-_____

_____

(If no) Had he/she ever been in contact with people who developed polio? **YES / NO**

23.14) As we are particularly interested in neurological problems, please would you tell me whether your twin ever suffered from any of the following disorders:-

a] Physical Tremor of any muscle group eg. in arm or hand? **YES / NO / DON'T KNOW**

  b] Parkinson's Disease? **YES / NO / DON'T KNOW**

  c] Excess Forgetfulness? **YES / NO / DON'T KNOW**

Specify:_____

  d] Motor Neurone Disease? **YES / NO / DON'T KNOW**

  e] Nervous Problems? **YES / NO / DON'T KNOW**

Specify:_____

f] Any other Neurological Disorder?   YES / NO / DON'T KNOW

Specify:_____

Please would you give some details about the onset and symptoms of your twins (......)?

_____

_____

_____

_____

23.10) Did he/she ever suffer from any other troublesome health problems or medically diagnosed condition? Please try to remember when these were and how long they lasted.

                                                    YEAR DURATION

a] _____

b] _____

c] _____

d] _____

e] _____

f] _____

g] _____

h] _____

i] _____

j] _____

23.15) Did your twin ever require hospital treatment as an in-patient?   YES // NO

Please list each admission stating the reason why, the year, and the length of stay.

Reason for In-patient Hospital Treatment	Age / Year
a] _____	
b] _____	
c] _____	
d] _____	
e] _____	

f] _____

g] _____

h] _____

23.16) Please can you tell me how many times your twin received any of the following items:-

	Never .	Once .	Twice.	3-5 . Times	>5 Times

A Blood Transfusion
or equivalent:
Severe Electric Shock
or Lightning Stroke:
A General Anaesthetic:

(If get a positive reply:)
     Did he/she suffer from any immediate complications or long-term consequences after
     receiving .....? (depends on their answers to above)

a] Blood Transfusion:_____
     (or equivalent)

b] Severe Electric Shock:_____
     (Lightning Strike/ECT)

c] General Anaesthetic:_____

26.17) Many medical and surgical treatments are carried out in out-patient clinics <u>ONLY</u>.
Was your twin ever treated as a hospital out-patient?
                                YES // NO

     Please give below details of the out-patient treatments you consider important.

Reason for Out-patient Visit	. Age / Year
a]	
b]	

c] _____

d] _____

e] _____

23.18) Most of the illnesses, injuries or health problems we experience throughout our lives are never treated at a hospital, and sometimes not even seen by our G.P. Can you remember any such illness, injury or health problem that your twin experienced, that has not already been mentioned. (Include any flu-like illnesses, but not common colds)

Illness, Injury or Health Problem   . Year/Age . Duration

a] _____

b] _____

c] _____

d] _____

e] _____

f] _____

23.19) Was your twin ever immunised against any of the following diseases or conditions?

a] _____ Measles: b] _____ German Measles: c] _____ Mumps:

d] _____ Whooping Cough: e] _____ Tetanus: f] _____ Small Pox:

g] _____ Polio (Sugar Lump):   h] _____ Polio (Injection):   i] _____ Cholera

j] _____ Yellow Fever: k] _____ Typhoid: l] _____ Hepatitis:

m] _____ Other Condition: (Please name):_____

Did your twin ever experience an **adverse** reaction following any vaccination?

YES // NO /or **DON'T KNOW** (go to question 23.20)

Can you remember which ones and the reaction?

_____

_____

23.20) Did your twin ever suffer from any allergies?

YES / /   NO /or **DON'T KNOW** (go to question 24)

Was your twin allergic to any of the following **groups of substances**, as a child or adult?

a] FOODS: (ie. fruits, meats, shell fish, dairy products) .... _____

b] ANIMALS, BIRDS or INSECTS: (ie. feathers, stings, fur) .... _____

c] PLANTS AND PLANT EXTRACTS: (ie. hay, grass, oils) .... _____

d] AIRBORNE SUBSTANCES: (ie. dust pollen) .... _____ e]

DRUGS or MEDICAL TREATMENTS: (ie. penicillin, aspirin, anaesthetics) _____

f] CHEMICAL SUBSTANCES: (ie. bleach, washing powder, paint, fertiliser) . _____

g] NON-CHEMICAL MANUFACTURED MATERIALS OR GOODS .... _____

Can you name any **specific** substances that your twin felt he/she was allergic to as a child or adult?

a] _____   b] _____

c] _____   d] _____

e] _____   f] _____

What do you feel are/were the major symptoms of each of your allergies?

Allergy a] - Symptoms _____

Allergy b] - Symptoms _____

Allergy c] - Symptoms _____

Allergy d] - Symptoms _____

Allergy e] - Symptoms _____

Allergy f] - Symptoms _____

FAMILY MEDICAL HISTORY

I would now like to ask you some questions about the health of other members of your family.

These are not only concerned with the members of your immediate family, but also your more distant family and those who may no longer be living. This diagram will hopefully help you to locate yourself in relation to the other members of your family who I would like you to think about.

There is a factor that is important for us to know about when we come to compare your medical history with that of your family, please could you tell me whether your parents were your natural parents or adoptive.

Natural Parents _____ // Adoptive Parents _____

<u>MEDICAL HISTORY OF MOTHER & FATHER</u>

Please would you first think about your parents:

25.0) Are they still alive?   MOTHER - **YES / NO**   FATHER - YES / NO

    Can you remember their dates of birth?   MOTHER   FATHER

                                    DOB / /   DOB / /

Do you know whether your parents were in any way related to one another before they got married? For instance first or second cousins.

No Relation:_____   Probable Relat:_____   Definite Relat:_____   Don't Know:_____

Please specify:_____

25.1) As we are particulary interested in neurological problems, can you try and remember whether your parents ever experienced any of the following kinds of neurological conditions?

		MOTHER	FATHER
a]	Poliomyelitis: (or anything vaguely similar)	**YES / NO**	**YES / NO**
a]	Physical Tremor:	**YES / NO**	**YES / NO**
b]	Parkinson's Disease:	**YES / NO**	**YES / NO**
c]	Excess Forgetfulness:	**YES / NO**	**YES / NO**
Specify:_____			
d]	Motor Neurone Disease:	**YES / NO**	**YES / NO**
f]	Nervous Problem:	**YES / NO**	**YES / NO**
Specify:_____			
g]	Other Neurological Disorder:	**YES / NO**	**YES / NO**

Symptoms/onset:_____

_____

_____

25.2) Please could you tell me about any other troublesome health problems or medically diagnosed conditions your parents are presently suffering or experienced during their lifetime. (Include flu-like illnesses; but exclude common colds)

    MOTHER

Health Problem /Medical Condition	.Year/age.Duration
a]	

b] _____

c] _____

d] _____

e] _____

f] _____

FATHER

Health Problem /Medical Condition	.Year/age.Duration

a] _____

b] _____

c] _____

d] _____

e] _____

f] _____

MOTHER:
Full Name _____ Date of Death: / /

Cause of Death: _____ Place Died: _____

FATHER:
Full Name _____ Date of Death: / /

Cause of Death: _____ Place Died: _____

25.3) Now can you try and think about the health problems / illnesses other members of your immediate and more distant family in relation to any of the following neurological conditions.

Relationship to Twin	Phy .Trm	.Park. .Dis	MND. .	Sen. Dem.	Pol. io	Sch. izo.	Oth. Neu.	Specific Details

25.4) Please could you now think about any other health problems or medical conditions that your family members have suffered. Please include those members of your family who have died.

Relationship of Family Member	. Dead/. Alive.	Age or Age Died	. Health Problems/Medical . Condition / Cause Death
a]			
b]			
c]			
d]			
e]			
f]			
g]			
h]			
i]			
j]			
k]			
l]			
m]			
n]			
o]			
p]			
q]			
r]			
s]			
t]			

NATIONAL MOTOR NEURONE DISEASE STUDY

Confidentiality

The questions in this section are very personal. Your answers
will be treated in strict confidence; the interviewer does not
need to see them. Your name will not be on the answer sheet.

This section will be left with you to answer in your own time.
When you have finished please return your answer sheet in the
envelope provided.

How to answer

Just put a tick on the line opposite the appropriate answer

or write on a number like this _____

Please read the questions carefully before answering. Not all
the questions will apply to you: follow instructions.

Importance

It is very important to the whole study that you answer these
questions honestly and as accurately as you can.

Some things may be hard to remember, so please take your
time.

Code Number

FOR MEN ONLY

This is concerned with your own sexual experiences.

QUESTION 1 - FEMALE PARTNERS

Please include everyone you have ever had sex with at any time in your life, whether it was just once, a few times, a regular partner or your wife.

a) Altogether in you life so far, with how many **women** have you had sexual intercourse (vaginal, oral or anal)?

NONE _____ (go to question 2)

OR, IF ANY WRITE IN THE NUMBER HERE _____

OR, GIVE YOUR BEST ESTIMATE HERE _____

QUESTION 2 - MALE PARTNERS:

a) Have you ever had ANY kind of sexual experience or sexual contact with a **male**?

Please answer 'yes' here, even if it was a long time ago. Answer 'yes' even if it did **not** involve contact with the genital area (penis).

YES _____ - go to question 2 b)

NO _____ - go to question 3
(overpage)

b) Have you ever had sex with a man involving the genital area / penis contact?

YES _____ - go to question 2 c)

NO _____ - go to question 3

c) Altogether, in your life so far, with how many **men** have you had sex (that is oral, anal or other forms of genital contact)?

PLEASE WRITE IN NUMBER HERE _____

OR, GIVE YOUR BEST ESTIMATE HERE _____

QUESTION 3

a) Have you ever attended a sexually transmitted disease (STD) clinic or special (VD) clinic?

YES _____   NO _____

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CONCERNING YOUR TWIN IF MALE

This is concerned with the sexual experiences of your twin.

QUESTION 1 - FEMALE PARTNERS

Please include everyone you are aware of that your twin ever had sex with at any time in his life, whether it was just once, a few times, a regular partner or his wife.

a) Altogether during your twin's life, with how many **women** did he have sexual intercourse (vaginal, oral or anal)?

NONE _____ (go to question 2)

OR, IF ANY WRITE IN THE NUMBER HERE _____

OR, GIVE YOUR BEST ESTIMATE HERE _____

QUESTION 2 - MALE PARTNERS:

a) Did your twin ever have ANY kind of sexual experience or sexual contact with a **male**?

Please answer 'yes' here, even if it was as a very young man. Answer 'yes' even if it did **not** involve contact with the genital area (penis).

YES _____ - go to question 2 b)
                        (overpage)

NO _____ - go to question 3
                      (overpage)

b) Did your twin ever have sex with a **man** involving the genital area / penis contact?

YES _____ - go to question 2 c)

NO _____ - go to question 3

c) Altogether, during your twin's life, with how many **men** did he have sex (that is oral, anal or other forms of genital contact)?

PLEASE WRITE IN NUMBER HERE _____

OR, GIVE YOUR BEST ESTIMATE HERE _____

QUESTION 3

a) Did your twin ever attend a sexually transmitted disease (STD) clinic or special (VD) clinic?

YES _____   NO _____

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This is concerned with your own sexual experiences.

QUESTION 1 - MALE PARTNERS

Please include everyone you have ever had sex with at any time in your life, whether it was just once, a few times, a regular partner or your husband.

a) Altogether in you life so far, with how many **men** have you had sexual intercourse (vaginal, oral or anal)?

NONE _____ **(go to question 2)**

OR, IF ANY WRITE IN THE NUMBER HERE _____

OR, GIVE YOUR BEST ESTIMATE HERE _____

QUESTION 2

a) Have you ever attended a sexually transmitted disease (STD) clinic or special (VD) clinic?

YES _____   NO _____

CONCERNING YOUR TWIN IF FEMALE

This concerns the sexual experiences of your twin

QUESTION 1 - MALE PARTNERS

> Please include everyone you are aware of that your twin ever had sex with at any time in her life, whether it was just once, a few times, a regular partner or her husband.

a) Altogether during your twin's life, with how many **men** did she have sexual intercourse (vaginal, oral or anal)?

<div align="center">

NONE _____ (go to question 2)

OR, IF ANY WRITE IN THE NUMBER HERE _____

OR, GIVE YOUR BEST ESTIMATE HERE _____

</div>

QUESTION 2

a) Did your twin ever attend a sexually transmitted disease (STD) clinic or special (VD) clinic?

<div align="center">

YES _____   NO _____

</div>

<div align="right">

Continues overleaf....

</div>

FOR ALL

QUESTION 4

a) Have you ever injected yourself with any drugs or other substances, medical or otherwise?

YES _____ – **go to question b)**

NO _____ – **go to question 5**

b) Were any of these drugs or other substances prescribed by a doctor for a medical condition?

YES - ALL PRESCRIBED _____

SOME PRESCRIBED, SOME NOT _____

NO - NONE PRESCRIBED _____ – **go to question c)**

What was the condition ? _____
(PLEASE WRITE IN)

c) What drugs / substances have you taken this way? _____

d) Have you ever shared a needle – used for injecting – with someone else?

YES _____   NO _____

QUESTION 5

Have you ever taken any drugs or other substances, **not** prescribed by a doctor or available from a chemist, that you swallowed or inhaled.

(eg. Cannabis, Marijuana, Amphetamines, Speed, Angel Dust, Skol Bandits, LSD, Magic Mushrooms, Glue Sniffing, Cocaine, Crack, Morphine)

YES _____   NO _____

If 'yes'; please specify _____

QUESTION 6

a) Did your twin at any time during his/her life ever inject his/herself with any drugs or other substances, medical or otherwise?

YES _____ – **go to question b)**

NO _____ – **go to question 7**

b) Were any of these drugs or other substances prescribed by a doctor for a medical condition?

YES – ALL PRESCRIBED _____

SOME PRESCRIBED, SOME NOT _____

NO – NONE PRESCRIBED _____ – **go to question c)**

What was the condition ? _____
      (PLEASE WRITE IN)

c) What drugs / substances did your twin take this way? _____

_____

d) Did your twin ever share a needle – used for injecting – with someone else?

YES _____ NO _____

QUESTION 7

Did your twin at any time ever take any drugs or other substances, **not** prescribed by a doctor or available from a chemist, that you swallow or inhale.

(eg. Cannabis, Marijuana, Amphetamines, Speed, Angel Dust, Skol Bandits, LSD, Magic Mushrooms, Glue Sniffing, Cocaine, Crack, Morphine)

YES _____ NO _____

If 'yes'; please specify _____

_____