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The E. Graeme Robertson Lecture

MITOCHONDRIAL GENES AND NEUROLOGICAL DISEASE

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Mitochondria possess their own genetic material, each mitochondrion containing 2 to 10 double stranded circular DNA molecules about 16.6 kilobases (kb) in length. Mitochondrial DNA (mtDNA) is transmitted only by females. This part of the human genome was neglected in relation to disease until recently, but in the last few years the study of defects of mtDNA has become an important area of human genetics, particularly neurogenetics, and this has important implications in neurological practice.

In the mid 1980s, 2 observations related to coding function and mode of transmission pointed to mtDNA as a potential culprit in the pathogenesis of a clinically heterogeneous group of diseases, mitochondrial myopathies and encephalomyopathies (MEM). Another disorder, Leber's hereditary optic neuropathy, was also a good candidate for a mitochondrial genetic disorder as it exhibits maternal inheritance. This review will discuss mtDNA defects in relation to these 2 groups of disorders, and also more speculative data concerning their role in neurodegenerative disorders and ageing.

MITOCHONDRIAL STRUCTURE AND FUNCTION

Mitochondria are cylindrical organelles with a diameter of 0.5–1.0 μm. They comprise an outer membrane, an inner membrane and 2 internal compartments, the intermembranous space and the matrix. The outer membrane is permeable to ions and small proteins, whereas the highly convoluted inner membrane has a highly selective permeability[1] .

Many mitochondrial enzymes are concentrated in the matrix, including those catalysing the oxidation of pyruvate and fatty acids, and acetyl CoA in the Krebs' cycle, the main products of which are carbon dioxide and NADH. NADH is the

principal substrate for the respiratory chain, which comprises 4 major enzyme complexes (Complexes I–IV) embedded in the inner mitochondrial membrane. Electrons from NADH (and $FADH_2$) pass along the electron transfer chain, gradually releasing energy which pumps protons across the inner membrane. This process utilises oxygen and creates an electrochemical proton gradient across the inner membrane. This drives the production of ATP from ADP and phosphate by the enzyme complex ATP synthase (complex V) bound to the inner mitochondrial membrane. This energy is also used to transport pyruvate and other mitochondrial enzyme substrates, and nuclear encoded mitochondrial proteins, into the mitochondrial matrix[1,2].

MITOCHONDRIAL DNA

MtDNA differs from nuclear DNA in that it contains hardly any non-coding sequence, and to some extent in its genetic code. Each strand of mtDNA is transcribed from a single promotor site and then processed. The heavy (H) strand transcripts consist of 2 ribosomal RNAs, 14 transfer RNAs (tRNAs), and 12 protein reading frames, whereas the light (L) strand codes for 8 tRNAs and one protein reading frame sequence. Each strand replicates from its origin of replication using a method called displacement synthesis. The heavy strand origin is in a region of mtDNA called the displacement (D-) loop, and this region contains, or is near to, several regulatory elements involved in transcription and translation[3-5].

MtDNA encodes for 13 of the 80 or so subunits of the mitochondrial respiratory chain and oxidative phosphorylation system: 7 subunits of complex I; cytochrome b (complex III); subunits I, II, and III of cytochrome oxidase (complex IV); and subunits 6 and 8 of ATP synthetase[6,7]. Nuclear genes encode the remaining polypeptides in the respiratory chain, and control their transport into the mitochondrion[1]. Transcription, translation and replication of the mitochondrial genome are also dependent on nuclear products such as ribosomal proteins and polymerases[4].

Mitochondria divide by binary fission, at a rate similar to that of division of their parent cells. MtDNA molecules also replicate with every cell cycle in most circumstances, keeping the amount of organelle DNA per cell constant (about 1% of total cellular DNA). MtDNA is effectively transmitted only by females. The cytoplasm of the ovum forms a part of the developing embryo. Some paternal mitochondria penetrate the ovum, but these appear to degenerate subsequently, and only extremely low abundance paternal mtDNA can be detected in offspring[8]. Thus each mammal has the same mtDNA as any

individual related to it through females. However, extensive nucleotide sequence divergence in mtDNA occurs between different maternal lines, and its mutation rate is high. Despite this, all the mtDNA in an individual normal human appears to be identical, although extensive sequence data comparing different tissues have not been reported. MtDNA heteroplasmy, the presence of 2 different populations defined by sequence variation, has been described in cows[9] and is common in the presence of harmful mutations (see below).

Mitochondrial encephalomypathies (MEM)

Clinical and biochemical features

These are clinically and biochemically heterogeneous disorders which usually exhibit structural abnormalities of skeletal muscle mitochondria[10,11]. Ragged red fibres (RRF), containing peripheral and intermyofibrillar accumulations of abnormal mitochondria, are seen with the modified Gomori trichrome or succinate dehydrogenase stains. RRF were initially observed in patients presenting with syndromes of chronic progressive external ophthalmoplegia (PEO) and/or proximal myopathy. They were subsequently described in children and adults with complex multisystem disorders predominantly or exclusively affecting the central nervous system (CNS). These present with psychomotor retardation, dementia, ataxia, seizures, movement disorders, stroke-like episodes, pigmentary retinopathy, deafness, and peripheral neuropathy in various combinations. Involvement of other systems, such as the heart, endocrine system and kidney has also been described[10,11].

Some cases of MEM can be classified into distinctive clinical syndromes. These include: the Kearns-Sayre syndrome, a combination of PEO and pigmentary retinopathy developing before the age of 20 years, associated with ataxia, cardiac conduction defects, and a raised cerebrospinal fluid protein concentration[12]; mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS)[13]; and myoclonic epilepsy with ragged red fibres (MERRF)[14]. Although there is considerable clinical overlap between these syndromes, they represent combinations of some of the more striking features of these disorders, and are useful as pointers to the underlying diagnosis. They also tend to share the same molecular genetic, but not biochemical, basis.

Most patients with MEM have a pathological increase in serum lactate concentration during and after exercise. Polarography and enzyme assays have identified a variety of single or multiple defects of the respiratory chain and oxidative phosphorylation system in patients with MEM, all of which are associated with a wide range of clinical syndromes. Conversely, symptoms and

signs may be similar in patients with different biochemical defects[10,11].

Genetic features

The majority of patients with MEM do not have a history of affected relatives, but when individuals are affected in more than one generation, maternal transmission to offspring is far more frequent than paternal transmission[15]. Mitochondrial inheritance of MEM was proposed[16], a hypothesis supported by the fact that respiratory chain complexes contain subunits encoded by mtDNA. Several defects of the mitochondrial genome have since been described in patients with MEM (Table 1).

MtDNA deletions and duplications

Some patients with MEM have large single deletions of muscle mtDNA, 2–9 kb in length, which may contribute up to 80% of the total mtDNA population. Nearly all patients with deletions have PEO, and some have the KSS, although not all cases of PEO and KSS have mtDNA deletions[17-19]. Virtually all patients with deletions are sporadic cases, and it seems most likely that deletions originate during oogenesis in the patients' mothers[18]. Deletions are present in much lower abundance in blood in patients with neurological disease, only rarely being detectable by Southern blot.

Pearson's syndrome presents in infancy with pancytopaenia, exocrine pancreatic failure and hepatic dysfunction. MtDNA deletions are present in blood. Infants who survive later develop the features of KSS, indicating that this syndrome is a severe phenotype of the same molecular defect[20].

Autopsy studies have shown widespread tissue distribution of deleted mtDNA in KSS[21], but no data from patients with PEO alone have been reported. In situ hybridization studies of muscle of patients with mtDNA deletions show that deleted mtDNA is largely confined to histochemically abnormal fibre segments (RRF and cytochrome oxidase-negative fibres) and is transcribed, whereas normal mtDNA is depleted in these segments[22,23].

Over 100 patients with mtDNA deletions have been described to date. About ⅓rd of deletions are identical, flanked by a 13 base pair (bp) direct repeat existing at bp 8470–8482 and bp 13447–13459, and referred to as the common deletion[24]. It is thought that such deletions arise from slippage during replication. Some deletions are not flanked by repeats, suggesting heterogeneous pathogenetic mechanisms. Brockington *et al*[15] showed that some patients with deletions also have a tandem duplication in the D-loop region of mtDNA in a small proportion

of molecules. This may reflect an inherent tendency towards mtDNA instability secondary to another factor, such as impaired function of one of the nuclear encoded proteins involved in mtDNA replication.

Table 1 Defects of mitochondrial DNA associated with neurological disease and ageing

	Disease
Primary mtDNA defects	
Large deletions (sporadic)	Mitochondrial myopathies (PEO, KSS), age+
Duplications (most sporadic)	Mitochondrial myopathies (PEO, KSS)
Point mutations* (may be maternal FH)	
in tRNA genes (bp):	
8344, 8356	MERRF
3243, 3250, 3271, 11084	MELAS/other encephalomyopathies
3302, 3250, 15990	myopathy
3260	myopathy/cardiomyopathy
3251	PEO, early death
in reading frame:	
8993	neurogenic weakness, ataxia, retinitis pigmentosa*
11778, 3460, 4160, 14484	Leber's hereditary optic neuropathy*
in ribosomal RNA gene:	
1555	non-syndromic and antibiotic induced deafness
MtDNA defects secondary to presumed nuclear mutations:	
Multiple deletions (AD)	PEO, myopathy, deafness, neuropathy
Depletion (varies between tissues)(AR)	myopathy/renal/hepatic failure

*detectable in blood samples, hence useful screening test + = detected in small amounts only
FH = family history; AD = autosomal dominant; AR = autosomal recessive

Duplications of mtDNA exhibit a similar phenotype to mtDNA deletions and are usually sporadic, but maternal transmission may occur[28,29]. They may represent deleted mtDNA dimers; duplications probably play a role in the pathogenesis of deletions[27]. Ballinger *et al* described a family with maternally inherited diabetes and deafness stated to have a large deletion of mtDNA, but the report is more compatible with the presence of a mtDNA duplication, rather than a deletion[27].

Point mutations of mtDNA

Several point mutations of mtDNA, most in tRNA genes, have been reported in MEM syndromes, the most frequent of which are at bp 8344 and 3243 (Table 1). The 8344 bp mutation, which is in the lysine tRNA gene, was initially demonstrated in a large kindred with maternally inherited MERRF[29,30]. It is the major, but not the sole, cause of MERRF and all index patients reported to date have had the core clinical features of myoclonus, ataxia, and a variety of types of seizure[31,32]. Additional features include optic atrophy, peripheral neuropathy, deafness, dementia, ophthalmoplegia, stroke-like episodes and Leigh's syndrome. Relatives may have only one of these features or be asymptomatic. The mutation is heteroplasmic, and disease severity shows some correlation with the proportion of mutant mtDNA[30,32]. A further mutation, at bp 8356 (also in the lysine tRNA gene) has been reported in 2 families with MERRF[33,34].

The phenotype associated with the MELAS mutation, at bp 3243 in the leucine tRNA gene[35], is much more variable, only approximately half of patients presenting with multiple stroke-like episodes. Others may have a combination of myopathy, ataxia and deafness, PEO, myopathy alone[36,37], or even diabetes and/or deafness without neurological disease[38,39]. Two further mutations associated with the MELAS syndrome are at bp 3271[40] and 11084[41]. The latter is unusual in that it is in complex I subunit gene, rather than a tRNA. Some patients with either the MERRF (8344) or MELAS (3243) mutations do not have ragged red fibres on muscle biopsy.

Other pathogenic mtDNA point mutations are listed in Table 1. It appears that the leucine$^{(UUR)}$ tRNA gene is a hotspot for pathogenic mtDNA mutations, although this could be an artefactual observation due to investigators focusing on this tRNA. The phenotypic heterogeneity observed with 7 different mutations spanning only 28 bp of this gene is difficult to explain. This ranges from myopathy alone (bp 3250, 3302) [42,43], through myopathy and cardiomyopathy (bp 3260) [44,45], proximal and truncal myopathy and sudden early death (bp 3251)[46], to a disorder which often predominantly affects the central nervous system

including the MELAS syndrome (bp 3243, 3271, 3252)[35,41,47]. A point mutation in the proline tRNA gene, converting the anticodon to serine, has been reported in a patient with myopathy[48]. It is likely that other rare point mutations underly many cases of MEM. About 40% of adult patients with MEM in our series do not have a known mtDNA defect (unpublished data). It is probable that some of these will have as yet undefined point mutations, although some may have nuclear defects.

MtDNA defects presumed secondary to nuclear mutations

Some mtDNA defects are inherited as mendelian traits, suggesting a nuclear mutation with secondary effects on mtDNA. One is the syndrome of multiple mtDNA deletions[49,50], which is most commonly of autosomal dominant inheritance. The clinical features characteristically comprise adult onset PEO, proximal myopathy and deafness. Cataracts, ataxia and peripheral neuropathy also occur. RRF are present in muscle biopsies. Southern blot analysis of muscle shows several deleted mtDNA fragments in addition to the normal one(s). The deletions are not detectable in blood. Two siblings born to consanguineous healthy parents with similar clinical features may demonstrate autosomal recessive inheritance[51]. Other phenotypes described in association with multiple deletions include ataxia, ketoacidotic comas, PEO, deafness and spasticity[52], and recurrent exertional myoglobinuria was described in 2 brothers[53]. It is thought that the nuclear mutation in these disorders involves a gene encoding a factor involved in mtDNA replication[50].

In 2 cousins with a fatal mitochondrial disease, affecting muscle in one and liver in the other, there was severe depletion of mtDNA in affected tissues[54]. MtDNA depletion in affected tissues (muscle, muscle and kidney) was also observed in two other, unrelated, infants. Tritschler *et al* [55] studied 5 further children with mitochondrial myopathy manifesting within or soon after the first year of life, all of whom had depletion of muscle mtDNA (2–34% of normal). Inheritance of this syndrome is probably autosomal recessive, and pathogenesis may involve failure of the resumption of mtDNA replication after early embryogenesis.

Clinical applications and genetic counselling

The bp 3243 and 8344 mutations can be detected in leukocyte DNA from at least 95% of patients[36]. Such analysis is useful in screening patients with myoclonic syndromes or early stroke for mitochondrial disease. The other point mutations described above are also generally detectable in blood samples, and are

worth investigating in patients with the appropriate phenotype. Screening for mtDNA mutations in blood may establish a diagnosis in patients with MEM and remove the need for muscle biopsy. Failure to detect a mutation does not of course exclude a diagnosis of MEM.

Genetic counselling in mitochondrial myopathies is often difficult since a similar clinical presentation may have different recurrence risks to offspring, and these risks are highly dependent on molecular genetic analyses. The demonstration of a large single mtDNA deletion confers a very low recurrence risk to relatives, including the offspring of females. Autosomal dominant inheritance of documented multiple deletions can be assumed if there is paternal transmission in the pedigree, and this disorder appears to be fully penetrant. The presence of a point mutation of known pathogenicity confers no risk to the offspring of affected males, but counselling affected or asymptomatic females is not straightforward. In families with the bp 8344 mutation, there is some correlation between clinical severity and the proportion of mutant mtDNA in blood, and it also appears that females with affected children have a higher proportion than those who do not[32]. However, there is considerable overlap, and it is not clear whether the proportion of mutant mtDNA in blood alters with age. Prenatal diagnosis is not advisable for the same reasons. The situation is more complex with the most common bp 3243 mutation, as there is little correlation between the proportion of mutant mtDNA and the severity[37].

Neurogenic muscle weakness, ataxia and retinitis pigmentosa

A maternally transmitted syndrome with variable features, but including neurogenic muscle weakness, ataxia, and retinitis pigmentosa (NARP) in adult patients, is associated with a point mutation of mtDNA at bp 8993[56]. Dementia and seizures also occur. Leigh's syndrome, defined as progressive or relapsing brainstem dysfunction with characteristic pathology, is the most severe phenotype of this genetic defect[57]. It has become clear that the 8993 mutation is a common cause of Leigh's syndrome even in the absence of affected relatives[58]. The mutation is detectable in blood samples[56]. It is unusual in that it is in a protein coding gene (in ATPase subunit 6) rather than in a tRNA gene. No ragged red fibres are seen on muscle biopsy. It is of interest that the mitochondrial proliferation which gives rise to ragged red fibres occurs in defects of mtDNA involving transfer RNAs (deletions or point mutations), but generally not with mutations involving mitochondrial genes encoding proteins, as in this syndrome and LHON (see immediately below).

LEBER'S HEREDITARY OPTIC NEUROPATHY

Leber's hereditary optic neuropathy (LHON) causes severe visual loss in young adults, predominantly males. LHON is exclusively maternally transmitted, suggesting an underlying mutation of mtDNA, and two common pathogenic mutations, exclusively found in affected families, have been described. The most frequent is a point mutation at position 11778 of mtDNA which leads to an amino acid change in a mitochondrial respiratory chain complex I subunit (ND4)[59]. Patients with this mutation rarely recover useful visual function[60]. The mutation at position 3460 is in the ND1 subunit gene and confers a slightly better visual prognosis[61-63]. A third mutation, at position 4160 (also in the ND1 gene), has been reported exclusively in a large unusual Australian pedigree in which some members have encephalopathy[64]. A further mutation at position 14484 (ND) is probably pathogenic, and appears to be associated with a relatively good prognosis for visual recovery[65,66]. Other mtDNA mutations have been described in LHON pedigrees, particularly one at base pair (bp) 13708, but their significance is unclear as they also occur in the normal population and mtDNA is highly polymorphic[67-69].

There are several features of LHON which are not wholly explained by a defect of mtDNA. Many males with a high proportion of mtDNA remain unaffected, and a mtDNA mutation does not explain the predominance of affected males. It has been suggested that these observations can be accounted for by a gene on the X chromosome which determines visual loss susceptibility in LHON families[70], but this has not been confirmed[71].

The identification of pathogenic mtDNA mutations in LHON families, detectable in blood samples, has provided diagnostic markers for the disease which are very useful in clinical practice. They have also changed the definition of its phenotype to some extent. Previously it was difficult to make a diagnosis of LHON with certainty in the absence of affected relatives, but about half of our patients with LHON, as defined by subacute optic neuropathy in the presence of the 11778 or 3460 bp mtDNA mutations, have no known affected relatives (unpublished data). The range of age of onset is also wider than generally thought. Although this is usually between the ages of 16 and 37 years, it varies from 8 to 60 years[60]; one patient had an onset at the age of 63 years and was thought initially to have an anterior ischaemic optic neuropathy[72].

An association between LHON and multiple sclerosis (MS) was suggested in the past, but this was difficult to substantiate without modern investigative techniques. We have recently reported 6 females with a family history of LHON

who presented with bilateral optic neuropathy but later developed other neurological features compatible with a diagnosis of MS. This diagnosis was supported by magnetic resonance imaging (MRI); MRI is normal in males with LHON. A further 2 females with LHON in the absence of other neurological symptoms had the characteristic features of MS on MRI. All had the 11778 bp mtDNA mutation[73].

The identification of mtDNA mutations has made it possible to identify families who need genetic counselling for LHON which previously may have gone unrecognized because there was only one affected member. Molecular genetic analysis has not made a major impact on refining the genetic risks in LHON families, because some males with a high proportion of mutant mtDNA never develop the disease and genetically similar females do not always transmit it. Based on pedigree data, it has been estimated that about 50% of the sons of a normal female who is an obligate carrier (with an affected son and brother or uncle) are affected, and about 50% of her daughters transmit the disease themselves. These risks are rather higher (probably about 75%) in the offspring of affected females[74]. However, these figures are probably artificially high, as no genetic counselling data have been provided from a large series of unselected families, including those containing singleton cases, taking age related penetrance into account. The risk of developing the disease is more difficult to estimate in women, but in the sisters of male patients and the daughters of obligate carriers it is about 12% in European populations, and twice this if the individual's mother is affected[74].

DEAFNESS

Deafness is a common feature of MEM, and may be seen as an isolated feature in members of families with the bp 8344 and 3243 mutations[32,39]. Pedigree analysis of an inbred Arab-Israeli family with deafness, and Chinese kindreds containing more than one individual with aminoglycoside-induced deafness, suggested that there was mitochondrial genetic susceptibility. The same point mutation of mtDNA, at position 1555 in one of the ribosomal RNA genes, has been identified in both syndromes[75].

NEURODEGENERATIVE DISORDERS AND AGEING

Mitochondrial complex I deficiency has been reported in both the substantia nigra and platelets of patients with Parkinson's disease (PD)[76]. The finding of deleted mtDNA in the brains of patients dying with PD is almost certainly an age related phenomenon[77]. It has also been proposed that point mutations of mtDNA

may be relevant to the pathogenesis of PD[78,79], but it is questionable whether such mutations are of any pathological significance. The same applies to observations in Alzheimer's disease[79]. It is clear that there is no pedigree evidence of mitochondrial inheritance in PD; there is a slight excess of paternal transmission[80].

There is evidence that respiratory chain activity in muscle decreases with age[81], and the hypothesis that there may be a contribution to the ageing process from accumulating mtDNA mutations has attracted great interest[82]. The presence of the common deletion of mtDNA was demonstrated in heart muscle from elderly, but not fetal, heart muscle[83]. In the brain, the ratio of deleted to normal mtDNA increases with age, particularly after the age of 80 years. Deleted mtDNA is most prevalent in the striatum and substantia nigra, and of low abundance in the cerebellum[84,85]. It is not as yet clear whether this regional variation has any significance in relation to the ageing process or neurodegenerative disease[86].

It seems unlikely that low abundance deleted mtDNA alone accounts primarily for the biological effects of ageing, or that it necessarily accounts for declining respiratory chain function in the elderly. A possible contribution of multiple mtDNA defects, including point mutations, to ageing requires investigation, although the results would be difficult to interpret. One point against the role of mtDNA defects as a major contributor to ageing is that patients with recognized mtDNA diseases do not exhibit any of the features of progeria. It is, however, clear that studies of mtDNA defects in neurodegenerative diseases must take account of findings in the normal elderly population.

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REFERENCES

1. Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD. Molecular biology of the cell. Garland, New York, 1983
2. Hatefi Y. The mitochondrial electron transport and oxidative phosphorylation system. Annual Review of Biochemistry 1985; 54:1015–1069.
3. Anderson S, Bankier AT, Barrell BG *et al.* Sequence and organization of the human mitochondrial genome. Nature 1981; 290:457–465
4. Tzagoloff A, Myers AM. Genetics of mitochondrial biogenesis. Annual Review of Biochemistry 1986; 55:249–285.
5. Clayton D. Replication and transcription of vertebrate mitochondrial DNA. Annual

Review of Cell Biology 1991; 7:453–478.
6. Chomyn A, Mariottini P, Cleeter MWJ *et al.* Six unidentified reading frames of human mitochondrial DNA encode components of the respiratory chain NADH dehydrogenase. Nature 1985; 314:592–597.
7. Chomyn A, Mariottini P, Cleeter MWJ *et al.* Functional assignment of the unidentified reading frames of human mitochondrial DNA. In: Quagliariello E *et al*, eds. Achievements and perspectives of mitochondrial research, vol II: Biogenesis. Amsterdam: Elsevier, 1985:259–75.
8. Gyllensten U, Wharton D, Josefssoon A, Wilson AC. Paternal inheritance of mitochondrial DNA in mice. Nature 1991; 352:255–257.
9. Hauswirth WW, Laipis PJ. Mitochondrial DNA polymorphism in a maternal lineage of Holstein cows. Proceedings of the National Academy of Science, USA 1982; 79:4686–4690.
10. DiMauro S, Bonilla E, Zeviani M, Nakagawa M, DeVivo DC. Mitochondrial myopathies. Annals of Neurology 1985; 17:521–538.
11. Petty RKH, Harding AE, Morgan-Hughes JA. The clinical features of mitochondrial myopathy. Brain 1986; 109:915–938.
12. Berenberg RA, Pellock JM, DiMauro S *et al.* Lumping or splitting? "Ophthalmoplegia-plus" or Kearns-Sayre syndrome? Annals of Neurology 1977; 1:37–54.
13. Pavlakis SG, Phillips PC, DiMauro S, De Vivo DC, Rowland LP. Mitochondrial myopathy, encephalopathy, lactic acidosis and strokelike episodes: a distinctive clinical syndrome. Annals of Neurology 1984; 16:481–488.
14. Fukuhara N, Tokiguchi S, Shirakawa K, Tsubaki T. Myoclonus epilepsy associated with ragged red fibers (mitochondrial abnormalities): disease entity or syndrome? Journal of Neurological Sciences 1980; 47:117–33.
15. Harding AE, Petty RKH, Morgan-Hughes JA. Mitochondrial myopathy: a genetic study of 71 cases. Journal of Medical Genetics 1988; 25:528–535.
16. Egger J, Wilson J. Mitochondrial inheritance in a mitochondrially mediated disease. New England Journal of Medicine 1983; 309:142–145.
17. Holt IJ, Harding AE, Morgan-Hughes JA. Deletions of mitochondrial DNA in patients with mitochondrial myopathies. Nature 1988; 331:717–719.
18. Holt IJ, Harding AE, Cooper JM *et al.* Mitochondrial myopathies: clinical and biochemical features of 30 patients with major deletions of muscle mitochondrial DNA. Annals of Neurology 1989; 26:699–708.
19. Moraes CT, DiMauro S, Zeviani M *et al.* Mitochondrial DNA deletions in progressive external ophthalmoplegia and Kearns-Sayre syndrome. New England Journal of Medicine 1989; 320:1293–1299.
20. McShane MA, Hammans SR, Sweeney M *et al.* Pearson syndrome and mitochondrial encephalomyopathy in a patient with a deletion of mtDNA. American Journal of Human Genetics 1991; 48:39–42.
21. Shanske S, Moraes CT, Lombes A *et al.* Widespread tissue distribution of mitochondrial DNA deletions in Kearns-Sayre syndrome. Neurology 1990; 40:24–28.
22. Shoubridge EA, Karpati G, Hastings KE. Deletion mutants are functionally dominant over wild-type mitochondrial genomes in skeletal muscle fiber segments in mitochondrial disease. Cell 1990; 62:43–49.

23. Hammans SR, Sweeney MG, Wicks DAG, Morgan-Hughes JA, Harding AE. A molecular genetic study of focal histochemical defects in mitochondrial encephalomyopathies. Brain 1992; 115:343–365.
24. Mita S, Rizzuto R, Moraes CT *et al.* Recombination via flanking direct repeats is a major cause of large-scale deletions of human mitochondrial DNA. Nucleic Acids Research 1990; 18:561–567.
25. Brockington M, Sweeney MG, Hammans SR, Morgan-Hughes JA, Harding AE. A tandem duplication in the D-loop of human mitochondrial DNA is associated with deletions in mitochondrial myopathies. Nature Genetics 1993; 4:67–71.
26. Ballinger SW, Shoffner JM, Hadaya EV *et al.* Maternally transmitted diabetes and deafness associated with a 10.4kb mitochondrial DNA deletion. Nature Genetics 1992; 1:11–15.
27. Poulton J, Deadman ME, Bindoff L, Morten K, Land J, Brown G. Families of mtDNA rearrangements can be detected in patients with mtDNA deletions: duplications may be a transient intermediate form. Human Molecular Genetics 1993; 2:23–30.
28. Poulton J, Deadman ME, Gardiner RM. Duplications of mitochondrial DNA in mitochondrial myopathy. Lancet 1989; 1:236–240.
29. Rosing HS, Hopkins LC, Wallace DC, Epstein CM, Weidenheim K. Maternally inherited mitochondrial myopathy and myoclonic epilepsy. Annals of Neurology 1985; 17:228–237.
30. Shoffner JM, Lott MT, Lezza AM, Seibel P, Ballinger SW, Wallace DC. Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA(Lys) mutation. Cell 1990; 61:931–937.
31. Zeviani M, Servidei S, Bresolin N *et al.* Rapid detection of the A->G(8344) mutation in Italian families with myoclonus, epilepsy and ragged red fibres (MERRF). American Journal of Human Genetics 1991; 48:203–211.
32. Hammans SR, Sweeney MG, Brockington M *et al.* The mitochondrial DNA transfer RNA^{Lys} A->$G^{(8344)}$ mutation: Clinical phenotype and relationship to proportion of mutant mitochondrial DNA. Brain 1993; 116:617–632.
33. Silvestri G, Moraes CT, Shanske S, Oh SJ, DiMauro S. A new mtDNA mutation in the tRNALys gene is associated with myoclonic epilepsy and ragged red fibres (MERRF). American Journal of Human Genetics 1992; 51:1213–1217.
34. Zeviani M, Muntoni F, Savarese N *et al.* A MERRF/MELAS overlap syndrome associated with a new point mutation of mitochondrial $tRNA^{Lys}$ gene. European Journal of Molecular Genetics 1993; 1:80–87.
35. Goto Y, Nonaka I, Horai S. A mutation in the tRNALeu(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature 1990; 348:651–653.
36. Hammans SR, Sweeney MG, Brockington M, Morgan-Hughes JA, Harding AE. Mitochondrial encephalopathies: molecular genetic diagnosis from blood samples. Lancet 1991; 337:1311–1313.
37. Ciafaloni E, Ricci E, Shanske S *et al.* MELAS: Clinical features, biochemistry, and molecular genetics. Annals of Neurology 1992; 31:391–398.
38. van Ouweland JMW, Lemkes HHPJ, Ruitenbeek W *et al.* Mutation in mitochondrial

tRNALeu(UUR) gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. Nature Genetics 1992; 1:368–371.
39. Reardon W, Ross RJM, Sweeney MG *et al.* Diabetes mellitus associated with a pathogenic point mutation in mitochondrial DNA. Lancet 1992; 340:1376–79.
40. Goto Y, Nonaka I, Horai S. A new mtDNA mutation associated with mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS). Biochimica Biophysica Acta 1991; 1097:238–240.
41. Lertrit P, Noer AS, Jean-Francois MJB *et al.* A new disease-related mutation for mitochondrial encephalopathy lactic acidosis and strokelike episodes (MELAS) syndrome affects the ND4 subunit of respiratory complex I. American Journal of Human Genetics 1992; 51:457–468.
42. Goto Y, Tojo M, Tohyama J, Horai S, Nonaka I. A novel point mutation in the mitochondrial tRNALeu(UUR) gene in a family with mitochondrial myopathy. Annals of Neurology 1992; 31:672–675.
43. Bindoff LA, Howell N, Poulton J *et al.* Abnormal RNA processing associated with a novel tRNA mutation in mitochondrial DNA: a potential disease mechanism. Journal of Biological Chemistry 1993. *In press.*
44. Zeviani M, Gellera C, Antozzi C *et al.* Maternally inherited myopathy and cardiomyopathy: association with mutation in mitochondrial DNA tRNALeu(UUR). Lancet 1991; 338:143–147.
45. Sweeney MG, Brockington M, Weston MJ, Morgan-Hughes JA, Harding AE. Mitochondrial DNA transfer RNA mutation $LEU^{(UUR)A\cdot\cdot G\ 3260}$: a second family with myopathy and cardiomyopathy. Quarterly Journal of Medicine 1993; 86:435–438.
46. Sweeney M, Bundey S, Brockington M, Poulton KR, Winer JB, Harding AE. Mitochondrial myopathy associated with sudden death in young adults and a novel mutation in the mitochondrial DNA transfer RNA(UUR) gene. Quarterly Journal of Medicine. *In press.*
47. Morten KJ, Cooper JM, Brown GK, Lake BD, Poulton J. A new point mutation associated with mitochondrial myopathy and diabetes mellitus. *In press.*
48. Moraes CT, Ciacci F, Bonilla E, Ionasescu V, Schon EA, DiMauro S. A mitochondrial tRNA anticodon swap associated with a muscle disease. Nature Genetics 1993; 4: *In press.*
49. Zeviani M, Bresolin N, Gellera C *et al.* Nucleus-driven multiple large-scale deletions of the human mitochondrial genome: A new autosomal dominant disease. American Journal of Human Genetics 1990; 47:904–914.
50. Zeviani M. Nucleus driven mutations of human mitochondrial DNA. Journal of Inherited Metabolic Diseases 1992; 15:456–471.
51. Yuzaki M, Ohkoshi N, Kanazawa I, Kagawa Y, Ohta S. Multiple deletions in mitochondrial DNA at direct repeats of non-D-loop regions in cases of familial mitochondrial myopathy. Biochemical and Biophysical Research Communications 1989; 164:1352–1357.
52. Cormier V, Rotig A, Tardieu M, Colonna M, Saudubray J-M, Munnich A. Autosomal dominant deletions of the mitochondrial genome in a case of progressive encephalopathy. American Journal of Human Genetics 1991; 48:643–648.
53. Ohno K, Tanaka M, Sahashi K *et al.* Mitochondrial DNA deletions in inherited

recurrent myoglobinuria. Annals of Neurology 1991; 29:364–369.
54. Moraes CT, Shanske S, Tritschler HJ *et al.* mtDNA depletion with variable tissue expression: a novel genetic abnormality in mitochondrial diseases. American Journal of Human Genetics 1991; 48:492–501.
55. Tritschler HJ, Andreetta F, Moraes CT *et al.* Mitochondrial myopathy of childhood associated with depletion of mitochondrial DNA. Neurology 1992; 42:209–217.
56. Holt IJ, Harding AE, Petty RKH, Morgan-Hughes JA. A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. American Journal of Human Genetics 1990; 46:428–433.
57. Tatuch Y, Christodoulou J, Feigenbaum A *et al.* Heteroplasmic mtDNA mutation (T-G) at 8993 can cause Leigh disease when the percentage of abnormal mtDNA is high. American Journal of Human Genetics 1992; 50:852–858.
58. Santorelli FM, Shanske S, Macaya A, DeVivo DC, DiMauro S. The mutation at nt 8993 of mitochondrial DNA is a common cause of Leigh syndrome. Annals of Neurology. *In press.*
59. Wallace DC, Singh G, Lott MT *et al.* Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 1988; 242:1427–1430.
60. Newman NJ, Lott MT, Wallace DC. The clinical characteristics of pedigrees of Leber's hereditary optic neuropathy with the 11778 mutation. American Journal of Ophthalmology, 1991; 111:750–762.
61. Howell N, Bindoff LA, McCullough DA *et al.* Leber hereditary optic neuropathy: identification of the same mitochondrial ND1 mutation in six pedigrees. American Journal of Human Genetics 1991; 49:939–950.
62. Huoponen K, Vilkki J, Aula P, Nikoskelainen EK. A new mtDNA mutation associated with Leber hereditary optic neuroretinopathy. American Journal of Human Genetics 1991; 48:1147–1153.
63. Johns DR, Smith KH, Miller NR. Leber's hereditary optic neuropathy. Clinical manifestations of the 3460 mutation. Archives of Ophthalmology 1992; 110:1577–1851.
64. Howell N, Kubacka I, Xu M, McCullough DA. Leber hereditary optic neuropathy: involvement of the mitochondrial NDI gene and evidence for an intragenic suppressor mutation. American Journal of Human Genetics 1991; 48:935–942.
65. Mackey D, Howell N. A variant of Leber hereditary optic neuropathy characterized by recovery of vision and by an unusual mitochondrial genetic etiology. American Journal of Human Genetics 1992; 51:1218–1228.
66. Johns DR, Heher KL, Miller NR, Smith KH. Leber's hereditary optic neuropathy. Clinical manifestations of the 14484 mutation. Archives of Ophthalmology 1993; 111:495–498.
67. Johns DR, Berman J. Alternative, simultaneous, complex I mitochondrial DNA mutations in Leber's hereditary optic neuropathy. Biochemical and Biophysical Research Communications 1991; 174:1324–1330.
68. Brown MD, Voljavec AS, Lott MT, Torroni A, Yang CC, Wallace DC. Mitochondrial DNA complex I and III mutations associated with Leber's hereditary optic neuropathy. Genetics 1992; 130:163–173.
69. Johns DR, Smith KH, Savino PJ, Miller NR. Leber's hereditary optic neuropathy.

Clinical manifestations of the 15257 mutation. Ophthalmology 1993; 100:981–986.
70. Vilkki J, Ott J, Savontaus ML, Aula P, Nikoskelainen EK. Optic atrophy in Leber hereditary optic neuroretinopathy is probably determined by an X-chromosomal gene closely linked to DXS7. American Journal of Human Genetics 1991; 48:486–491.
71. Sweeney MG, Davis MB, Lashwood AM *et al.* Evidence against a locus close to DXS7 determining visual loss in Italian and British families with Leber's hereditary optic neuropathy. American Journal of Human Genetics, 1992; 51:741–748.
72. Borruat F-X, Green WT, Graham EM, Sweeney MG, Morgan-Hughes JA, Sanders MD. Late onset Leber's optic neuropathy: a case confused with ischaemic optic neuropathy. British Journal of Ophthalmology 1992; 76:571–573.
73. Harding AE, Sweeney MG, Miller DH *et al.* Occurrence of a multiple sclerosis-like illness in women who have a Leber's hereditary optic neuropathy mitochondrial DNA mutation. Brain 1992; 115:979–989.
74. Harding AE, Sweeney MG. Leber's hereditary optic neuropathy. In: DiMauro S and Schapira AHV (eds). Mitochondrial diseases. Butterworth International Medical Reviews, London. *In press.*
75. Prezant TR, Agapian JV, Bohlman C *et al.* Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. Nature Genetics 1993; 4:289-294.
76. Mann VM, Cooper JM, Krige D *et al.* Brain skeletal muscle and platelet mitochondrial function in Parkinson's disease. Brain 1992; 115:333–342.
77. Mann VM, Cooper JM, Schapira AHV. Quantitation of a mitochondrial DNA deletion in Parkinson's disease. FEBS Letters 1992; 209:218–222.
78. Ikebe S, Hattori N, Mizuno Y *et al.* Point mutations of mitochondrial DNA in Parkinson's disease. Movement Disorders 1992; 7 (Suppl. 1):71.
79. Shoffner JM, Brown MD, Torroni A *et al.* Mitochondrial DNA varaiants observed in Alzheimer disease and Parkinson disease patients. Genomics 1993; 17:171–184.
80. Maraganore DM, Harding AE, Marsden CD. A clinical and genetic study of familial Parkinson's disease. Movement Disorders 1991; 6:205–211.
81. Trounce I, Byrne E, Marzuki S. Decline in skeletal muscle mitochondrial respiratory chain function: possible factors in ageing. Lancet 1989;i:637–9.
82. Linnane AW, Marzuki S, Ozawa T, Tanaka M. Mitochondrial DNA mutations as an important contributor to ageing and degerative diseases. Lancet 1989;i:642–645.
83. Cortopassi GA, Arnheim N. Detection of a specific mitochondrial DNA deletion in tissues of older humans. Nucleic Acids Research 1990; 18:6927–6933.
84. Soong NW, Hinton DR, Cortopassi G, Arnheim N. Mosaicism for a specific somatic mitochondrial DNA mutation in adult human brain. Nature Genetics 1992; 4;318–323.
85. Corral-Debrinski M, Horton T, Lott MT, Shoffner JM, Beal MF, Wallace DC. Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age. Nature Genetics 1992; 4:324–329.
86. Harding AE. Growing old: the commonest mitochondrial disease of all? Nature Genetics 1992; 4:251–252.

THE *EXPLOSIVE COPULA* OF THOMAS WILLIS

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SUMMARY

Thomas Willis (1621–1675), arguably the founding father of neurology, devised an interpretation of neurophysiology which involved motor function being mediated by explosions in nerve tissue and muscle, facilitated by the temporary development of an *explosive Copula* comprising short-lived aggregates of 'nitrous' and 'sulphur' particles i.e. the components of gunpowder. Seen from a modern standpoint, such a concept is manifestly absurd. However, seen from the standpoint of the Paracelsian iatrochemistry to which Willis subscribed, and understood in the spirit of analogy which he probably intended, Willis' interpretation can be regarded as the beginning of the application of bioenergetics to neural function.

In neurology, the name of Thomas Willis is a very great one indeed. In fact, the very term 'neurology', i.e. 'the doctrine of the nerves', is of his creation, as pointed out by Feindel[1] in 1965, and every medical student must surely have encountered mention of the arterial circle at the base of the brain to which Willis' name is so enduringly attached. His is the priority in describing both narcolepsy and myasthenia gravis. There has been considerable recent interest in his life and thought and he has been the subject of several biographies, including those of Feindel[1], Dewhurst[2] and of Trevor Hughes[3].

Willis can with some justification be regarded as the founding father of neurophysiology, and his concepts of the function of the nervous system have been discussed in some detail e.g. by Meyer and Hierons[4]. However, there is one element of Willis' thought on nervous system function that has often either been ridiculed, as it was by many of his contemporaries and immediate successors, or tacitly ignored, as by some later writers who have behaved as if tactfully sweeping under the carpet an intellectual aberration which might have otherwise sullied the reputation of a most considerable thinker. The concept in question is Willis' notion that motor function and muscle contraction were mediated through the agency of an *explosive Copula* which produced gunpowder explosions in the brain and in muscle, respectively. (According to Willis' translator, Pordage, a *Copula* is 'a joining or fastening together, a fettering').

Surely one has a duty towards Willis' memory to ask why he chose to devise and promulgate such an outlandish, indeed seemingly absurd, idea which appears to have totally failed to find favour with thinking men. In attempting to answer this question one needs to have some appreciation both of Willis' life and times, and of his philosophical position.

THOMAS WILLIS – LIFE (1621–1675)

A younger contemporary of the great William Harvey (1578–1657), Thomas Willis spent most of his life in and around Oxford, which was the Royalist capital for much of the English Civil War. Willis served for a time in the King's army, and pursued medical studies in Christ Church College, Oxford, graduating MA and MB (later MD). He practiced as a physician in Oxford during the Commonwealth and, at the time of the Restoration of the Monarchy (1660) was appointed Sedleian Professor of Natural Philosophy in the University, replacing the Cromwellian appointee who had been dismissed from the Chair. Willis built up the largest medical practice of his day in Oxford, and was a driving figure in a group which included the microscopist Robert Hooke, Robert Boyle (of Boyle's law fame), Richard Lower, the philosopher John Locke and the architect Christopher Wren, who then held the Chair of Astronomy at Oxford. Willis carried out numerous animal dissections and human post-mortems, endeavouring to discern how the body and particularly the nervous system functioned, utilising what would now be called clinico-pathological correlations and a comparative anatomical approach, though he also indulged in a certain amount of physiological experimentation. Willis' most influential works probably were his *'Cerebri Anatome'*, *'Pathologiae Cerebri'* and *'De Anima Brutorum'*, plus his writings on pharmacology in his *'Pharmaceutice Rationalis'*. In later life he moved to London, where he acquired a huge medical practice and was elected Fellow of the Royal College of Physicians, as well as being a founding Fellow of the Royal Society. He died in his 54th year, probably from pulmonary tuberculosis, and was buried in Westminster Abbey. Within a few years of his death his collected works[5], all originally written in Latin, had been 'English'd' by the unsuccessful playwright Samuel Pordage, and were published in London in 1681.

THOMAS WILLIS – PHILOSOPHY

To understand Willis' interpretation of neurophysiology, it is necessary to appreciate that he was a Paracelsian in a medical community that was dominantly

Galenist and Aristotlean in its attitude. Paracelsus (Phillipus Theophrastus Bombastus von Hohenheim, 1493–1541), a peripatetic, wayward and erratic genius, was the originator of the chemical approach to medicine. He regarded man as a microcosm, and believed that anything which occurred in the wider macrocosm had its parallel in the microcosm of human life. Thus, because of the macrocosm–microcosm relationship, astral events could influence the human situation. Paracelsus interpreted many of the events of nature in terms of the chemistry of his time, but this chemistry was a mixture of late medieval alchemy and the rudimentary metallurgical chemistry which Paracelsus knew from first–hand experience. His therefore was mainly an inorganic chemistry, but one in which there was no distinction between what we now know as elements and compounds, and one in which relatively few substances were characterised as they now are. There was no notion at all of our present day organic chemistry. Paracelsus recognised that animal and plant life depended on the availability of air and he reasoned that 'vital spirits' from air must enter the animal body and maintain its life. Because inorganic chemical reactions occurred both in the macrocosm, and in the microcosm, and because certain chemical mixtures are explosive, yielding energy whose presence is revealed only by its manifestations, Paracelsus reasoned that the 'vital spirits' must be chemical in nature and that the animal body must function chemically. Therefore health might be restored by appropriate (often inorganic) chemical treatments. On this basis Paracelsus began to employ what he regarded as such measures. To Paracelsus[6] we owe the great pharmacological dictum:

> 'All things are poison and nothing is without poison: it is only the *Dosis* which makes a thing not a poison.'

In *'The Diseases that Deprive Man of his Reason'* Paracelsus[7] likened an epileptic seizure to an earthquake occurring within the brain, based on his microcosm-macrocosm analogies.

> 'If the *spiritus vitus* is shifted from its right disposition, it boils and effervesces, and this happens so quickly that memory and reason are destroyed. This boiling may be compared with the approach of an earthquake, which makes the whole earth tremble. Earthquakes and the falling sickness have the same causes.'

Such an interpretation is not dissimilar to Willis' concept of epileptogenesis (in *'De Morbus Convulsivis'*):

> 'And indeed, I think it very likely so, that the Epileptik Paroxism is stired up, from a certain suddain rarefication, and explosion of the animal spirits,

inhabiting the Brain, . . .'

The Paracelsian iatrochemical approach evolved further in western Europe in the latter half of the 16th Century and, from the end of that century onwards, in Britain where its story has been told by Debus[8]. There it existed in tension with the dominantly Aristotlean and Galenian interpretations of physiology and disease. Probably Robert Fludd (1574–1637) was the most notable of the English iatrochemists, and Willis was the last of them to be discussed by Debus[8], the progressively modified Paracelsian ideas having by his time begun to achieve an increasing measure of reconciliation with the older Galenian pattern of thinking.

Willis' commitment to Paracelsian concepts led him to seek to understand bodily events in terms of iatrochemical ideas. However, the chemical knowledge of his day was woefully inadequate to interpret the situation in a way that would convince his contemporaries or subsequent generations.

WILLIS' NEUROPHYSIOLOGY

The central element in Willis' neurophysiology was his 'animal spirits', which in effect mediated both what would now be regarded as neurotransmission and also nervous system nutrition. Willis believed the 'animal spirits' were derived from chemical materials which entered the blood from outside the body, in a manner akin to the Paracelsian 'vital spirits' from the macrocosm. In the blood, itself the 'vital liquor' and one of the two component parts of the 'corporeal soul', these chemicals burned with a 'gentle and friendly heat', but not all of them were consumed (in *'De Anima Brutorum'*).

> '. . . it follows thence, that some particles being burnt, others of a various Kind being manumitted or let go, they are Variously imployed in the offices of the others; but of these, those which are chiefly Subtil, as it were Beams of light sent from a flame, are, as it were distilled into the Brain and Cerebel. These most subtil particles are called the Animal Spirits, and first of all entering the Cortical Substances of those parts, and from thence flowing into the *Meditullia* or middle parts of either of them, and into the Oblong and Spinal Marrow, and further into all the Nerves and Nervous Fibres, dispersed thorow the whole Body, Constitute the other and more noble part of the Corporeal Soul, . . .'

Willis elsewhere (in *'Cerebri Anatome'*) explained that both a nervous juice (or 'latex') with a nutritional function and the animal spirits were distilled (a chemical analogy for a process which separated these materials from other blood

constituents) into the brain and cerebellum. In the nervous system the nervous juice and the 'animal spirits' generally moved about together, though the animal spirits could move more rapidly when the circumstances required it:

> 'The animal spirits being left to themselves, follow the motion of this Juyce, and flowing together with it in the same course, are pleasingly or quietly expatiated; but in the mean time, as occasion is offered, the same Spirits, as a breath moving on those waters conceive other spreadings abroad, and those more rapid.'

The movement of the animal spirits along predetermined pathways consisting of ultramicroscopic pores in the nervous parenchyma subserved the function of neurotransmission. Centrifugal movement of the spirits into the medulla, then the cord, peripheral nerves and muscles (Willis had no notion of the existences of synapses and myo-neural junctions and considered all these structures to be in continuity) mediated muscle contraction, and their centripetal movement produced sensation, while intracerebral movement between particular regions was responsible for higher functions such as thought and memory.

It was the natures of the chemical substances constituting the animal spirits that gave subsequent generations difficulty in accepting Willis' ideas. For he considered that these chemicals that were essential for continuing life as well as for neural function were firstly nitre, or 'nitrous particles', absorbed from the atmosphere or, in the case of aquatic creatures, from the surrounding water, and secondly sulphur which was obtained from the diet and taken into the blood from the alimentary tract. As Willis put it, in *'De Anima Brutorum'*

> '. . . there is need constantly of an Internal Sulphureous Food, together with an External nitrous;'

He then took the matter rather further, recognising that nitre and sulphur were the ingredients of gunpowder:

> '. . . That altho Fire and Flame necessarily require, besides Sulphureous food from the matter of the Subject, something nitreous from the Air, which being denyed or withdrawn, they are suddenly extinguished; yet, if that the matter be inkindled of Sulphur and Nitre (as is wont to be in Gun-Powder) together mixed with the Concrete, that Fire or Flame will burn in the midst of the Waters, or in a place Empty of Air;'

Willis (in *'Cerebri Anatome'*) developed the gunpowder analogy to greater lengths in writing of powerful muscle contraction:

> '. . . so that their force being stirred by a strong endeavour, it seems like an explosion of Gun-powder; and also the same Spirits being continually consumed within the Muscles more profusely than is wont to be in the Membranes and other parts, are in some measure made up or repaired from the bloody sustenance: because whenas the arterious Juyce joyns more plentifully with the nervous flowing within the sanguineous parts, it may well be thought, that it also lays upon the Spirits brought thither with it, as it were some nitrosulphureous particles, and intimately fixes them on them; and so, by reason of this Copula, highly flatuous and apt to be rarified, the Spirits themselves become there more active, so that in every motive endeavour, whereby the Muscle is suddenly intumified, they, as if inkindled, are exploded.'

Thus the *Copula* was, as it were, a linking of nitrous and sulphur particles with the local animal spirits, producing a high concentration of potentially explosive matter. Once the explosive particles separated from this combination and came in contact with each other, an explosion like that of gunpowder occurred.

$$4KNO_3 + 2S + 6C \rightarrow 2K_2S + 2N_2 - 6CO_2$$

This explosion expanded the girth of the muscle in which it took place, thus drawing the ends of the muscle together to produce muscle contraction and movement. Particularly in *'De Morbus Convulsivis'*. Willis also applied this *explosive Copula* idea to the central nervous system events that initiated voluntary movement. It was reasonable that he should do so, given that he believed that the animal spirits of the central and peripheral nervous systems were in direct continuity with those of muscle. In discussing the role of the animal spirits within the nerves in producing convulsive movement, he wrote in relation to physiological movement (in *'De Morbus Convulsivis'*):

> '. . . to wit, so long as they are imbued, with a fit and moderate explosive *Copula,* and are moved to that striking forth, only by the Command of the Appetite, or instinct of Nature, they bring forth motions altogether regular;'

and invoked the idea of a *heterologous* (and excessively) *explosive Copula* which was the basis of epileptogenesis.

Thus what had begun as analogy to the behaviour of gunpowder seemed to become an acceptance that gunpowder and its explosion were actually involved in motor events. Yet Willis (in *'De Morbus Convulsivis'*) acknowledged the danger in writing of such 'explosions', realising that this might seem

> 'only *ignoti per ignotius explicatio,* an explication of unknown things by more unknown things;'

and in various places in his writings seemed to infer that he was dealing in analogies rather than in real processes. That he was doing so becomes clearer when one remembers his thought was based on Paracelsian iatrochemical concepts.

The alchemists and their iatrochemical successors did not understand 'nitre' or 'nitrous particles' and 'sulphur' as we understand these words today. The 'nitrous particles' of the atmosphere comprised the material in the air that maintained life and combustion, and thus was tantamount to our oxygen. This may not seem so preposterous if one recalls that heating nitre (saltpeter) yields oxygen. 'Sulphur' to them was not the chemical element itself, but originally referred to fire. The history of the idea of the 'Paracelsian aerial niter' was traced by Debus[9], who showed that with time the iatrochemists tended to assign to atmospheric 'sulphur' the life-maintaining properties earlier ascribed to the 'aerial nitre'. Where Willis departed from the usual iatrochemical interpretation was in his belief that bodily 'sulphur' was of dietary rather than immediate atmospheric origin. In view of the presuppositions underpinning Willis' thought, it seems likely that all Willis' writing was predicated about an unstated iatrochemical understanding of the use of the concepts of 'nitre' and 'sulphur'. To do Willis justice then, we should interpret all his neurochemistry in the light of analogy. If his atmospheric 'nitre' is seen to be oxygen, and his dietary 'sulphur' to be glucose, Willis' 'animal spirits' become equivalent to the energy source of neural tissue, and the *explosive Copula* is simply the enhanced energy consumption involved in bursts of motor activity. However, Willis had no good chemical analogy for the mechanisms of nerve conduction and synaptic neurotransmission in the functions he allocated to his 'animal spirits' and his *explosive Copula.*

Hence, given the conceptual background to his hypotheses, Willis' notion of intraneural and intra-sarcoplasmic gunpowder explosions facilitated by an *explosive Copula* deserves better than the ridicule or polite disbelief so long apportioned to it. The idea represents the beginnings of the application of bioenergetics to neural function.

REFERENCES

1. Feindel, W. The origin and significance of *Cerebri Anatome.* In: Feindel W (ed) Thomas Willis. The anatomy of the brain and nerves. Tercentenary Edition. Montreal, McGill University Press 1965. pp 5–104.
2. Dewhurst, K. Thomas Willis as a physician. Los Angeles. William Andrew Clark Memorial Library, University of California. 1964.
3. Hughes, JT. Thomas Willis 1621 – 1675. His life and work. Royal Society of

Medicine Services, London 1991.
4. Meyer A, Heirons R. On Thomas Willis' concepts of neurophysiology. Medical History 1965; 9:1–15 & 142–155.
5. Pordage S. The remaining medical works of that famous and renowned physician Dr Thomas Willis of Christ Church in Oxford, and Sidley Professor of Natural Philosophy in that famous University. London. Dring, Harper, Leigh & Martyn. 1681. (All quotations in the present text employ the spelling of this edition, except that the 'f' has been replaced with the modern 's').
6. Paracelsus. Seven defensiones. In: Siegerist H.E.(ed) Four treatises of Theophrastus von Hohenheim called Paracelsus. Translated Temkin, C.L. Baltimore. Johns Hopkins Press. 1941.
7. Paracelsus. The diseases that deprive man of his reason. In: Siegerist H.E.(ed) Four treatises of Theophrastus von Hohenheim called Paracelsus. Translated Zilboorg, G. Baltimore. Johns Hopkins Press. 1941.
8. Debus AG. The English Paracelsians. New York. Franklin Watts. 1965.
9. Debus AG. The Paracelsian aerial niter. Isis 1964; 55:43–61. Reprinted in: Debus A.G. Chemistry, alchemy and the new philosophy, 1550–1700. London, Variorum Reprints.

MOTOR NEUROPATHIES AND ANTIGLYCOLIPID ANTIBODIES

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SUMMARY

This paper describes patients with demyelinating motor neuropathies associated with conduction blocks, pure motor neuropathies and intermediate forms with resemblances to amyotrophic lateral sclerosis, in persons with raised titres of anti-GM_1 antibodies. The specificity of the abnormal anti-GM_1 antibody titres is discussed, and the possibilities of immunosuppressive therapy mentioned.

The possibility of effective therapy for neuropathies has been of considerable interest in recent years. In relation to this possibility, we have obtained valuable experience in managing motor neuropathies associated with antiglycolipid antibodies, especially anti-GM_1 antibodies, in a series of 30 patients.

IgM anti-GM_1 antibodies were first detected in patients with IgM monoclonal gammopathy and amyotrophic lateral sclerosis (ALS) or motor neuropathy. Monoclonal IgM antibodies were generally non-malignant and most often lambda light chain ones. Later anti-GM_1 antibodies were detected in the absence of any monoclonal gammopathy[15]. Moreover, sensorimotor and subsequently motor demyelinating neuropathies with conduction blocks were described, often associated with antiglycolipid antibodies. More and more cases have since been reported, clinically similar to ALS, and possibly induced by an immunological mechanism. Despite some uncertainty, they are likely to benefit from treatment.

The anti-GM_1 antibody titre is frequently raised in these disorders. Gangliosides form a group of glycolipids composed in part of carbohydrates, including glucose, and molecule(s) of sialic acid and also of a lipid, ceramid, a fatty acid bound to sphingosine. They are concentrated on the external

surface of plasma membranes, mainly in the central and peripheral nervous systems. These antibodies bind mainly to GM_1- and to asialo-GM_1-gangliosides. The antigen target is a carbohydrate, a disaccharide which is a component of GM_1, but which is also present in other gangliosides antigens (asialo-GM_1, GD_1 and GM_2). This epitope is called Gal (β1-3) Gal Na C.

Anti-GM_1 antibody binding to peripheral nerve is inhibited by cholera toxin which is specific for GM_1 sites, but not by peanut agglutinin, which recognizes Gal (β 1–3) Gal Na C.

DEMYELINATING MOTOR NEUROPATHY WITH MULTIFOCAL PERSISTENT CONDUCTION BLOCKS

We have observed such cases with conduction blocks (CB). The diagnostic criteria for conduction block are not universally agreed. A stringent definition would require a reduction of compound action potential (CMAP) amplitude and negative peak area of over 50% after proximal stimulation, in the absence of any temporal dispersion. Conduction block, involving motor nerves exclusively, determines the diagnosis and reveals the presence of focal demyelination. In fact, with temporal dispersion and reduced conduction velocity, the amplitude of the negative potential is decreased and the duration of the negative peak is increased through a phase cancellation process. The existence of misleading blocks must also be recognised; they can be observed in the reinnervating process in ALS. Conduction blocks are characterized by several features: they are limited to 30 to 100 mm long segments of nerves, and are distributed randomly on various part of the peripheral nerve but occur predominantly at its proximal part. The motor axon is selectively involved without any reduction in sensory conduction. There is no explanation for this selectivity of involvement and the hypothesis that it is due to antigenic differences between motor and sensory nerves remains unproven. Finally, the persistence of such a block distinguishes it from the block observed in chronic polyradiculoneuritis, which usually regresses with time.

Clinically, the symptoms are quite uniform despite some unusual variations. The main clinical features are[14]: a male predominance, the disease frequently occurring before 45 years of age: progressive motor involvement beginning distally, predominantly in the upper limbs, extending to the hands and usually being asymmetrical. In some cases, the disease pattern suggests a multiple mononeuropathy involving, for instance, the radial, ulnar and peroneal nerves. In other cases, it resembles a monomelic amyotrophy. A case of tongue hemiatrophy has been observed[5]. Muscle weakness is often more

marked than amyotrophy. Wasting is in the distribution of peripheral nerves rather than being segmental. For instance, among the thenar muscles innervated by C8 and T1, those supplied solely by the median nerve are involved, whereas those innervated by the ulnar nerve are spared. Cramps and fasciculations are frequently present. Myokymia is less frequent. Reflexes are reduced or absent, never brisk. Sensory disorders are absent or not significant, being limited to paresthesias or slight hypoaesthesia, a difference between these pure motor forms of the disorder and the forms previously described[7]. The CSF is more often normal and the course is very slowly progressive over several years. The occurrence of quadriplegia without any pyramidal or bulbar signs is rare. These clinical features allow the disorder to be distinguished from true ALS, with which it can be confused for some time.

In our experience there are 2 main forms of the disorder, viz. asymmetrical and symmetrical. For example, an asymmetrical case was observed in a 32–year old man with a left foot drop and left biceps brachii atrophy and cramps. Examination showed fasciculations and areflexia. The CSF was normal; the EMG showed neurogenic muscle involvement with conduction blocks in the relevant nerves. Elisa testing showed a high titre of anti-GM_1 antibodies. The patient's condition was improved by intravenous immunoglobulin injections. In contrast, another patient, a 40–year old man, complained of a 5–year history of ulnar atrophy which had later developed into bilateral lower and upper limb weakness with cramps and fasciculations. This was an example of the symmetrical form of the disorder. In both these cases the motor involvement was in a peripheral nerve distribution.

However there were several atypical cases. There were instances of hand weakness without atrophy, puzzling hand weakness with a 'preacher hand' and a case of monomelic weakness and atrophy, observed in a 48–year old man with right upper limb weakness and wasting, normal CSF findings, a normal cervical myelogram, CT scan, and MRI study but neurogenic EMG changes, nerve conduction blocks, high titres of anti-GM_1 antibodies and large diameter fibre reduction revealed by sural nerve biopsy. The patient's condition was stabilized with immunoglobulin treatment.

There is no general agreement about the best treatment. There has been a poor response to prednisone. Some improvement has been observed in patients treated with high doses of cyclophosphamide, administered for 5 days. A placebo-controlled double blind cross-over study with intravenous immunoglobulin injections permitted us to observe increased muscle strength for 1 or 2 months without any change in conduction block or in anti-GM_1 antibodies.

The relationship to chronic inflammatory demyelinating polyneuropathy (CIDP) should be discussed; the disorder could be a CIDP variant with a number of similar features or a distinct entity, as it is in our opinion. The arguments for considering these cases as a form of CIDP are doubtful, viz. there being a clinical continuum with cases presenting with sensory disorders, occasional conduction blocks and anti-GM_1 antibodies occurring in polyradiculoneuritis, similar appearances on nerve biopsy being present in both. If so, the disorder would be only a predominantly motor variety of chronic polyradiculoneuritis but the clinical pattern (exclusively motor, asymmetrical, involving multiple nerves, or monomelic), the electrophysiology finding (persistent conduction blocks, normal nerve conduction velocities, particularly sensory conduction velocities), the laboratory investigations (normal CSF, frequently high titres of anti-GM_1 antibodies) and the therapeutic response (resistance to corticosteroids with plasmapheresis being relatively ineffective) all differ.

PURE MOTOR NEUROPATHIES

Such cases should be distinguished from instances of demyelinating motor neuropathy with multifocal conduction block. The pathology is believed not to be a focal demyelination but a motor neuron lesion. In the absence of autopsy data it is difficult to differentiate between a simple axonopathy which spares the perikaryon, an acquired progressive spinal amyotrophy which spares the axon, and a neuronopathy involving the whole motor neuron. The differentiation between these 3 types of disorder is hard to make in practice though is often discussed[16], especially since some lesions of the neuron sometimes involve a distal axonopathy while, conversely, axonopathy can cause perikaryon damage through a dying back phenomenon. Moreover, the apparent absence of conduction blocks could be a result of their transient occurrence or of difficulty in detecting them. In pure motor neuropathies the age of onset is older, the male predominance is less, amyotrophy is more frequent, electrophysiological changes suggest an axonal neuropathy and the antiglycolipid antibody titre is raised. In some cases the amyotrophy is asymmetrical, and sometimes it is multifocal like that of a multiple mononeuropathy. It may be localized to the upper limbs as well as to the lower limbs, and may be unilateral or bilateral.

In our experience, the course is progressive (duration 10 years as compared with 5 years in cases with conduction blocks) and, in only 5 cases out of 13, the motor dysfunction involved peripheral nerves. Conversely, many cases resemble the entity of progressive spinal muscular atrophy. The main

examples were a 61–year old woman with distal progressive amyotrophy since the age of 44, a case of bilateral chronic hand amyotrophy reported as an example of the O'Sullivan and McLeod syndrome[13], in whom 11 years after the onset, high titres of anti-GM_1 antibodies were found, a case of Hirayama disease in a 24–year old (with 'oblique amyotrophy' corresponding to Hirayama's description of monomelic amyotrophy) and a case of bimelic amyotrophy predominantly involving the upper limbs. We have also observed a man with an associated inclusion body myositis.

'NON LATERAL AMYOTROPHIC SCLEROSIS'

We propose use of the term 'non lateral amyotrophic sclerosis' (NLAS) for intermediate cases, resembling ALS but without pyramidal or bulbar syndromes and with a very long course.

The following 2 cases illustrate this condition. Case 1 was a 42–year old man who, at the age of 33, had experienced a left radial nerve paralysis. He underwent surgery without any improvement. At 36, the disease developed into left hand amyotrophy with cramps and fasciculations. There was bilateral peroneal weakness. His MRI was normal and his EMG showed neurogenic changes. At 37, examination showed distal upper limb amyotrophy (left brachioradialis) with bilateral peroneal weakness and left ankle jerk loss. There was a bilateral Babinski sign. The EMG again was neurogenic and showed nerve conduction blocks. The CSF protein was slightly increased and the anti-GM_1 titre was very high. The patient's condition was improved by intravenous immunoglobulin infusion. At 43, his condition stabilised with lower limbs areflexia and normal anti-GM_1 titres. Case 2 was more characteristic. He was a 37–year old man who, at the age of 25, had distal upper limb amyotrophy which, by the age of 27 had progressed to cramps and fasciculations, distal weakness (upper and lower limbs) and ankle areflexia. The diagnosis made was ALS. Further examination showed normal CSF findings, neurogenic EMG appearances without conduction block and high anti-GM_1 titres. He was treated with intravenous immunoglobulin infusions. At 37 years of age he was confined to a wheel-chair, but was still able to stand. Examination then showed areflexia, the absence of pyramidal or bulbar signs, and very high anti-GM_1 titres.

These cases are obviously very different to patients with ALS. However high titres of anti-GM_1 antibodies are not rare in ALS. In true ALS, higher anti-GM_1, antibody titres are more frequent than in controls. High titres were reported in 9% of a series of 152 ALS cases[10], and were particularly frequent

in forms with bulbar and spinal involvement. In contrast, low titres are observed in half the cases of the disorder so that the raised titres are non-specific. Similar low titres can be found in about 50% of cases of autoimmune diseases and are also present in normal controls. Despite this, the antibody titre findings have given rise to excessive claims regarding the pathogenetic role of anti-GM_1 antibodies in ALS. Unfortunately, in true ALS, there is no improvement with cyclophosphamide or with immunoglobulin therapy.

SPECIFICITY OF ANTI-GM_1 ANTIBODIES

The presence of antiglycolipids antibodies, and particularly anti-GM_1 antibodies, is not specific for any disease. Such antibodies are reported particularly in severe axonal forms of Guillain-Barré syndrome, sometimes secondary to *Campylobacter jejuni* infection or to ganglioside injections. In this case, they are IgG or IgA antibodies rather than IgM ones. Anti-GM_1 antibodies are also found in various diseases e.g. multiple sclerosis, disseminated lupus erythematosus, IgM dysglobulinemia without neuropathy, Alzheimer's disease, polymyositis, myasthenia gravis, sometimes chronic polyradiculoneuritis, and in normal control subjects. Therefore it is difficult to assess the specificity of the antibodies. Their presence could be secondary to damage to motor neurons. However, there are a few indications that they may have a pathogenic role. Firstly, in the above-mentioned diseases, their titres are low and similar to those of controls, whereas in motor neuropathy the titres are frequently very high. Secondly, in some motor forms of the disorder with high titres, immunosuppression induces clinical improvement and lowered titres.

Experimental data are mainly in favour of anti-GM_1 antibody specificity, particularly immunostaining studies with combined preincubation with cholera toxin (specific for GM_1).

As regards demyelination with conduction block, the injection of cholera toxin and peanut agglutinin in the rat shows that anti-GM_1 antibodies are present at nodes of Ranvier[6]: this confirms the results of previous studies reporting the presence of antibodies in the nodes of Ranvier of rabbits immunized with anti-ganglioside antibodies[12]. However, such deposits are not constantly present in rat nerve even when conduction block develops[17].

A more recent experimental study[3] has reported that cholera toxin and peanut agglutinin are bound to the nodes of Ranvier. However, cholera toxin is localised to the compact paranodal myelin sheath, whereas peanut agglutinin

is bound to the nodes of Ranvier and concentrated in the nodal gap. Another recent study[17] has provided further data after injection of patients' sera with high titres of anti-GM_1 antibodies into the tibial nerve of the rat: using serum from cases with conduction block, a reduced CMAP was observed whereas with sera from cases without conduction block, no block could be obtained. A possible circulating factor different from anti-GM_1 antibodies could explain this finding.

As regards spinal motor neurons, immunostaining of the neuronal cell by means of anti-GM_1 antibodies, specifically bound to cholera toxin and not to peanut agglutinin, has been shown in bovine neurons[2], whereas binding to rat ganglia sensory neurons did not occur. These data are consistent with the predominantly motor involvement that occurs in the disease. Therefore, the specificity of anti-GM_1 antibodies is more obvious than that of cross reactive glycolipids, especially as the intensity of the immunostaining is proportional to the anti-GM_1 antibody titres.

Without drawing any conclusion as to their pathogenic role, the obvious increase in anti-GM_1 antibodies in pure motor neuropathy justifies the trial of immunosuppresive therapy which, in a number of cases, has improved what had appeared to be a bad prognosis.

REFERENCES

1. Azulay JP, Blin 0, Billé F, Cades G, Boucraut J, Pouget J, Serratrice G. High dose intravenous human immunoglobulin is effective in the treatment of lower motor neuron syndrome associated with elevated serum anti-GM_1 antibody titres. A double blind placebo controlled study. Neurology 1992; 42(suppl 3), 334.
2. Corbo M, Quattrini A, Lugaresi *et al.* Patterns of inactivity of human anti-GM_1 antibodies with spinal cord and motor neurons. Annals of Neurology 1992; 32:487–493.
3. Corbo M, Quattrini A, Latov N, Hays AP. Localization of GM_1 and Gal (beta 1–3) Gal Nac antigenic determinants in peripheral nerve. Neurology 1993; 43:809–814.
4. Freddo L, Yu RK, Latov N *et al.* Gangliosides GM_1 and GD_{1b} are antigens of IgM M-protein in a patient with motor neuron diseases. Neurology 1986; 36:454–458.
5. Kaji R, Shibasaki H, Kimura J. Multifocal demyelinating motor neuropathy: cranial nerve involvement and immunoglobulin therapy. Neurology 1992; 42:506–509.

6 Latov N. Antibodies to GM_1 gangliosides in neuropathy and motor neuron disease. In: Serratrice G *et al* (eds) Système nerveux, muscles et maladies systémiques. Vol 1. Expansion Scientifique française 1993; 238–243.
7. Lewis RA, Sumner AJ, Brown MI, Asbury AK. Multifocal demyelinating neuropathy with persistent conduction block. Neurology 1982; 32:958–964.
8. Parry GJ, Clarke S. Multifocal acquired demyelinating neuropathy masquerading as motor neuron disease. Muscle and Nerve 1988; 11:102–107.
9. Parry GJ. Motor neuropathy with multifocal conduction block. In: Dyck PJ *et al* (eds) Peripheral neuropathy. 3rd ed. Saunders. Philadelphia. 1993 pp. 1518–1524.
10. Pestronk A, Li F. Motor neuropathies and motor neuron disorders: association with antiglycolipid antibodies. Advances in Neurology 1991; 56:427–432.
11. Sadiq SA, Thomas FP, Kilidireas K *et al.* The spectrum of neurological disease associated with anti-GM_1 antibodies. Neurology 1990; 40:1067–1072.
12. Santoro M, Uncini A, Corbo M *et al.* Experimental conduction block induced by serum from a patient with anti GM_1 antibodies. Annals of Neurology 1992, 31, 385–390.
13. Serratrice G. Amyotrophie spinale distale chronique localisée aux deux membres supérieurs (type O'Sullivan et Mc Leod). Revue Neurologique 1984; 140: 368–369.
14. Serratrice G. Neuropathies motyrices avec anticorps antigangliosides. Presse Medicale 1993; 22:705–707.
15. Shy ME, Evans VA, Lublin PD *et al.* Autoantibodies to GM_1 and GD_{1b} in patients with motor neuron disease without plasma cell dyscrasia. Annals of Neurology 1989; 25:511–513.
16. Thomas PK. Separating motor neuron diseases from pure motor neuropathies. Advances in Neurology 1991; 56:381–384.
17. Uncini A, Santoro M, Corbo M *et al.* Passive transfer of conduction abnormalities with serum from patients with anti GM_1 antibodies and neuropathy. Neurology 1992; 42(suppl 3):178.
18. Uncini A, Santoro M, Corbo M, Lugaresi A, Latov N Conduction abnormalities induced by sera of patients with multifocal motor neuropathy and anti-GM_1 antibodies. Muscle and Nerve 1993; 16:610–615.

MOTOR NERVE BIOPSY: FEASIBILITY AND SAFETY

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SUMMARY

Motor nerve biopsy was attempted on 19 occasions in 18 patients. Nerve tissue was obtained in 16. The nerves biopsied included those to the anconeus (4 times), palmaris longus (6), flexor sublimus (1), triceps (2), extensor carpi radialis (1), quadriceps (1) and gastrocnemius (1). Attempts to biopsy the radial nerve, the nerve to plantaris and the left common peroneal nerves failed in 2 patients.

The lengths of nerve obtained varied from 1 to 3 cm, and from 1–5 fasciculi were present in the specimens. Sufficient material for both electron microscopy and teasing was present in 11. No patient experienced increased weakness, but one had transient paraesthesiae in the distal forearm following biopsy of the nerve to the palmaris longus.

We conclude that motor nerve biopsy as described is both feasible and safe. The nerve to the palmaris longus, where that muscle was present, provided the optimum specimen for pathological studies.

Until recently few attempts have been made to biopsy motor nerves[1-3]. In 1990 one of the authors (PG) obtained a biopsy of the nerve to the extensor carpi radialis in a patient with asymmetrical weakness and wasting of the forearms with mild sensory symptoms, no sensory signs but abnormalities on sensory nerve conduction studies. The biopsy demonstrated a demyelinating peripheral neuropathy and the patient responded to corticosteroid and intravenous γ-globulin treatment[4].

The present paper describes a 3 year experience of motor nerve biopsy in patients with suspected motor neurone disease or peripheral neuropathy.

PATIENTS AND METHODS

Eighteen patients (11 males, 7 females; average age 55 years; range 19-76 years) have undergone attempted motor nerve biopsies. The biopsies were performed by neurosurgeons except for those on the nerve to the palmaris longus, which was performed by plastic surgeons interested in studying this nerve and its use in plastic surgery, and on the nerve to the quadriceps which was biopsied by a general surgeon.

The nerves were prepared for pathological examination using the methods described by Dyck *et al*[5] for preparation of sural nerve biopsies.

All nerves were subjected to electron microscopy (EM); teased fibre analysis was also performed when sufficient material was present.

RESULTS

Table 1 indicates the age and sex of the patients, the nerves biopsied, the adequacy of the tissue for electron microscopy and/or teased fibre analysis and the final clinical diagnosis.

The nerve to the palmaris longus, where present, provided the optimum amount of tissue for both EM and teased fibre study in all 6 patients. Biopsy of the palmaris longus muscle also provided a large muscle biopsy specimen.

No patient experienced increased weakness following the biopsy. One patient experienced transient paraesthesiae of the distal forearm lasting 6 weeks following biopsy of the nerve to palmaris longus; the sensory disturbance was presumably due to trauma to one of the superficial sensory nerves.

In 2 patients the results of the biopsy altered the clinical diagnosis to that of a multifocal motor neuropathy. In one there was significant segmental demyelination and in the other there was an increased number of thin myelin sheaths.

Although the nerve to the anconeus was biopsied in 4 patients, this nerve was difficult to find and provided suboptimal material for subsequent pathological study. The biopsy of the nerve to the quadriceps also provided insufficient tissue but this may have been due to the reluctance of the general surgeon involved to take a large enough specimen.

Table 1 Patients age, sex, nerve biopsied, number of fasciculi, length of nerve, suitability for electron microscopy (EM) and teased fibre analysis (TF)

Sex	Age (yrs)	Nerve biopsied	Fasci-culi	Length (cm)	EM	TF	Final clinical diagnosis
F	50	Radial	3	1.3	+	+	Demyelinating peripheral neuropathy
M	63	Anconeus	1	1.6	+	–	Multifocal motor neuropathy
F	58	Anconeus	1	1.2	+	+	Axonal peripheral neuropathy
M	51	Anconeus	1	?	+	+	Multifocal motor neuropathy
M	52	Anconeus	1	1.3	+	–	Motor neuron disease
M	54	Plantaris	No nerve				Motor neuron disease
M	76	Radial(T)	1	2.0	+	+	Motor neuron disease
F	72	Radial(T)	No nerve				Motor neuron disease
M	49	Radial(T)	3	1.0	+	–	Motor neuron disease
M	49	Femoral	2	1.0	+	–	Motor neuron disease (familial)
M	19	Tibial	4	2.0	+	_	Axonal peripheral neuropathy
F	55	Peroneal	No n erve				Motor neuron disease
F	58	Flexor sublimis	1	1.0	+	+	Peripheral neuropathy
F	66	Palmaris	2	1.0	+	+	Peripheral neuropathy
F	72	Palmaris	2	1.3	+	+	Motor neuron disease
F	57	Palmaris	5	?	+	+	Motor neuron disease
F	60	Palmaris	3	1.5	+	+	Motor neuron disease
M	67	Palmaris	1	3.0	+	+	Multifocal motor neuropathy
M	42	Palmaris	2	?	+	+	Motor neuron disease

Abbreviations: EM = Electron microscopy, TF = teased fibre, ? = length not stated, peroneal = common peroneal, palmaris = palmaris longus, radial (T) = branch of radial nerve to triceps, + = suitable, – = not suitable

DISCUSSION

There are available limited data for normal motor nerves which can be used to help interpret the findings of the present study. Swallow[6] studied 23 anterior tibial nerves taken from the dorsum of the foot at autopsy. The postmortem interval was not stated, teased fibre analysis was not performed and Wallerian degeneration was not discussed. Fibre densities of 3923 to 7565 myelinated fibres per mm^2 in a bimodal distribution were found. This is much lower than the figures obtained by Greenfield and Carmichael[7]. In a subsequent study O'Sullivan and Swallow[8] examined the radial nerve near the wrist. Again the postmortem interval was not discussed, but the fibre density was again bimodal in distribution and varied from 7440 to 5980 myelinated fibres per mm^2 in patients 17 to 39 and 60 to 80 years respectively. Wallerian degeneration and segmental demyelination were not discussed. Stevens *et al*[3] studied 5 nerves to the peroneus brevis and 18 deep peroneal nerves. Internodal diameter, internodal length and fibre density were analysed using teased fibre preparations but segmental demyelination and Wallerian degeneration were not studied. Increased variability in the parameters with increasing age was noted.

We have limited normal control data available at present but have found that artefactual changes in myelin appear in nerves where the postmortem interval exceeds 15 hours. However, sufficient data have been obtained to allow determination of the myelinated fibre diameter distribution histograms for the nerve to the palmaris longus (unreported observations).

Biopsies of the lateral popliteal and radial nerves (site not stated) in 10 patients with motor neurone disease were studied by Dayan *et al*[2]. They found thin myelin and small numbers of naked internodes typical of active segmental demyelination. In 10 patients with suspected amyotrophic lateral sclerosis, biopsy of the lateral fascicle of the deep peroneal nerve just above the ankle was reported by Dyck *et al*[9]. An abnormal frequency of fibres showing segmental demyelination and remyelination occurred but the difference was not statistically significant compared with the controls[3]. Auer *et al*[10] biopsied the proximal ulnar nerve (in the axilla) and found onion bulb formation in a patient with a pure motor neuropathy resembling motor neurone disease clinically. Recently Kaji *et al*[11] reported a patient with an enlarged brachial plexus on a MRI scan. The plexus was explored surgically. The nerves were swollen and a biopsy of the medial pectoral nerve demonstrated scattered demyelination and onion bulb formation. None of these recent studies has commented on any worsening of weakness following the biopsy.

Our initial impression was that the nerve to palmaris longus was the optimal motor nerve to biopsy and perhaps in patients with peripheral neuropathy this may still be true. On the other hand, more proximal[10] or more directed biopsy[11] may be more appropriate in the investigation of motor neurone disease.

Despite the feasibility and safety of motor nerve biopsy there are 2 major areas of concern, viz. the small numbers of normal controls (currently being accumulated) and the more important possibility that sampling a small portion of a distal peripheral nerve may not detect pathology in the proximal nerve or the nerve root. This concern is highlighted by a patient who underwent biopsy of the nerve to the palmaris longus. Teased fibre analysis showed 5% Wallerian degeneration and 8% segmental demyelination while 87% of the fibres were normal. This was interpreted as a primary axonal neuronopathy. The patient responded to treatment with intravenous γ-globulin and corticosteroids, which was given because he had electrophysiological evidence suggesting conduction block and more importantly he had remitted spontaneously with identical symptoms some 12 months before presenting again[4].

Thus we have demonstrated that motor nerve biopsies are both feasible and safe. Although motor nerve biopsy did confirm the diagnosis in cases of peripheral neuropathy, the nerves biopsied in the series did not allow distinction between instances of motor neurone disease and cases of multifocal motor neuropathy. The value of motor nerve biopsy in clinical practice needs to be more clearly defined.

REFERENCES

1. Dyck PJ, Lofgren EP. Method of fascicular biopsy of human peripheral nerve for electrophysiologic and histologic study. Mayo Clinic Proceedings 1966; 41:778–784.
2. Dayan AD, Graveson GS, Robinson PK. Schwann cell damage in motoneuron disease. Neurology 1969; 19:242–246.
3. Stevens JC, Lofgren EP, Dyck PJ. Histometric evaluation of branches of peroneal nerve: technique for combined biopsy of muscle nerve and cutaneous nerve. Brain Research 1973; 52:37–59.
4. Gates PC, Byrne E. Gammaglobulin responsive peripheral neuropathy resembling motor neurone disease. Presented at the Australian Association of Neurologists, Annual General Meeting Cairns, 1993.
5. Dyck PJ. Pathological alterations of nerves. In: Dyck PJ Dyck PJ, Giannini C, Lais A (eds). Peripheral neuropathy. Saunders, Philadelphia. 1993 3rd ed. pp 514–596.
6. Swallow M. Fibre size and content of the anterior tibial nerve of the foot. Journal of Neurology, Neurosurgery and Psychiatry 1966; 29:205–213.
7. Greenfield JG, Carmichael EA. The peripheral nerves in cases of subacute combined degeneration of the cord. Brain 1935; 58:483–491.

8. O' Sullivan DJ, Swallow M. The fibre size and content of the radial and sural nerves. Journal of Neurology, Neurosurgery and Psychiatry 1968; 31:464–470.
9. Dyck PJ, Stevens JC, Mulder DW, Espinosa RE. Frequency of nerve fibre degeneration of peripheral motor and sensory neurons in amyotrophic lateral sclerosis. Neurology 1975; 25:781–785.
10. Auer RN, Bell RB, Lee MA. Neuropathy with onion bulb formations and pure motor manifestations. Canadian Journal of Neurological Science 1989; 16:194–197.
11. Kaji R, Oka N, Tsuji T, Mezaki T, Nishio T, Akiguchi I, Kimura J. Pathological findings at the site of conduction block in multifocal motor neuropathy. Annals of Neurology 1993; 33:152–158.

THE ROLE OF SKIN NOCICEPTIVE AFFERENT NERVES IN BLISTER HEALING

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SUMMARY

Because sensory neuropeptides improve survival of critical skin and muscle flaps in rats, skin nociceptive sensory nerve function in blister healing was examined. Sensory nerve ablation by unilateral hindlimb denervation or cutaneous axon reflex enhancement by 14 days systemic nicotine treatment (5 mg $kg^{-1}day^{-1}$) decreased and increased, respectively, peripheral motor functions of nociceptive (peptidergic) skin nerves. Effects on nociception were measured by a radiant heat tail-flick test. Axon reflex flares were evoked by transdermal iontophoresis of acetylcholine or noxious electrical stimulation under pentobarbitone 40 mg kg^{-1} anaesthesia. Resultant changes in cutaneous microvascular blood flux were measured non-invasively by laser Doppler flowmetry. In nicotine-treated rats compared with placebo-treated controls, acetylcholine-evoked axon reflex flare was enhanced by 240% ($p<0.01$) without enhancement of electrically evoked flare. Thus, nicotine-sensitized nociceptors show stimulus specificity in their enhancement of neurogenic flare responses. No significant changes were seen in other endothelial-dependent or smooth muscle-dependent microvascular dilator responses. Nicotine-treated rats had prolonged tail-flick withdrawal latencies to noxious radiant heat stimuli compared with placebo-treated controls ($p<0.05$), suggesting an anti-nociceptive or analgesic effect of nicotine-treatment. Neurogenic effects on wound healing rate were assessed by measuring the dimensions of standardized blisters twice daily. The blisters were raised on hindpaw glabrous skin using a constant weight and diameter of compressed dry ice pellet applied for 30 secs at constant force. Dry-ice blisters raised on the hindpaw 14 days post-denervation were significantly slower to heal completely (42 days) than controls (30 days: $P<0.05$) and the surrounding inflammation was reduced. By contrast, nicotine-treated rats showed more rapid blister healing (25 days) than controls (30 days), seen only in the later phase after day 15. Finally, resting substance P release from blisters, after direct cutaneous nerve stimulation, appears to be enhanced in nicotine-treated rats. Thus nociceptive innervation appears critical for inflammation and rapid healing of blisters in rat skin. The

data signal a possible important role for neuropeptides in these processes and question the function of nicotinic receptors on sensory nerves.

Lewis[1] and more recently Lembeck[2] described neurogenic inflammation and its possible 'nocifensor' or protective actions. When a noxious stimulus impinges on the skin, polymodal nociceptors respond with a discharge both orthodromically to the spinal cord and also antidromically along collateral branches (the 'axon reflex') generating a local flush[1,2,3]. The axon reflex results in release of neuropeptides eg, substance P (SP) and calcitonin gene related peptide (CGRP), from arborised nerve terminals[2,3,4]. Subsequent vasodilatation occurs through their direct actions on vascular endothelium[5] or smooth muscle or their actions via the liberation of vasoactive substances from mast cells[6] (Fig 1). The neuropeptides, tachykinins and eicosanoids involved in this cascade trigger the following spectrum of defensive and healing processes: macrophages become phagocytic; lymphocytes in regional nodes are activated; microvessels dilate and plasma extravasation occurs; the kinin and complement systems are activated. Fever, leucocytosis and altered erythrocyte sedimentation rate may follow if tissue damage results in accumulation of lysosomal products, pyrogens and leucocytosis factors. Important roles of nociceptive primary afferent skin nerves in the axon reflex flare[1], microvascular reactivity[6] and wound healing[7] have been reported recently in a study[7] which used unilateral denervation as the primary strategy to in effect ablate the skin sensory nerves. In that study, denervation adversely affected all measured nocifensor functions of skin sensory nerves.

Based on these data, the present study tested the hypothesis that increases or decreases in sensory nerve responsiveness will alter the neuropeptide release and therefore the rate of wound healing. Its strategy employed the effect of systemic nicotine treatment, which enhances axon reflexes[8,9], to putatively increase sensory nerve responsiveness. Thus, it was proposed that chronic nicotine exposure should (i) increase neurogenic inflammation, (ii) alter nociception, (iii) enhance neuropeptide release and (iv) alter the wound healing rate. The present paper reports the testing of these proposals.

METHODS

Animals used

The rat husbandry, anaesthesia, neurovascular testing by noxious electrical transcutaneous nerve stimulation (TNS), iontophoretic application of acetylcholine (ACh) and sodium nitroprusside (SNP), and the use of laser Doppler velocimetry to measure the microvascular blood flux responses to noxious stimulation of paw skin have been described in detail in preceding papers[7,8,9]. Rats received electrical and iontophoretic stimulation on days 0, 14, and 28.

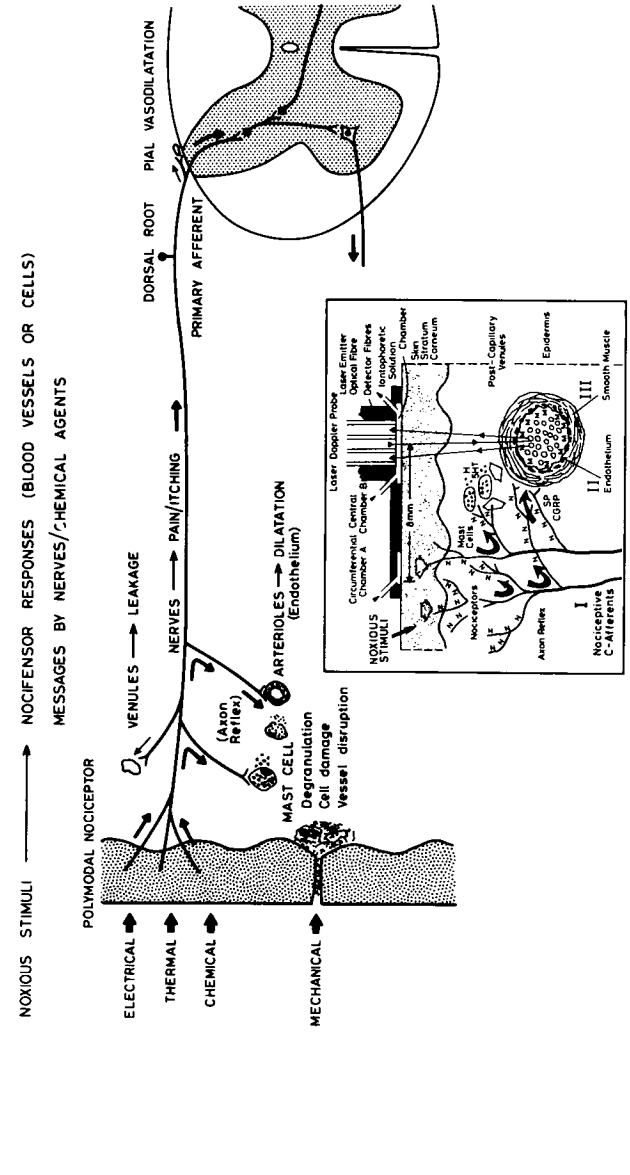

Fig 1 Neurovascular interactions in skin. When a noxious stimulus, either electrical, chemical, thermal or mechanical impinges on the skin, polymodal nociceptors respond with a discharge both orthodromically to the spinal cord and antidromically along collateral branches generating the so-called 'axon reflex'. Nocifensor responses to noxious stimulation listed on diagram are triggered by neuropeptide release and are categorised as cellular, humoral, vascular, immunological & systemic. **INSET:** Indirect stimulation via noxious electrical pulses at the arrow, or by iontophoresis of ACh at A, evokes an axon reflex. This results in neuropeptide release from arborised sensory C-fibre terminals and subsequent vasodilatation through an action directly on vascular endothelium or smooth muscle or via the liberation of vasoactive substances from mast cells. Laser Doppler velocimetry (LDV) was used to assess cutaneous vasodilatation in response to specific quantified stimuli as an indicator of neural and vascular function. The responses to direct vascular stimuli applied by iontophoresis via chamber B were also recorded in this way.

Nicotine administration

The naturally occuring isomer (-)-nicotine (hydrogen tartrate salt) dissolved in sterile 0.9% saline at a dose of 5 mg $kg^{-1}day^{-1}$ was administered systemically by an Alzet mini-osmotic pump[9]. This device, implanted subcutaneously between the scapulae, delivered the solution over a period of 14 days at a fixed rate of 0.5 ± 0.1 µl/hr. A perspex pellet, identical in size to the Alzet-2002 pump, served as a placebo implant[17].

Tail-flick *(Noxious Thermal Latency Test)*

Each animal was tested with the tail flick test on days 0, 14 and 28 (Fig 2). The rat was placed in a darkened immobilising perspex chamber with its tail protruding. A K-type thermocouple provided feedback for temperature control; a halogen heat lamp (54°C) focussed onto the tail elicited a reflex tail-withdrawal (flick). The tail-flick, sensed optically by an infra-red beam chopping circuit and a stop watch, triggered synchronously with the heat lamp onset, was stopped automatically by a beam chopper pulse. Rats were placed in the perspex chamber for 20 mins acclimatisation, the noxious thermal stimulus was applied and the reflex tail-flick latency recorded. Tail temperature was recorded 10 cm from the tail tip using a digital thermometer. Stimulation at a distance 6 cm from the tail tip was performed twice on each rat at days 0, 14 and 28 (Fig 3A). In a small number of rats, a 3rd stimulus was applied randomly either 2 cm rostral or caudal to the initial stimulus site, i.e. 8 or 4 cm from the tail tip (Fig 3A). Based on these data, the order and site of stimulus application (2 x 6 cm, 1 x either 8 cm or 4 cm) was randomised.

Electrical nerve stimulation

Noxious transcutaneous pulses were applied as previously described[7,10,11]. The hind legs of rats anaesthetised with pentobarbitone (40 mg/kg, i.p.) were shaved. Intradermal needle electrodes were placed distal to the cathode, 1 cm apart, on the hair line of the heel, for stimulation of the area innervated by the posterior tibial and saphenous nerves. A heatpad maintained body temperature at 37°C. A series of rectangular electrical pulses (150V, 2 Hz, 1 ms duration) were delivered to the site while blood flow was concurrently recorded by laser Doppler immediately distal to the cathode[7]. In each animal, the electrical stimulation consisted of short trains of 2, 4, 16 pulses from a Grass S8 stimulator and SIU5A isolation unit[7].

Neurovascular testing

Dual probe-holder/iontophoretic electrode chambers[7,8,9] attached by double-sided adhesive discs on the medial aspect of the hindlimb (secured to a contoured foam pad) minimised fluid leakage without impeding blood flow. The 2 circular chambers (6 mm diameter) were separated by 6 mm, allowing remote stimulation of test skin areas and thus only nerve-mediated alterations in blood flow at the recording site[7,8,9]. This indirect test was performed using ACh on all rats in an attempt to reproduce enhanced nerve function mediated by nicotine, previously described[8,9].

Iontophoresis of vasoactive compounds

This was carried out as described previously[7,8,9,10,11] and was performed after electrical stimulation[7]. Acetylcholine chloride (ACh) and sodium nitroprusside (SNP) as

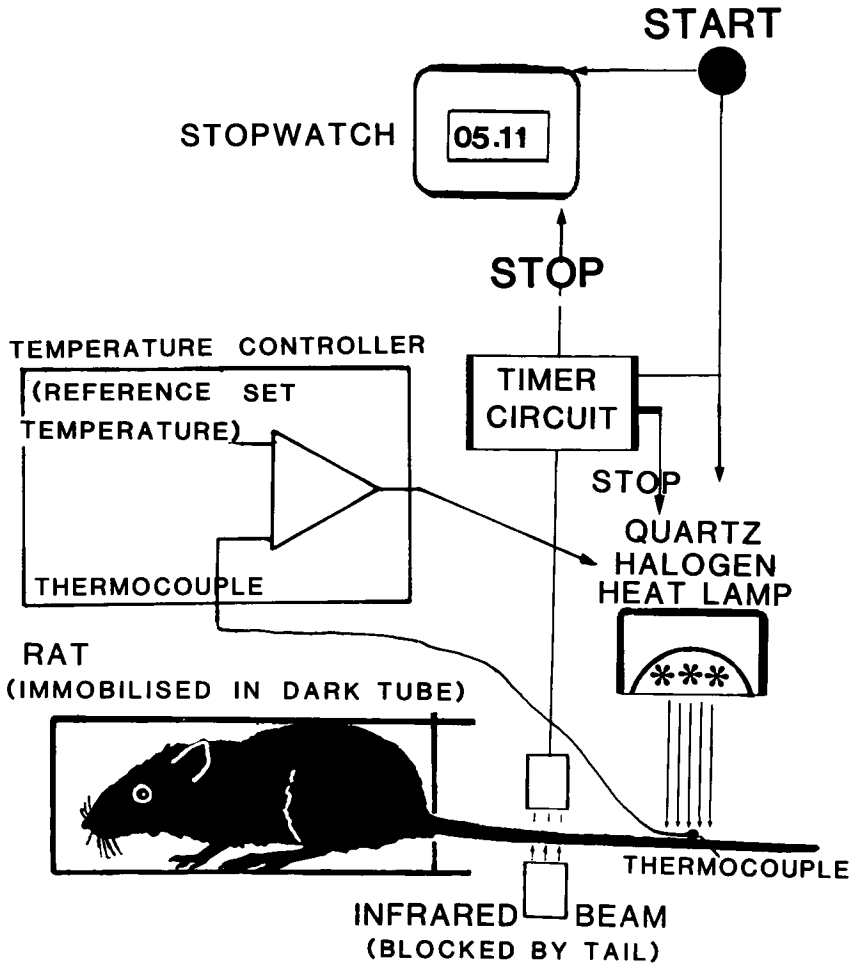

Fig 2 Tail-flick apparatus. A focused noxious radiant heat lamp was temperature regulated via a feedback thermistor and delivered a noxious heat pulse synchronously with the starting of a timer. Tail withdrawal was detected via an infra-red beam chopping circuit that stopped the timer and established a tail flick latency indicative of nociceptive sensitivity.

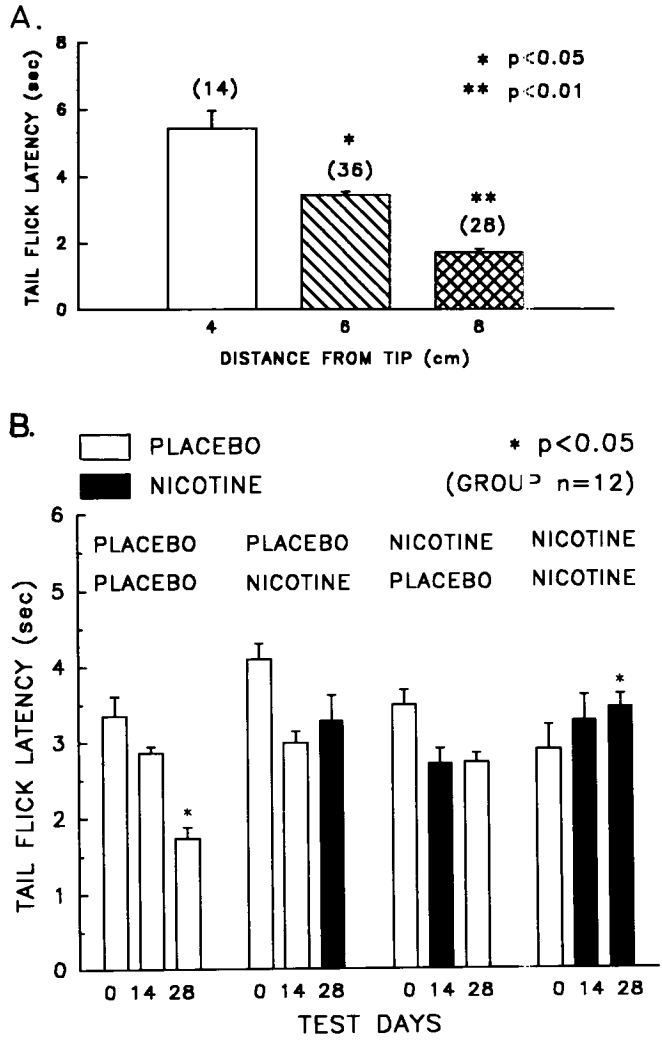

Fig 3 Tail flick withdrawal latency. A. Histograms showing tail-flick latencies (s) to 54°C noxious radiant heat stimuli at different locations (4,6,8 cm from the tip) along the rat's tail. All responses shown were obtained on day 0, before nicotine or placebo implants. "*" indicates a significant reduction in tail-flick latency compared with those obtained 4 cm from the tail tip ($p<0.05$): "**" indicates a significance of $p<0.01$. B. Time (s) to withdraw from a noxious stimulus of 54°C applied 6cm from tail tip on days 0, 14, and 28 shown in each group of placebo (28d); placebo (14d) - nicotine (14d); nicotine (14d) - placebo (14d) and nicotine (28d animals "*" indicates a significant reduction or increase in latency compared with the untreated day 0 value, $p<0.05$.

1% w/v solutions in inert 4% methyl cellulose gel in distilled water were each applied in a hemispherical 50 µl electrode chamber to the skin: a constant current stimulator provided a 0.2 mA galvanic (direct) current through the gel, with an indifferent electrode on the rat's forepaw[7,8,9]. Doses of ACh (direct) 4mC (0.2 mA X 20s), ACh (indirect) 16 mC (0.2mA X 80s), SNP (direct) 8mC (0.2 mA x 40) which produce sub-maximal responses were selected during pilot observations[7,8,10,11].

Blood flux responses

Laser Doppler velocimetry (LDV) with the Periflux PF1d (Perimed, Sweden) as previously described[10,11] was used non-invasively to assess cutaneous vasodilatation in response to specific quantified electrical or chemical vasodilator stimuli as an indicator of neural and vascular function. Typical dilator responses are shown in Fig 4 for iontophoretic stimuli applied either remotely from a circumferential chamber, or directly onto the skin (being monitored by LDV). Axon reflex dilator responses to noxious electrical stimulation have been shown in previous reports[7,10,11].

Wound healing: induction of blisters

Uniform, controlled blisters were created 14 days after implanting a nicotine or placebo pump. A standardised 7mm diameter pellet of solid carbon dioxide (dry ice) was applied for a period of 30 secs at a constant pressure of 0.25N. Blisters were induced in a concave part of the foot pad at the base of the toes to minimise post-wound trauma and lameness[7].

Assessment of blister-healing

Daily measurement of physical dimensions of the wound margin, delineated as the neurogenic flare border or the border of the scab, whichever was the larger, assessed the wound retraction/healing. Vernier calipers and a 6x lens with an overlay grid were used to determine the longitudinal and cross-sectional dimension and the total area, respectively[7].

Neuropeptide release study

To test the effect of nicotine treatment on neuropeptide release from skin sensory nerves, substance P (SP) release was measured during periods before, during, and after direct electrical stimulation of the sciatic nerve[4]. Using methods previously described[12], the SP was assayed in the fluid collected from paw suction blisters[4,13] raised after 14 days of systemic nicotine or placebo treatment. Twelve rats in each of the 2 groups (nicotine treated and control) were screened with chemical neurovascular tests and implanted with pumps. Fourteen days after implantation, animals were retested using the chemical neurovascular test battery.

Suction blister creation

Induction of bilateral suction blisters was performed with the animal under anaesthesia in a similar manner to that first described by Kiistala[13] in 1968. The device[4] consisted of a large brass plate with two 8mm diameter ports manifolded to a single outlet and attached to a venturi water pump for suction. The solid brass plate was heated with

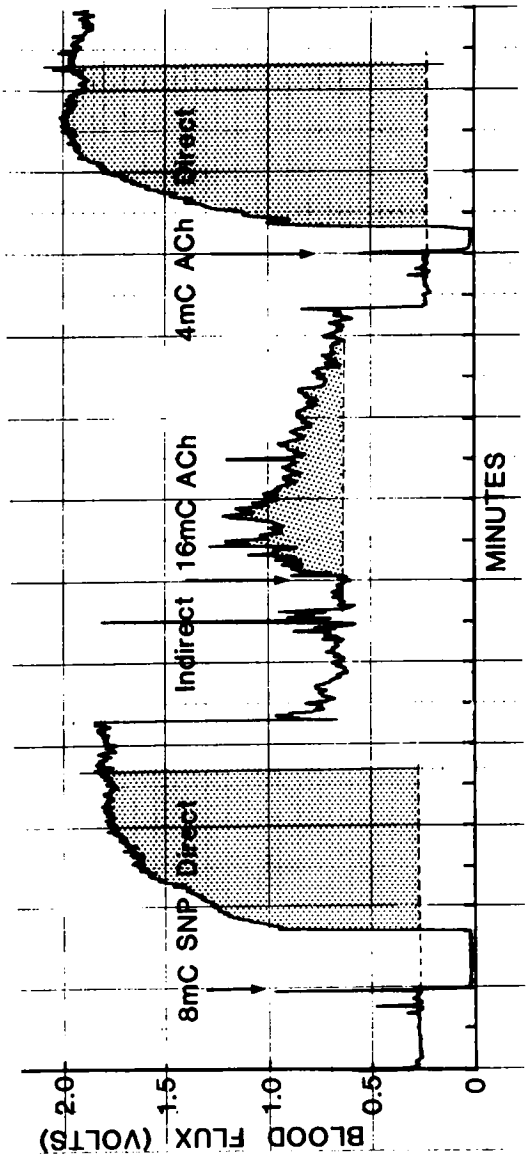

Fig 4 Blood flux responses. Recording of cutaneous blood flow by Periflux PF1d laser Doppler flowmeter (Perimed, Sweden), provided a continuous, real-time, non-invasive measure of evoked microvascular flux changes. Microvascular dilator responses to stimulation were quantified by determining the area beneath the voltage-time response recorded on an ICI DP600 chart recorder. Responses to indirect ACh iontophoresis[2,3] (centre) were integrated between the stimulus offset and the point at which the flux returned to baseline. Responses to iontophoresis of SNP (left) and ACh (right) directly under the laser probe were integrated over the first 4 mins of response.

a 10 ohm, 25W resistor to 42°C and maintained via a K-type thermocouple feedback. With the animal laid supine on a heated pad (37°C), the plantar aspects of the hind feet were placed over the suction ports and secured with an elastic strap and an airtight seal was assured with the use of high vacuum silicone grease (Ajax Chemicals). Application of a 30 kPa negative pressure (-220T) for a period of 30 mins (or to effect) resulted in an epidermal-dermal separation, and thus a blister. The epidermal layer was removed surgically. The foot was placed in a fluid-tight perspex clamp with the blister base exposed and the perspex cover forming a small fluid well.

Blister fluid collection

Standard Krebs-Ringer buffer (138 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM NaH_2PO_4, 11 mM $NaHCO_3$ 1 mM $C_{22}H_{38}O_7$, 11 mM $C_6H_{12}O_6$), 0.3% aprotinin (a protease inhibitor) and 0.25% bovine serum albumin were aliquoted as 300 µl samples into the blister well. Fluid was aspirated from the blister well after 15 mins using a Terumo µ-100 insulin syringe with a 29G needle (<1µl dead space), and collected into acetic acid (final concentration 0.1 M). Samples were centrifuged at 12,000 rpm for 2-3 minutes to remove any contaminants (especially blood cells or platelets) and 220 µl of supernatant was aspirated for assay. Samples were collected serially in triplicate for the resting, stimulation and recovery periods.

Nerve stimulation

The sciatic nerve was isolated surgically and transected in the upper thigh. To evoke peptide release the nerve was stimulated using two platinum electrodes (cathode distal to anode), at 50 V, 8 Hz, 1 ms duration[4,16]. Periods of 15 mins were used, after which blister fluid was removed, frozen and stored for assay.

Neuropeptide assay – substance P

Radioimmunoassay of samples was performed by a method similar to that previously detailed by Morilak *et al*[12] in which samples were collected into polyethylene tubes on ice, centrifuged, aspirated vortexed then frozen and allowed to stand at -80°C. Samples were thawed, reconstituted in 100 ml of buffer and lyophilised. The substance P (SP) content was determined by radioimmunoassay using an antiserum raised against SP in the rabbit, and iodinated SP labelled with the Bolton and Hunter reagent. The SP detection limit was 0.3 – 0.4 pcg per sample.

Statistics

Data were expressed as means and standard errors of the mean (sem). Statistical analysis was performed using analysis of variance (ANOVA) with post-hoc analysis, for both paired and unpaired data. Statistical significance was taken as $p<0.05$ for ANOVA or t-tests. Pearson's r value was used as an indicative measure of significance for linear correlations where appropriate.

Table 1 Summary of sensory nerve functions tested

TEST NAME	STRUCTURE TESTED	RESPONSE	MEASURED
AXON REFLEX			
Electrical Chemical Iontophoresis Indirect ACh	I Nociceptor, primary afferent, microvascular Endothelium II & Smooth muscle III	Neurogenic flare (vasodilation)	Laser Doppler Flux rise
IONTOPHORESIS ACh direct	Microvascular Endothelium II & Smooth muscle III (+ nerves under probe)	Vasodilation → EDNO, & indirect flush (+ nerve flare)	Laser Doppler Flux rise
IONTOPHORESIS SNP direct nitrodilator	Microvascular Smooth muscle III	Smooth muscle relaxation → direct flush	Laser Doppler Flux rise
TAIL FLICK	Nociceptor, primary afferent, CNS (perception)	Reflex tail withdrawal to noxious heat	Latency of withdrawal sec-2
WOUND HEALING RATE (suction blister)	Microvessels, blood cells, epidermal cells, nerves	Angiogenesis, fibroblast grow, new skin growth	Blister area (2 diameters of blister base)
SUBSTANCE P Release to stimulation	Peptidergic nerves in epidermis	Release of SP to electrical K+ stimulation	SP content in perfusate by RIA

RESULTS

Systemic effects of nicotine treatment

Urinary cotinine (μg/l) was compared in 3 placebo and 3 nicotine-exposed animals (5 mg $kg^{-1}day^{-1}$) as an indicator of osmotic pump function and systemic nicotine distribution (Table 1). As expected, urinary cotinine was high in rats which received a nicotine implant for 7 days when compared to controls in which levels were undetectable.

Table 2 Results from random testing of 6 rats for nicotine exposure. Urinary cotinine levels (μgl^{-1}) tabulated for 2 groups of rats (n=3) with placebo exposure and 7 days nicotine exposure.

TREATMENT GROUP	PLACEBO (14 days)	NICOTINE (14 days)
cotinine (μg/l)	83.33 ± 34.75	4140 ± 1099

Mean body weights of rats (n=12)

The mean body weight of each of the 4 groups of animals viz. placebo-placebo; placebo-nicotine; nicotine-placebo and nicotine-nicotine measured at days 0, 14 and 28 is shown in Table 3. No statistically significant differences ($p>0.05$) were found when tested by multiple ANOVA.

Table 3 Mean body weights of rats (n=12) in each of the four groups viz. placebo-placebo; placebo-nicotine; nicotine-placebo and nicotine-nicotine measured at days 0, 14 and 28. No statistically significant differences ($p>0.05$) were found when tested by multiple ANOVA.

TABLE OF BODY WEIGHT (grams)	PLACEBO (28d)	PLACEBO (14d) NICOTINE (14d)	NICOTINE (14d) PLACEBO (14d)	NICOTINE (28d)
DAY 0	213.83 ± 3.35	213.33 ± 5.01	211.27 ± 3.63	215.92 ± 3.01
DAY 14	225.42 ± 3.78	225.00 ± 4.24	219.83 ± 3.51	227.75 ± 2.60
DAY 28	231.36 ± 3.91	223.91 ± 3.23	226.00 ± 3.07	229.42 ± 2.09

Tail-flick test

Results from the 4 nicotine treatment regimens in rats on days 0, 14 and 28 are shown in Fig 3B.

Placebo-implanted animals showed a significant reduction in tail-flick latency over the experimental period but the reduction was significant only by the 3rd testing (post hoc analysis $p<0.05$). In contrast, animals which received nicotine throughout the 28 day period showed a progressive statistically significant increase in withdrawal latency ($p<0.05$). The 2 combinations of placebo and nicotine showed no consistent trend.

Neurovascular testing

Fig 4 shows typical responses to neurovascular tests. Fig 5 shows the mean blood flux response to a chemically-evoked axon reflex (AR) measured at days

0, 14 and 28 with the 4 nicotine treatment regimens. In each case placebo implants had no effect on the AR response to ACh. However, a statistically significant increase ($p<0.05$) in the size of the AR response was observed after 14 days of nicotine treatment. Further, this nicotine-induced AR enhancement reverted to normal following cessation of nicotine therapy.

By contrast, none of the nicotine treatment schedules produced a significant change in the AR responses to electrical stimulation ($p>0.05$, Fig 6) or to directly applied microvascular dilators SNP or ACh ($p >0.05$, see Figs 7A,7B).

Stimulus specificity was tested, but no correlation was found between thermal and chemical nociceptive function tests at day 0 ($r=-0.187$, $n=32$, $p>0.05$) or following 14 days of nicotine treatment ($r=0.139$, $n=33$, $p>0.05$).

Wound healing

Fig 8B shows the healing rate profiles of nicotine pre-treated rats (circles) and placebo treated or control rats (triangles). No significant difference was found for orthogonal comparisons of profile shape between groups ($F_{(1,22)} = 2.13$, $p > 0.05$). However, a divergence of the profiles is apparent at approximately day 16 after blister induction, highlighted by the enlarged inset (B), indicating a trend towards decreased healing time from this point onwards. On repeated measures analysis this is significantly different ($p<0.05$).

Neuropeptide release

Substance P (SP) release (pg/200µl) was assayed in blister fluid during rest, stimulation and recovery, samples being collected over 15 minute periods (Fig 9). Control rats showed a stimulus dependent increase in SP levels but this was not statistically significant. In rats treated with nicotine no such stimulus dependent increase was observed - instead there was a tendency for the absolute level of SP to be elevated in basal terms but the change fell short of statistical significance ($p=0.061$). By contrast, Table 4 shows that the increase in SP release with direct nerve stimulation correlated significantly with an indicator of nociceptor function, viz, the size of the chemical axon reflex response in control animals ($r=0.945$, $n=6$, $p<0.01$). However, there was no significant correlation for stimulated SP release levels and tests of vascular endothelial reactivity and smooth muscular function.

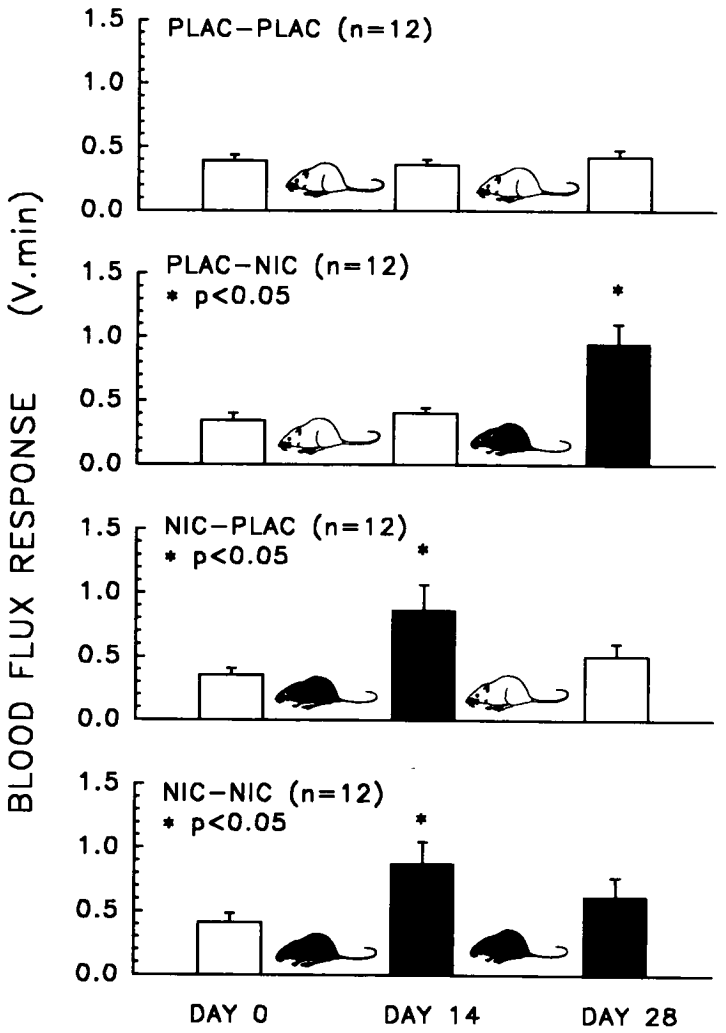

Fig 5 Nicotine effects on chemical axon reflex. Nicotine Treatment Schedule. In a 2 way cross-over arrangement, rats were on a 28 day schedule and received either a nicotine or placebo implant for the first 14 days followed by a reimplant of either placebo or nicotine for the remainder. The histograms show the effect of nicotine on the chemically evoked axon reflex response, measured at days 0, 14 and 28, in the 4 treatment regimens. Open bars represent responses following 14 days of placebo implant and shaded bars represent those following 14 day nicotine treatment. Black filled rats indicate nicotine treatment during the corresponding 14 day period, while unfilled rats indicate placebo. "*" indicates a significant increase in size of the axon reflex compared to day 0 ($p<0.05$).

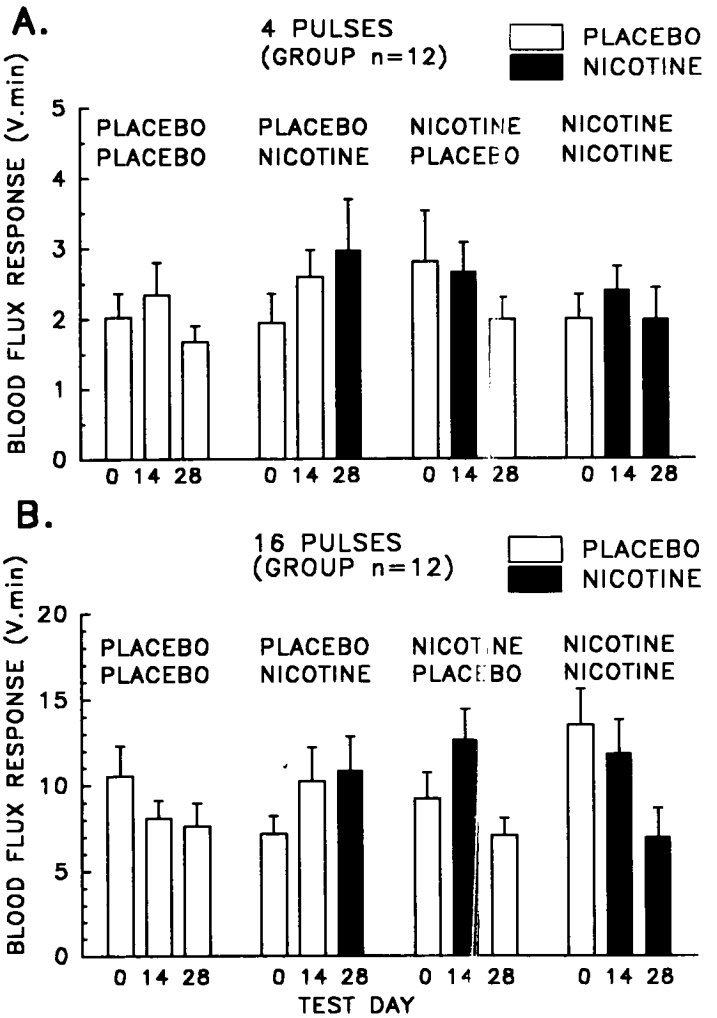

Fig 6 Nicotine effects on electrical axon reflex. Blood flux dilator responses are shown to noxious electrical stimulation (150V, 2Hz, 1msec) of (A) 4 pulses and (B) 16 pulses in the 4 nicotine treatment groups, placebo (28d); placebo (14d) – nicotine (14d); nicotine (14d) – placebo (14d) and nicotine (28d), on each test day, 0, 14 and 28. Stimuli were administered by a cathode remote from the LDV probe, the anode being 2cm further away (see methods). Open histograms show dilator responses after placebo treatment; filled histograms depict responses following 14 days of systemic nicotine. There was no significant difference between responses in either group on any test day ($p>0.05$).

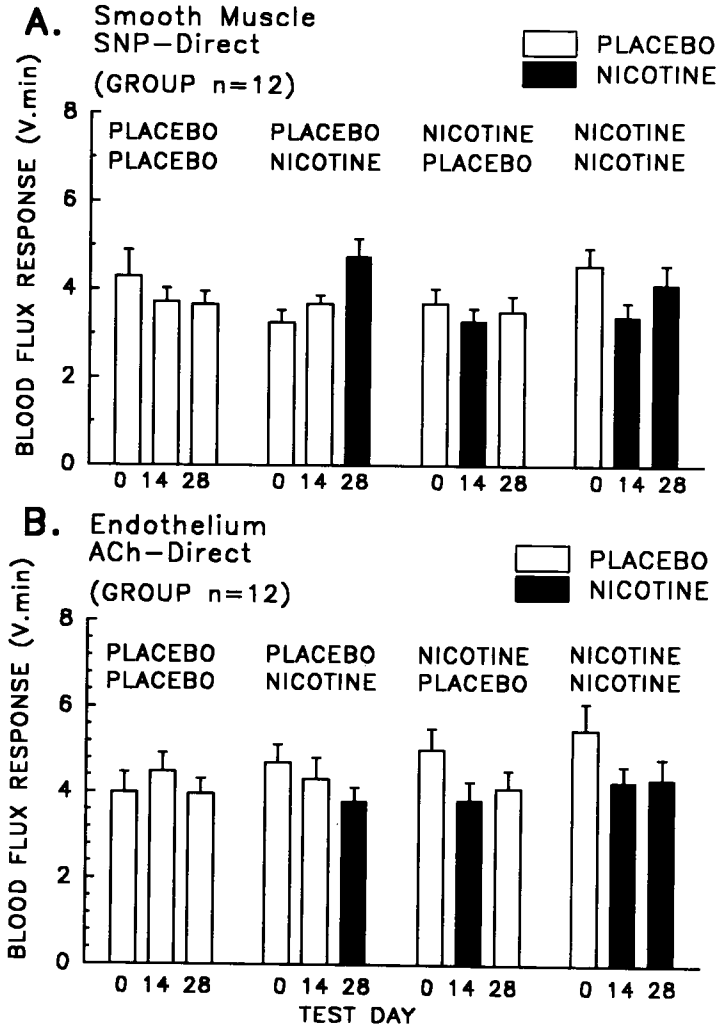

Fig 7 Nicotine microvascular effects. Histograms showing blood flux dilator responses (volt.min ± sem) in each nicotine treatment group, placebo (28d); placebo (14d) - nicotine (14d); nicotine (14d) - placebo (14d) and nicotine (28d) on each test day 0,14 and 28. Open histograms show responses in untreated animals or following placebo treatment. Solid histograms show responses after 14 days of preceeding nicotine treatment. The graphs show responses elicited with (A) the endothelium independent nitro-dilator, SNP (8mC) and (B) the endothelium-dependent dilator, ACh (4mC).

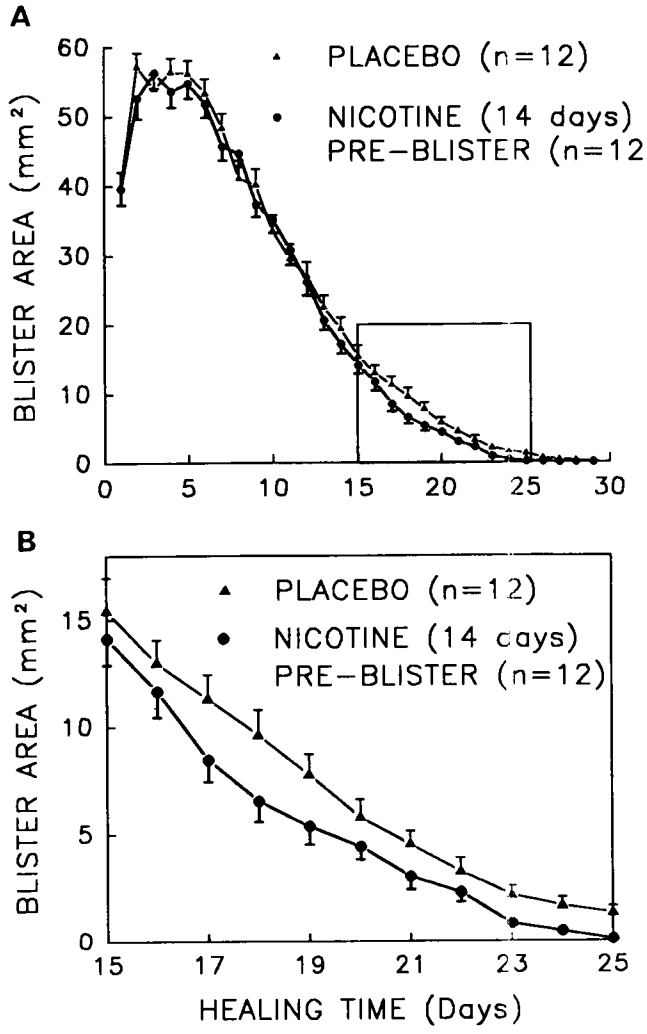

Fig 8 Blister healing rate after nicotine treatment. **A:** Healing rate profiles of nicotine pre-treated rats (circle) and placebo treated or control rats (triangle). No significant difference was found for orthogonal comparisons of profile shape between groups ($F_{(1,22)} = 2.13$, $p > 0.05$). However, a divergence of the profiles is apparent at approximately day 16 after wounding, highlighted by the enlarged inset **(B)**, indicating a trend towards decreased healing time from this point onwards. On repeated measures analysis this portion is significantly different ($p<0.05$).

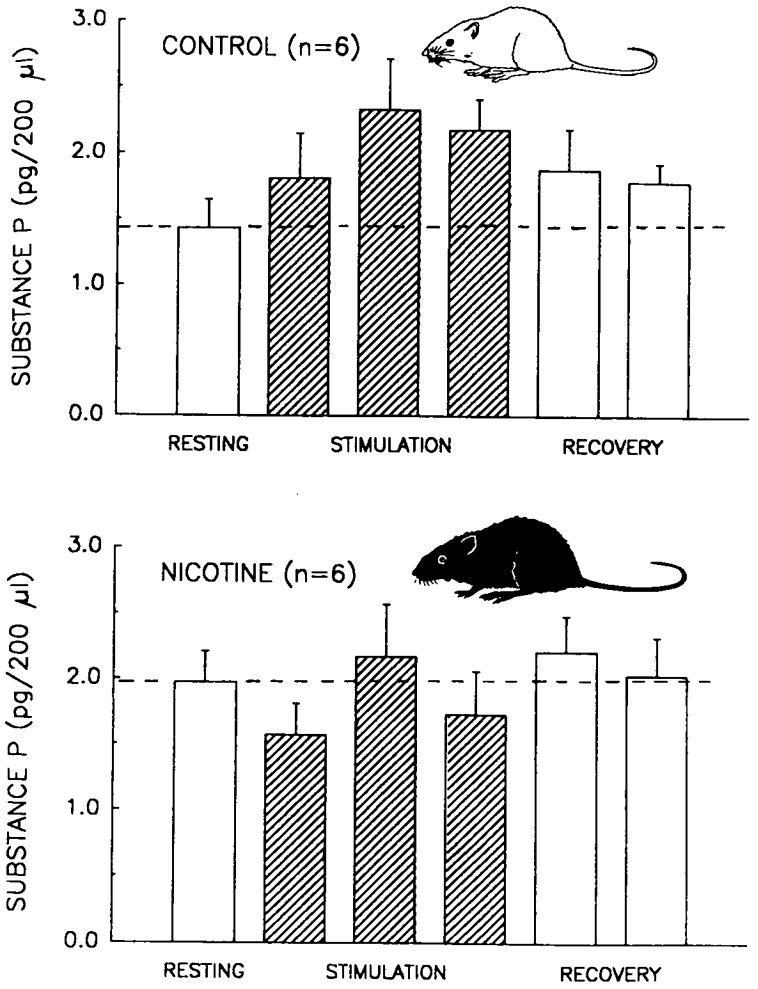

Fig 9 Substance P release. Levels of SP (pcg/200µl) assayed in blister fluid during rest, stimulation and recovery. Samples were collected over 15 min periods. Dotted lines show the initial (resting) level of SP release (n=6). Control rats showed a stimulus dependent increase in SP levels which was not statistically significant. In rats treated with nicotine no such stimulus dependent increase was observed. Instead there was a tendency for the absolute level of SP to be elevated in basal terms but there was not significant (p=0.061). The SP levels are near the lowest detection limits of the assay and the data should be considered indicative only.

Table 4 Relationship between substance P (SP) release in response to direct electrical stimulation of the sciatic nerve, and axon reflex blood flux responses to indirectly applied ACh. The correlation between the size of the chemical axon reflex and the net increase in SP release upon stimulation (ie. amount released upon stimulation minus amount released during rest periods) is statistically significant (r=0.945, n=6, p<0.01)

Net SP release (pg/200 µl)	Chemical Axon Reflex (V.min.)
0.185	0.319
0.245	0.252
0.620	0.293
0.635	0.329
0.780	0.396
1.810	1.116

DISCUSSION

The overall project strategy was to alter sensory nerve function in 2 ways: viz. (i) denervation – to impair axon reflexes, neurogenic inflammation, nociception, wound healing rate (reported by Carr *et al*[7]) and (ii) nicotine treatment – to enhance axon reflexes[8,9], neurogenic inflammation, nociception, neuropeptide release and wound healing rate.

Denervation has adverse effects on all nocifensor functions of skin sensory nerves, which clearly are important for inflammation and speed the late phase of blister-healing[7,17]. The present study provides evidence that systemic nicotine-treatment enhances neurogenic axon reflex flare if evoked by ACh (Fig 5), but not the axon reflex evoked by noxious TNS (Fig 6) – i.e., the effect is stimulus specific. This may be interpreted in terms of the present understanding of cutaneous neurovascular mechanisms[2,3,6,7,8] (Fig 1). All noxious stimuli impinging on the skin evoke axon reflexes resulting in neuropeptide release[3,6,7] from nerve terminals, leading to vasodilatation evident as skin reddening[7,8]. Dilator responses for noxious electrical stimulation[7] were not enhanced (Fig 6), which suggests that nicotinic acetylcholine receptors (nAChr) are not involved in producing the electrically-evoked axon reflex flare. Such stimulus specificity (Table 5) also points to different transduction mechanisms being involved in initiating the electrical and chemical axon reflex flare. Maintained electrical depolarization induces a large electrical gradient across nerve terminals producing a receptor

potential and thus a response[14]. Chemical stimuli such as ACh bind to specific receptors to open cationic channels[14], whilst thermal and mechanical stimulus transduction involve different mechanisms[14]. Furthermore, there are no microvascular effects of short nicotine exposure (Fig 7) which is explicable by the presence of nAChr on primary sensory nerves[7], but only muscarinic AChr on microvasculature[5].

Table 5 Stimulus specificity: Correlation between thermal and chemical (ACh) nociceptive function. Tail flick latency (sec) against integrated area of chemical axon reflex response (V.min.). First row shows results for rats on day 0 before nicotine or placebo implant (r=0.187, n=32, p>0.05) and second row shows results for the same animals after 14 days nicotine treatment (r-0.139, n=33, p>0.05). Linear regressions and p values indicate lack of statistical significance for both correlations.

Day	Correlation coefficient (r)	Number of animals	Significance (p)
0	–0.187	32	>0.05
14	0.139	33	>0.05

It is believed that synthesis of neural nAChr is regulated by phosphorylation[15], and is dependent on such factors as activity levels, and desensitisation by chronic exposure to agonist or antagonists[16]. Thus the enhanced flare response is best interpreted in the light of nAChr upregulation[7,8,16]. In Figure 3B the effect of prolonged tail-flick latency seen with N-treatment is significant and indicates some form of anti-nociception. Regional variation in sensory acuity or innervation density is excluded by testing at the same level in all rats[17], and the test is not dependent on basal skin temperature[17]. There are published data suggesting that such an action may be mediated centrally, peripherally, or both[18,19,20].

The nAChr blocker hexamethonium, which does not cross the blood brain barrier, has no effect on the anti-nociceptive effect of nicotine[19,20,21,22]. This suggests a central site of N-anti-nociceptive action. The mechanism underlying N-analgesia may involve participation of endogenous opioid peptides, since naloxone (opiate antagonist) blocked the anti-nociceptive effect of systemic nicotine in rats[22]. Supporting this, are the observations of Eiden *et al*[23] demonstrating release of metenkephalin from adrenal chromaffin cells after N-treatment. Another possible mechanism involves nicotine interaction with substance P (SP) , which has anti-nociceptive properties[24]. SP does not act on opioid receptors[22], but produces naloxone-reversible anti-nociception in rats after both intracerebral and systemic administration[20,22]. SP may induce release of metenkephalin from nociceptive brain regions[25], but SP-induced anti-nociception does

not block N-induced anti-nociception[22,24]. In spite of these findings[20-25],it is still possible that nicotine may partly mediate its effects at the periphery. Noxious stimuli induce peptide release from skin nerves[25], as does nicotine. Interactions between the nicotine-evoked actions on eicosanoids[26], peptides and tachykinins modulate nociceptor sensitivity[27]. Nevertheless, in spite of N-enhanced neurogenic inflammation[8,9], N-induced changes in the amount of peptide released in response to measured stimuli have not previously been reported. Therefore the trend shown in Fig 9 needs to be confirmed.

Denervation has been shown to prolong blister healing time in recent reports by Carr *et al*[7,17]. The effect is noted as a reduction of the acute inflammation surrounding the blister during the first 7 days after induction. The healing profiles diverge dramatically after 10 days, with a significant prolongation in healing time from 26 days (sham) to 42 days for the denervated hindlimb. This conforms with the finding of decreased survival of experimental critical flaps after sensory denervation with capsaicin[28]. The converse also applies, in that calcitonin gene related peptide (CGRP) increases survival of such flaps[29]. In the case of nicotine treatment, the only group of N-treated animals which showed a significant decrease in the blister-healing rate was the 14 day pre-blister nicotine treatment group. The effect was significant during the latter part of the healing profile, after day 15. In addition, healing was shortened by 5 days. Although SP release correlated significantly with an index of sensory nerve function, the axon reflex flare (Table 4), it is difficult to imagine that the mechanism underlying this effect is simply enhanced neuropeptide release at the time of blister induction. This would be expected to be manifest as a divergence of the healing profile early, analogous with the early reduction in inflammation acompanying denervation[7]. Since the divergence in healing profiles occurs late, it is presumed that some other mechanisms may be involved. These may involve increased local blood flow[30], alterations in the local turnover of eicosanoids[31], or modulation of growth factors[32]. Furthermore, there may be dose-dependent and/or time-dependent processes such as changes in SP or neuropeptide kinetics[33], which could mediate the nicotine effect. If higher doses are effective, the topical application of nicotine may be a better method of achieving appropriate levels locally[34]. The present result is tantalising, and in conflict with the observed adverse effects of smoking on skin flap healing[35,36], although Hahn[37] has shown that smoking also increases axon reflexes, presumably via nicotine. If these effects are nicotine-dependent, how are the nAChr on sensory nerves involved in the process? Clearly these questions should be explored further.

Primary skin sensory nerves which mediate neurogenic inflammation (axon reflex flare) are sensitised by chronic exposure to nicotine. Axon reflex responses

to ACh applied by iontophoresis are enhanced, but not those evoked by noxious transcutaneous electrical nerve stimulation. This indicates stimulus specificity of the polymodal nociceptors which may respond to both stimulus modalities by different excitation mechanisms. Also it suggests that nicotinic ACh receptors are not involved in the electrical axon reflex response. Nicotine treatment is anti-nociceptive for noxious thermal stimuli, and this may reflect either a central or a peripheral desensitisation effect. Nicotine treatment appears to produce a small alteration in the substance-P release during resting, nerve-stimulated and recovery collection periods. Nictotine pretreatment also accelerated the late phase of blister healing by 5 days.

REFERENCES

1. Lewis T. The nocifensor system of nerves and its reactions. I. British Medical Journal 1937; 1: 431-434; II. ibid. 1937; 1:491–494.
2. Lembeck F. Sir Thomas Lewis's nocifensor system, histamine and substance P-containing primary afferent nerves. Trends in Neurological Science. 1983; 6:106–108.
3. Foreman JC, Jordan CC. Neurogenic inflammation. Trends in Pharmacological Science 1984; 5:116–119.
4. White DM, Helme RD. Release of substance P from peripheral nerve terminals following electrical stimulation of the sciatic nerve. Brain Research 1985; 336:27–31.
5. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature (Lond.) 1980; 288:373–376.
6. Devillier P, Regoli D, Asseraf A *et al.* Histamine release and local responses of rat and human skin to substance P and other mammalian tachykinins. British Journal of Pharmacology 1986; 32:340–347.
7. Carr RW, Delaney CA, Westerman RA, Roberts RGD Denervation impairs cutaneous microvascular function and blister healing in the rat hindlimb. NeuroReport 1993; 4/5:467–470.
8. Grunfeld JA, Tiedemann GJ, Westerman RA. Chronic nicotine exposure enhances cutaneous axon reflexes in the rat. NeuroReport 1991; 2:421–424.
9. Grunfeld JA, Tiedemann GJ, Westerman RA. Maternal nicotine exposure enhances cutaneous axon reflexes in the neonatal rat. NeuroReport 1993; 4:635–638.
10. Low A, Westerman RA. Neurogenic vasodilatation in the rat hairy skin measured using a laser Doppler. Life Sciences 1989; 45:49–57.
11. Westerman RA, Low AM, Widdop RE, Neild TO, Delaney CA. Non-invasive tests of neurovascular function in human and experimental diabetes mellitus. In: GM Molinatti, RS Barr, S Belfiore & M Porta (Eds). Frontiers in Diabetes. Karger, Basel 1990; 9:127–138.
12. Morilak DA, Morris M, Chalmers J. Release of substance P in the tractus solitarius measured by in vivo microdialysis: response to stimulation of the aortic depressor nerve in rabbits. Neuroscience Letters 1988; 94:131–137.
13. Kiistala U. Suction blister device for separation of viable epidermis from dermis. Journal of Investigative Dermatology 1968; 50:129–137.
14. Iggo A. Sensory receptors in the skin of mammals and their sensory functions. Reviews in Neurology 1985; 141:599–613.
15. Huganir RL, Delcour AH, Greengard P *et al.* Phosphorylation of the nicotinic acetylcholine receptor regulates its rate of desensitization. Nature 1986; 321:774–776.
16. Wonnacott S. The paradox of nicotinic acetylcholine receptor upregulation by nicotine. Trends in Pharmacological Science 1990; 11:216–219.

17. Carr RW. Sensory nerve function in cutaneous wound healing. BSc Hons Thesis, 1992, Monash University.
18. Bonnycastle DD, Cook L, Ipsen J. The action of some analgesic drugs in intact and chronic spinal rats. Acta Pharmacologica et Toxicologica 1953; 9:332–336.
19. Sahley TL, Berntson GG. Antinociceptive effects of central and systemic administration of nicotine in the rat. Psychopharmacology 1979; 65:279–283.
20. Tripathi HL, Martin BR, Aceto MD. Nicotine-induced antinociception in rats and mice: correlation with nicotine brain levels. Journal of Pharmacology and Experimental Therapeutics 1982; 221:91–96.
21. Mattila MJ, Ahtee L, Saarnivaara L. The analgesic and sedative effects of nicotine in white mice, rabbits and golden hamsters. Annals of Medicine and Experimental Biology Fenn 1986, 46:78–84.
22. Molinero MT, Del Rio J. Substance P, nicotinic acetylcholine receptors and antinociception in the rat. Neuropharmacology 1987; 26:1715–1720.
23. Eiden LE, Giraud P, Dave JR *et al.* Nicotinic receptor stimulation activates enkephalin release and biosynthesis in adrenal chromaffin cells. Nature 1984; 312:661–663.
24. Frederickson RCA, Burgis V, Harrell EC *et al.* Dual actions of substance P on nociception: possible role of endogenous opioids. Science 1978; 199:1359–1362.
25. Naranjo JR, Sanchez-Franco F, Garzon J *et al.* Analgesic activity of substance P in rats: apparent mediation by metenkephalin release. Life Sciences 1982; 30:441–446.
26. Sonnenfeld T, Wennmalm A. Inhibition by nicotine of the formation of prostacyclin-like activity in rabbit and human vascular tissue. British Journal of Pharmacology 1980; 71:609–613.
27. Khalil Z, Helme RD. Sequence of events in substance P mediated plasma extravasation in rabbit skin. Brain Research 1990; 527:292–298.
28. Kjartansson J., Dalsgaard C-J., Jonsson C-E. Decreased survival of experimental critical flaps in rats after sensory denervation with capsaicin. Plastic and Reconstructive Surgery 1987; 79, 218–221.
29. Kjartansson J, Dalsgaard C-J. Calcitonin gene-related peptide increases survival of a musculocutaneous critical flap in the rat. European Journal of Pharmacology 1987; 142:355–358.
30. Kjartansson J, Lundberg T, Samuelson U E, Dalsgaard C-J Heden P. Calcitonin gene-related peptide (CGRP) and transcutaneous electrical nerve stimulation (TENS) increase cutaneous blood flow in a musculocutaneous flap in the rat. Acta Physiologia Scandinavica 1988; 134: 89–94.
31. Nadler JL, Velasco JS, Horton R. Cigarette smoking inhibits prostacyclin formation. Lancet, 1983;1248–1250.
32. Nilson G, von Euler AM, Dalsgaard C-J. Stimulation of connective tissue cell growth by substance P and substance K. Nature, 1985; 315:61–63.
33. Nadel JA. Regulation of neurogenic inflammation by neutral endopeptidase. American Review of Respiratory Diseases 1992; 145 548–552.
34. Watcher MA, Wheeland RG. The role of topical agents in the healing of full-thickness wounds. Dermatological Surgery and Oncology 1989; 15:1189–1195.
35. Craig S, Rees TD. The effects of smoking on experimental skin flaps in hamsters. Plastic and Reconstructive Surgery 1985; 75:842–846.
36. Sherwin MA, Gastwirth CM. Detrimental effects of cigarette smoking on lower extremity wound healing. Journal of Foot Surgery 1990; 29:84–87.
37. Hahn A. The effect of smoking on small nerve and blood vessel function in man: a comparison with diabetes mellitus. Hons BSc. Thesis, Dept. of Physiology, Monash University, Clayton. 1990.

KENNEDY'S DISEASE: CLINICAL PRESENTATION AND LABORATORY DIAGNOSIS

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SUMMARY

Kennedy's disease is a form of progressive spinal and bulbar muscular atrophy of adult onset. This paper describes a case of Kennedy's disease and discusses the laboratory diagnosis and the underlying genetic mechanism. Three other neurological diseases, Huntington's disease, myotonic dystrophy and fragile X syndrome, which have similar genetic defects, are also discussed.

Kennedy's disease is an inherited form of motor neurone disease that affects adult males. Typical features of Kennedy's disease include spinal and bulbar involvement as well as gynaecomastia. Kennedy's disease is caused by a mutation on the androgen receptor gene, which results in the expansion of a triplet repeat sequence in the first exon of the gene. The androgen receptor is located on the X chromosome and affected males carry one copy of the mutant gene.

CASE HISTORY

MT was a 67 year old Vietnamese male. He had first noticed his weakness 25 years prior to presentation. The weakness began in his right arm. He also noticed muscular cramps at this time. Later he noticed weakness in all 4 limbs. After about 2 years he had trouble walking and later began using a walking stick. More recently he had begun to use a walking frame. He had had no problems with speech or swallowing. He had no known family history of neurological problems. On examination he had moderate gynaecomastia and his testicular volumes measured 10 ml on the right and 12 ml on the left. He had marked facial, tongue and limb girdle fasciculation. Examination of his cranial nerves demonstrated VIIth nerve weakness in a lower motor neuron distribution and a wasted tongue. His periphery showed mild wasting and symmetrical weakness. The weakness was

more marked proximally and he was areflexic. He had a waddling gait. A diagnosis of Kennedy's disease was considered. Amongst the investigations performed the creatine kinase was normal. A confirmatory test was performed to examine for the presence of the Kennedy's disease mutation. Using DNA isolated from whole blood, amplification of a section of exon A of the androgen receptor gene was performed using the polymerase chain reaction (PCR). PCR was performed across the region of the gene enlarged in Kennedy's disease and the DNA product was analysed by electrophoresis on an agarose gel. The mutation in the patient was identified by an enlargement of the specific PCR product amplified from exon A of the androgen receptor gene. One of the patient's daughters was tested and she had 2 PCR bands, one amplified from the normal androgen receptor gene and a 2nd larger band from the mutant gene, confirming that she was a carrier for the condition (Fig 1).

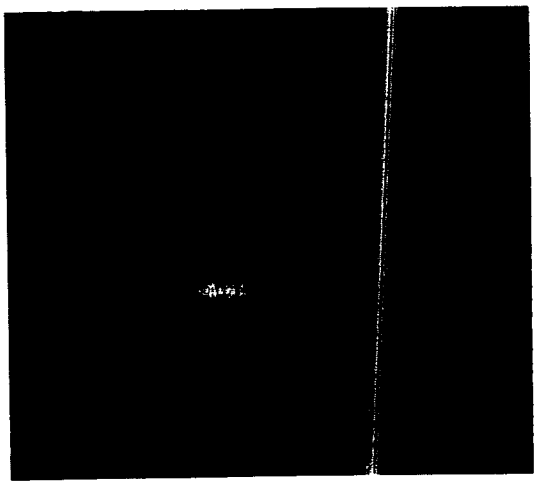

Fig 1 DNA amplified from exon A of the androgen receptor gene fractionated on a 1.4% agarose gel. The first lane shows a DNA size standard, the 2nd lane contains DNA from a normal patient, the 3rd lane contains DNA from the patient (enlarged band), and the 4th lane contains DNA from the patient's daughter (2 bands), indicating she is a heterozygote

DISCUSSION

Kennedy's disease was first described in 1968[1]. It is a syndrome comprising progressive proximal spinal and bulbar muscular atrophy of late onset. A patient afflicted with Kennedy's disease may have a normal life expectancy. The condition develops with cramps and later weakness and fasciculation. The weakness begins proximally. There is muscular wasting, tremor and decreased or absent reflexes. The patients often have dysarthria and dysphagia. On general examination moderate gynaecomastia is noted. Post-mortem examination shows decreased numbers of anterior horn cells throughout the spinal cord. Examination of skeletal muscle shows neurogenic atrophy. It is inherited as an X linked condition. In 1991 the genetic mutation was described. It involves a mutation in exon A of the androgen receptor. The mutation is an expansion in a triplet repeat sequence. In normal individuals there are 17 to 26 CAG repeats whereas in Kennedy's disease the number is 40 to 52[2].

The androgen receptor is the receptor for testosterone and 5α-testosterone. Testosterone is secreted by the Leydig cells of the testes and its release is controlled by the luteinising hormone (LH) of the pituitary gland. The main actions of androgens are the masculinisation of the developing foetus and the development and maintenance of secondary sexual characteristics in males. Along with follicle stimulating hormone (FSH) it stimulates spermatogenesis. It has a negative feedback effect on the release of pituitary LH. Its anabolic effect is ill defined but is thought to produce hypertrophy of skeletal muscle.

The androgen receptor is part of the steroid receptor super-family and, as such, is a nuclear transcription factor. Circulating testosterone diffuses through the cell membrane and binds to the androgen receptor in the cytoplasm. The testosterone/receptor complex migrates to the nucleus and binds to DNA in the promoter region of responsive genes. This complex then regulates the transcription of messenger RNA from these genes and hence the expression of protein translated from the messenger RNA.

Before Kennedy's disease, other androgen receptor disorders had been described. These are all disorders of masculinisation – androgen insensitivity syndromes. In the complete form, XY individuals have abnormal androgen receptors and are insensitive to the action of androgens and thus develop as phenotypic females. XY individuals who completely lack a functional androgen receptor show no signs of neurological disease. This is in stark contrast to Kennedy's disease in which a mutation in the androgen receptor leads to a form of motor neurone disease. Patients with Kennedy's disease show signs of mild

androgen insensitivity such as gynaecomastia but have a normal male phenotype. There are androgen receptors on motor neurones, but little is understood about their function.

In our patient with Kennedy's disease the diagnosis was confirmed by detecting an expansion in the relevant triplet repeat sequence using a PCR technique[3]. The test is highly specific – affected individuals can be separated from controls by detecting an enlarged band when the DNA is fractionated on an agarose gel. Many patients with sporadic motor neurone disease have been tested and none of these patients has been found to have the androgen receptor gene enlargement. This test has the potential to be used in symptomatic males, presymptomatic males, female heterozygotes and prenatally. We have used this test to diagnose 16 affected males and a similar number of carrier females.

Myotonic dystrophy[4], Huntington's disease[5], fragile X syndrome[6] and Kennedy's disease have all been shown to be caused by a similar genetic mechanism, viz. the expansion of triplet repeats in affected genes (Table 1). This mechanism had not previously been seen in humans. In Kennedy's disease the mutation involves the expansion of a CAG repeat in the coding sequence of the androgen receptor gene. In Huntington's disease the mutation is on chromosome 4 in a gene that has been termed huntingtin. Again the triplet repeat sequence is in the coding portion of the gene and involves a CAG repeat. Affected individuals have 42 to 66 repeats. In fragile X syndrome the mutated gene, termed FRM–1 is on the X chromosome.

Table 1 The genetic defects in the triplet repeat diseases

Disease	Chromosome	Gene	Intron/exon	Triplet	No. of repeats	
					Normal	Mutant
Kennedy's	X	AR	x	CAG	17–26	40–52
Huntington's[5]	4	hunt	x	CAG	11–34	42–66
Fragile X[6]	X	FRM1	i	CGG	6–54	>200
Myotonic dystrophy[4]	19	mpk	i	CTG	5–30	>50

AR = androgen receptor; x = exon; i = intron; hunt = huntingtin; mpk myotonin protein kinase

The gene has a CGG repeat and is in the 5' non-coding portion which is not translated into protein. Affected individuals have at least 200 repeats. It is not known how this expansion disrupts protein function.

In myotonic dystrophy the affected gene myotonin protein kinase is located on chromosome 19. Once again the CTG triplet repeat sequence is located in the non-coding portion of the gene. Affected patients have greater than 50 repeats.

In these conditions the number of repeats may vary between the generations. The variation in the number of repeats correlates with the variation in clinical expression between generations in affected families. In these conditions the greater number of repeats the more severe the clinical expression of the disease[7].

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REFERENCES

1. Kennedy WR, Alter M, Sung JH. Progressive proximal spinal bulbar muscular atrophy of late onset. A sex linked recessive trait. Neurology 1968; 18:671–680.
2. La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 1991; 352:77–79.
3. Choi WT, Warne GL, MacLean HE, Zajac JD, Chu S. Kennedy's disease: genetic diagnosis of an inherited form of motor neuron disease. Australian and New Zealand Journal of Medicine 1993; 23:187–192.
4. Mahadevan M, Tsilfidis C, Sabourin L *et al.* Myotonic dystrophy mutation: an unstable CTG repeat in the 3' untranslated region of the gene. Science 1992; 255:1253–1268.
5. The Huntington's disease collaborative research group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 1993; 72:971–983.
6. Kremer EJ, Pritchard M, Lynch M *et al.* Mapping of DNA instability at the fragile X to a trinucleotide repeat sequence p(CCG)n. Science 1991; 252:1711–1714.
7. Fu YH, Kuhl DPA, Pizzuti A *et al.* Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell 1991; 67:1047–1058.

ORTHOSTATIC TREMOR (SHAKY LEGS SYNDROME)

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SUMMARY

Nine patients (mean age 73 years: range 62–83 years) are described with a characteristic tremor or instability of the trunk and lower limbs which occurred when standing still, and which was either diminished or abolished by walking. Three had essential tremor of the upper limbs. The duration of the disorder ranged between 4 months and 20 years (mean 5 years). In all cases the condition worsened with time. Eight patients responded to clonazepam (0.5 to 2.0 mg per day) and one to chlordiazepoxide (30 mg per day). Orthostatic tremor is a disabling condition that responds to benzodiazepine treatment and may be more frequent than previously recognised.

Orthostatic tremor or shaky leg syndrome is a distinctive clinical entity comprising increasing instability during prolonged standing that resolves when the patient resumes walking or sits down[1-6]. Nine cases of orthostatic tremor are described, 3 of which have previously been reported[5].

CLINICAL FEATURES

CASE REPORT

A 62 year old woman presented with a 2 year history of increasing difficulty in standing still. This was particularly noticeable in the shower or when standing talking to friends. She had noticed that, the longer she stood still, the more she became unsteady. She realised that this was due to a problem with her legs and did not result from a sensation of vertigo or giddiness. The symptoms would come on within 20 seconds to a minute of her standing stationary and she would have to hold on to some support, sit down or commence walking.

She had noticed for many years a mild tremor of her hands, particularly noticeable when she was holding a cup of tea. Her 2 sons had a similar tremor. She

drank little alcohol and was unable to observe any benefit from it as regards her problem. In the past she had been given propranolol 60 mg 3 times a day for hypertension without any modification of the tremor in her arms or her instability on standing. On examination power, tone, reflexes and sensation were normal throughout and in particular proprioception was intact. There was no finger-nose or heel-shin ataxia, nor was there any nystagmus. When the patient was asked to stand still, she developed an increasing rocking of her body and legs. This was made worse by closing her eyes and also by standing with her feet closer together. Use of clonazepam 0.5 mg 3 times a day led to a complete resolution of her instability on standing.

The clinical picture described in the above case report is typical of the disorder. Some persons complain of an initial difficulty with standing, but whether this relates to the entity of orthostatic tremor is uncertain.

Table 1 summarises the clinical features of the 9 patients seen. Patient 5 was suffering from dementia and the history was obtained from nursing staff. The duration of her symptoms was many years and it was noticed that she was unable to stand without developing a rocking movement whenever the chlordiazepoxide that she was taking was withdrawn.

Table 1. Age, sex, duration of symptoms, coexisting or family history of essential tremor

Subject	Age (yrs)	Sex	Duration of symptoms	Family history of essential tremor	Coexisting esential tremor
1	62	F	2 years	yes	yes
2	83	M	2 years	no	no
3	79	F	4 months	no	yes
4	65	F	20 years	yes	yes
5	72	F	? years	?	no
6	71	F	4 years	yes	no
7	78	F	2 years	no	no
8	71	F	10 years	no	no
9	78	F	2 years	no	no

Eight patients have been treated with clonazepam in doses varying from 0.25 mg twice a day to 1.5 mg a day in two or three divided doses. One patient, as mentioned above, responded to chlordiazepoxide 20 mg twice a day.

The duration of follow up varied from 6 months to 5 years (mean 2.4 years). Table 2 indicates prognosis and therapy used at the time of writing.

Table 2. Duration of therapy, current treatment and response

Subject	Duration of follow-up	Current treatment and response
1	6 months	Clonazepam 0.5 mg bd: no tremor
2	5 years	Clonazepam 1 mg/day moderate tremor (partial control)
3	7 months	Clonazepam 1.25 mg/day: modest (incomplete) control of tremor
4	4 years	Deceased from carcinoma of lung
5	3 years	Chlordiazepoxide 10 mg tds plus clonazepam 0.5 mg tds: good control of tremor
6	1.25 years	Clonazepam 0.25 mg bd: good control of tremor
7	4.25 years	Bedridden due to multiple strokes
8	2.8 years	Clonazepam 0.25 bd: no tremor
9	6 months	Clonazepam 0.25 mg bd: moderate (incomplete) control

DISCUSSION

Orthostatic tremor is a distinctive clinical entity in which a sensation of increasing instability on the legs develops and worsens the longer the patient remains standing. Typically this occurs in the shower, standing at the kitchen bench or when sufferers stop walking and attempt to stand still. The tremor occurs within seconds to a minute of standing still and resolves quite promptly upon the patient either sitting or walking again.

Orthostatic tremor or the shaky legs syndrome has been recognised with increasing frequency in recent years[7-14]. Le Witte has used the alternative term paradoxical clonus[13]. Essential tremor, myoclonus, cerebellar ataxia and clonus due to spasticity may also produce a clinical picture of shaking and jerking of the legs that leads to unsteadiness when the patient is upright, with relief of symptoms on or after sitting down[7].

Although the condition was first described in the English literature by Heilman, there is an earlier report in the Italian literature[15]. It is thought to be related to familial essential tremor[12] and a co-existing essential tremor is common, as is a family history of essential tremor. Although orthostatic tremor is said to be associated with essential tremor the association was found in only 2.5% of patients[16]. It has also been described in association with a voice tremor[10,14], in which cases the frequency of the voice tremor coincided with that of orthostatic tremor (4.4–4.8 Hz) but was not influenced by a change in posture. No rhythmic movement was seen in the vocal cord during phonation but a fluoroscopic study of the diaphram revealed a synchronous tremor with a frequency of 4–5 Hz during phonation. The tremor in the subject's right leg could be evoked by passive flexion of the right ankle joint with the patient lying flat.

Gabellini *et al*[11] reported 8 cases of orthostatic tremor one associated with obstructive hydrocephalus in one chronic relapsing peripheral neuropathy. In both patients the tremor disappeared with appropriate treatment. In a personal patient the tremor preceded the onset of peripheral neuropathy and did not respond to corticosteroids.

With surface electromyography recordings in persons with orthostatic tremor the tremor discharge alternates between antagonist muscles at frequencies from 4.4 Hz[14] to 16 Hz[3,4,7,10] whereas essential tremor has a frequency of 6–8 Hz[7]. The tremor appears with any sustained and strong tonic muscle contraction, even in the upper limbs when the patient performs a hand stand[2,3] or pushes against the wall with the legs when supine[12]. Britton *et al*[7] proposed that the term primary orthostatic tremor should refer to a characteristic leg tremor that can be differentiated from other tremors by its rapid frequency of 16 Hz, its regularity and high degree of synchrony in different muscles, its being confined to the leg and trunk muscles, beginning a few seconds after standing and being relieved by walking or sitting. Using this definition, 4 of the patients described by Gabellini *et al*[11] and the case of Yokota[16] would not have had primary orthostatic tremor but probably essential tremor. However Fitzgerald and Jankovic[10] believed that orthostatic tremor

was a variant of essential tremor and that it had a variable frequency of 6–10 Hz. The tremor is believed to be of central origin because the frequency of the motor unit potential bursts is twice that expected from oscillations in an over-active spinal reflex and also because the stretch reflexes in the lower limbs are normal[3].

In 8 of the patients in the present study relief from clonazepam was either partial or complete, although in 2 patients the initial excellent response was followed after a variable period by some lessening of efficacy. There is no consistent response reported in the literature to any single drug, with some reports indicating benefit from clonazepam[1,6], sodium valproate[4], primidone[2,3,12] and phenobarbitone[8,14] whilst others have attributed no benefit to sodium valproate[8], levodopa[14], benzhexol hydrochloride[14] or clonazepam[5]. One patient in the present series responded to chlordiazepoxide, although without surface EMG recordings essential tremor could not be excluded in this case. Levodopa, bromocriptine and amitriptyline have all been tried without benefit[1,2]. Fitzgerald and Jankovic[10] claimed benefit from alcohol but their patient may have had essential tremor. It may be difficult to distinguish between orthostatic tremor and essential tremor so that therapeutic trials of a number of drugs may be necessary.

The large number of cases collected by the present author may reflect the peculiarity of his practice with a high number of retirees living locally. However the fact that more and more papers are appearing describing the entity of orthostatic tremor suggests that it has been under-recognised in the past.

REFERENCES

1. Heilman KM. Orthostatic tremor. Archives of Neurology 1984: 41;880–881.
2. Wee AS, Subramony SH, Currier RD. Orthostatic tremor in familial essential tremor. Neurology 1986: 36;1241–1245.
3. Thompson PD, Rothwell JC, Day BL. The physiology of orthostatic tremor. Archives of Neurology 1986: 43;584-587.
4. Kelly JJ, Sharbrough FW. EMG in orthostatic tremor. Neurology 1987: 37;1434.
5. Van der Zwan A, Verwey JC, Gijn J. Relief of orthostatic tremor by primidone. Neurology 1988: 38;1332.
6. Gates PC, Thyagarajan D. Orthostatic tremor: a cause of postural instability in the elderly. Medical Journal of Australia 1990: 152;373.
7. Britton TC, Thompson PD, Van der Kamp W, Rothwell JC, Day BL, Findley LJ, Marsden CD. Primary orthostatic tremor: further observations in six cases. Journal of Neurology 1992: 239;209–217.

8. Cabrera-Valvivia F, Jimenez-Jimenez FJ, Albea EG, Tejeiro-Martinez J, Ruiperez JAV, Ayuso-Peralta L. Orthostatic tremor: successful treatment with phenobarbital. Clinical Neuropharmacology 1191: 15;438–441.
9. Cristea RL, Goren H. Orthostatic tremor (letter). Archives of Neurology 1991: 48;1119.
10. FitzGerald P, Jankovic J. Orthostatic tremor: an association with essential tremor. Movement Disorders 1991: 6;60–64.
11. Gabellini AS, Martinelli P, Gulli P, Ambrosetto G, Ciucci G, Lugaresi E. Orthostatic tremor: essential and symptomatic cases. Acta Neurologica Scandinavica 1990: 81;113–117.
12. Walker F, McCormick GM, Hunt V. Isometric features of orthostatic tremor: an electromyographic analysis. Muscle & Nerve 1990: 13;918–922.
13. Le Witt PA. Orthostatic tremor: the phenomenon of "paradoxical clonus". Archives of Neurology 1990: 47;501–502.
14. Pazzaglia P, Sabattini L, Lugaresi E. Su di un singolare disturbo deura stazione eretta (osservazione di tri casi). Riv. Freniatr 1970: 96;450–457.
15. Martinelli P, Gabellini AS, Gulli MR, Lugaresi E. Different clinical features of essential tremor: a 200 patient study. Acta Neurolgica Scandinavica 1987: 75;106–111.
16. Yokota J, Imai H, Seki K, Ninomiya C, Mizuno Y. Orthostatic tremor associated with voice tremor. European Neurology 1992: 32;354–358.

ACUTE MYOPATHY IN STATUS ASTHMATICUS

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SUMMARY

An acute myopathy complicating life-threatening asthma has been reported with increasing frequency. We present a further 3 patients with this complication. Each patient had nerve conduction studies, electromyography and muscle biopsy performed.

The records of a cohort of 12 patients, ventilated in an intensive care unit over a 16 month period, were rviewed. Eleven out of the 12 patients developed an elevated creatine kinase level (median 1311 U/L, range 185–9973 U/L) and 4 developed symptomatic weakness.

The myopathy of status asthmaticus is not a homogeneous clinicopathological entity. Although myopathy is the predominant feature, there is a neuropathic component in some patients. Full recovery is usual.

The combination of corticosteroids and neuromuscular blocking agents has been proposed as the possible cause of the complication.

An acute myopathy in a patient with status asthmaticus was first reported in 1977 by MacFarlane[1]. Hydrocortisone was considered the possible cause of the myopathy. Sporadic cases were subsequently reported[2-6] during the 1980s.

Shee[7] described muscle weakness in 4 out of 9 patients ventilated for acute severe asthma. He considered that neuromuscular blockade may predispose to steroid-induced myopathy. A relatively high percentage of clinically detectable weakness was also noted in a study by Douglass *et al*[8]. Nine out of 25 asthma patients who were admitted to intensive care units for mechanical ventilation developed weakness.

In recent years there has been an increasing number of published reports of acute myopathy in severe asthma[9-15]. Despite this, we consider that the condition

remains under-recognised and report our experience with this complication of severe asthma.

PATIENTS AND METHODS

The clinical details, nerve conduction studies, EMG and muscle biopsy findings are described in 3 asthma patients who each developed an acute myopathy. In addition, a retrospective review of the records of 12 patients, admitted to the intensive care unit and ventilated for severe asthma during the period April 1991–August 1992, was carried out. This group included Case 3 described in the case presentations. Admission blood gases, daily creatine kinase levels, treatment details and clinical courses were analysed.

CASE REPORTS

Case 1

A 20 year old female was admitted on 5/12/90 with an acute exacerbation of asthma. Her admission blood gas results were pH 6.9, pCO_2 150 mm Hg and pO_2 400 mm Hg. Suxamethonium 100 mg was administered and the patient was ventilated. Paralysis was maintained with vecuronium at a rate of 4 mg/hr for the next 6 days. Methylprednisolone 1 gram statim was followed by hydrocortisone 200 mg 4th hourly. Other medications included intravenous salbutamol for 4 days (maximum rate 30 mg/day), isoflurane 1–3% followed by halothane inhalational anaesthesia for 2 days, and an aminophylline infusion from day 2 to day 9. Creatine kinase levels peaked on day 4 at 5,928 U/L (normal ≤125). Her serum K^+ was lowest on day 3, being 2.9 mmol/L (normal 3.4–4.8).

On day 7, while still being ventilated it was noted that she had generalised weakness and reduced reflexes. She was extubated on day 9. On day 12 her muscle power was graded with her neck flexors being 4/5 (MRC grade), upper limb muscles 1–2/5, proximal lower limb muscles 3–4/5 and distal lower limb muscles 2–3/5. Her reflexes were normal and there were no sensory abnormalities.

An EMG, performed on day 16, showed widespread fibrillations and early recruitment of short duration polyphasic potentials. Her sensory nerve action potential (SNAP) amplitudes were normal as were motor conduction velocities (MCV) and F wave latencies.

Percutaneous muscle biopsy (Fig 1) was performed on day 34. There was a slight increase in the number of internal nuclei. In a few fibres there was darkly staining material in the central cytoplasm. Electron microscopy (Fig 2) was performed to clarify the nature of the darkly staining material. The abnormal areas contained small lipid droplets, myofilaments and glycogen granules.

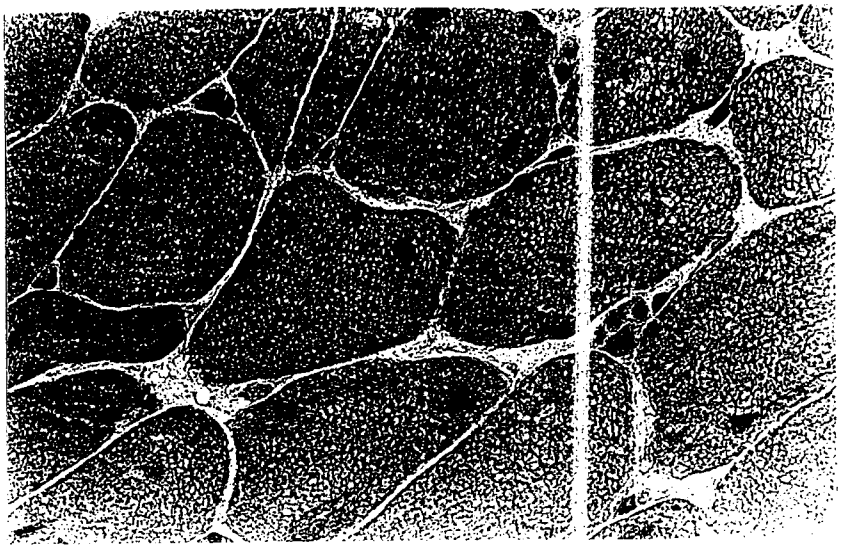

Fig 1 Muscle biopsy (Case 1) showing central nucleation and dark cytoplasmic material (H & E, paraffin section, x 125)

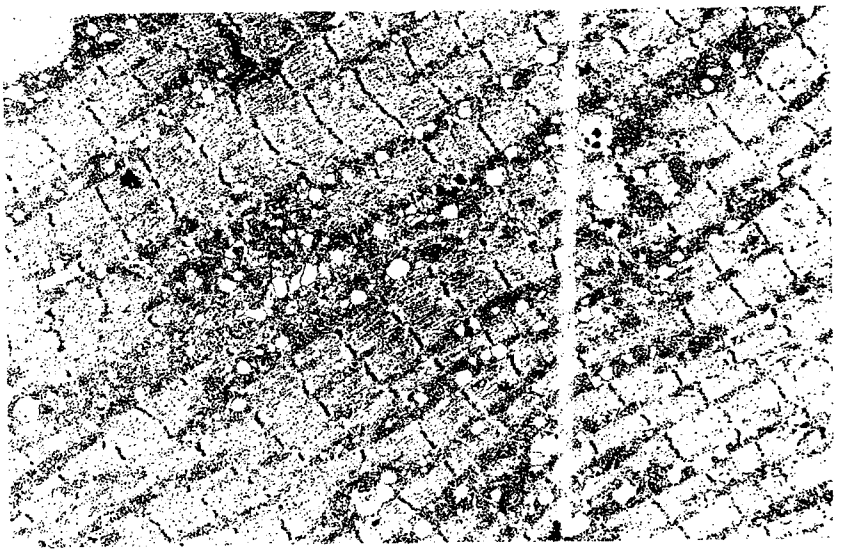

Fig 2 Electron microscopy (Case 1) showing abnormal areas containing lipid droplets, myofilaments and glycogen granules

On day 15 she was walking with assistance and by day 40 her power had returned to normal.

This patient had required ventilation for asthma on 2 previous admissions . In 1987 she was ventilated for 3 days and received hydrocortisone 400 mg/day and pancuronium 4 mg/hr. Her creatine kinase then peaked at 463 U/L on day 2. In 1989 ventilation was required for one day. Hydrocortisone 1200 mg/day and pancuronium 2–5mg/hour were administered. Her creatine kinase peaked on day 1 at 463 U/L. There was no weakness documented on either of these earlier admissions.

Case 2

A 28 year old female was admitted on 28/6/90 following a respiratory arrest for which she was resuscitated and intubated by paramedics. There was a history of asthma since childhood with multiple admissions, but none requiring intubation. Her admission blood gas findings were pH 7.1, pCO_2 20 mm Hg and pO_2 325 mm Hg. Ventilation was required for 6 days. She received a peak dose of hydrocortisone of 1700 mg/day, together with vecuronium 4 mg/hr and intravenous salbutamol 15 mg/day. Other drugs administered included adrenalin and 1% isoflurane.

Weakness was noted on day 9. On day 13 her muscle power was graded as 4/5 for the distal upper limb and proximal lower limb muscles and 4-/5 for the distal lower limb muscles. Her ankle reflexes were absent but there were no sensory signs. Her creatine kinase peaked on day 6 at 2389 U/L.

On day 17 her CSF examination was normal. On day 19 nerve conduction studies were mildly abnormal with slowing of the common peroneal MCV to 45 m/sec and the tibial nerve MCV to 43 m/sec (normal for both nerves ≥47). The right median F_2-wrist SNAP amplitude was reduced to 11 μV (normal >14). F wave latencies were normal. On EMG, sporadic fibrillations were noted in 2 of the 6 muscles sampled. All muscles had areas of short duration low amplitude polyphasic potentials consistent with a myopathy.

By day 36 her prednisone dose was reduced to 7.5 mg/day. Her muscle power did not return to normal until 4 months after admission.

Muscle biopsy (Fig 3) was performed on day 44. In the ATPase stain there was evidence of a marked selective atrophy affecting the Type II fibres.

Case 3

A 65 year old female was admitted on the 18/9/92 with an acute exacerbation of chronic obstructive airways disease. Her respiratory illness had involved a reversible component of airways obstruction. Her admission blood gas results were pH 7.2, pCO_2 63 mm Hg and pO_2 80 mm Hg. She was intubated and required a tracheostomy for prolonged ventilatory support over the next 24 days.

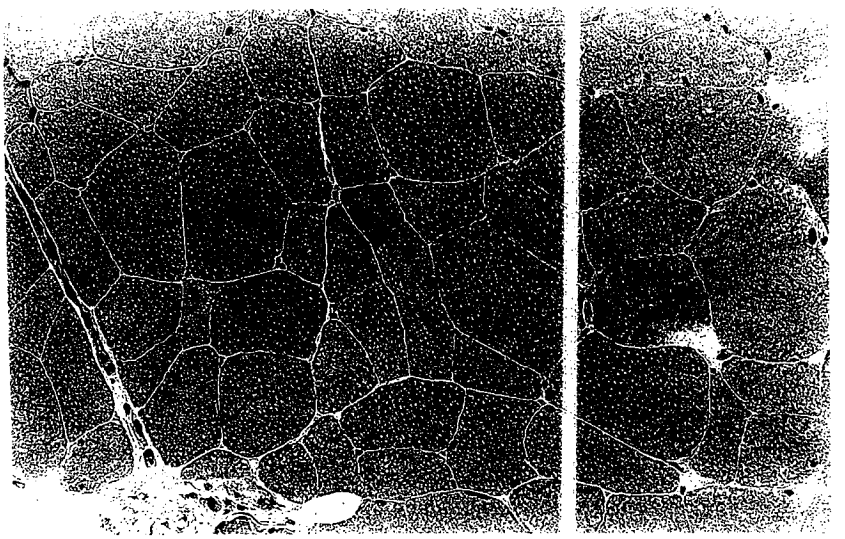

Fig 3 Muscle biopsy (Case 2) showing fibre size variation (H & E, paraffin section, x62.5)

Neuromuscular blocking agents were used on day 1 and then from days 4 to 7, with a peak dose of vecuronium 2–3 mg/hr. At other times sedation was maintained with a combination of pethidine and midazolam. The peak dose of hydrocortisone used was 800 mg/day, together with salbutamol 20 mg/day. An aminophylline infusion was administered from days 4 to 10 and gentamicin on days 0 to 2 and 8 to 14. Due to hypotension the patient was not able to tolerate isoflurane anaesthesia.

On day 9 weakness was noted. On day 10 the patient had a generalised tonic-clonic seizure. There was a quadriparesis on neurological examination on day 16 with muscle power graded as 1–3/5. Reflexes were present but reduced and there were no sensory changes.

There was an initial creatine kinase level peak of 1141 U/L on day 2, with a further second peak on day 17 at 708 U/L.

EMG and nerve conduction studies were performed on day 24. SNAPs were normal but the compound muscle action potential amplitudes were reduced. MCV and F wave latencies were normal. There were fibrillations and positive sharp waves and early recruitment of short duration low amplitude potentials consistent with a myopathy. Repetitive stimulation at 1 and 20 Hz did not result in a decremental or incremental response.

Muscle biopsy (Fig 4) on day 30 was consistent with a monophasic necrotising myopathy with almost all fibres showing evidence of necrosis and regeneration. Numerous

histocytes were noted throughout the biopsy.

At 2 months she was discharged home with a walking frame and muscle power at 4–4+/5. At 4 months there was mild residual weakness.

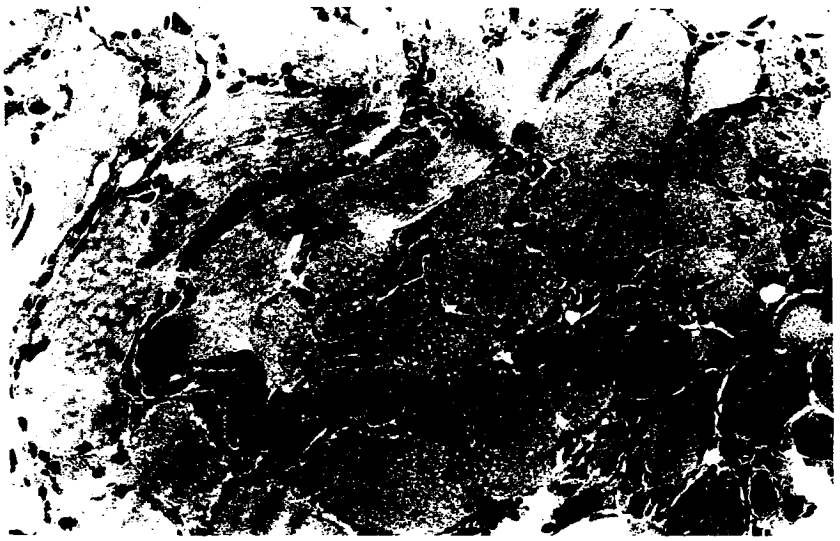

Fig 4 Muscle biopsy (Case 4) showing necrotic fibres and numerous histiocytes (H & E, paraffin section, x125)

RESULTS OF THE SURVEY

The cohort of 12 patients with severe asthma consisted of 5 males and 7 females. The mean age was 47 years (range 22–70). All patients were paralysed and ventilated. Treatment and clinical details are summarised in Table 1.

Creatine kinase levels were elevated in 11 patients, with a median value of 1331 U/L (range 185–9973 U/L; normal male ≤185, female ≤125). The time to peak creatine kinase level was 2.8 ± 1.2 days. One patient had a 2nd peak at day 17 (Case 3 in the case presentations).

Muscle weakness developed in 4 patients, all of whom were female. In 2 this was mild (MRC grade 4/5 or better in all muscle groups), in one moderate (at least one muscle <4/5) and in one marked (all limb muscles <4/5). The

weakness was global in 3, and more marked distally in one. Reflexes were transiently reduced in one patient and normal in the other 3. Sensation was normal. Muscle power returned to normal in 2 to 16 weeks. Myalgia was a feature in 2 patients with weakness and in one patient with no weakness but a creatine kinase peak of 4080 U/L.

Table 1 Summary of clinical and treatment details – 12 patients

	Muscle Weakness n = 16	No Weakness n = 8	Total n = 12
Male/Female	0/4	5/3	5/7
pH	7.12 ± 0.15	7.08 ± 0.08	7.10 ± 0.10
pCO_2	87 ± 41	92 ± 21	90 ± 27
Days paralysed*	4.2 ± 1.0	1.8 ± 1.5	2.6 ± 1.7
NMBA total mg[1]	198 ± 59	69 ± 85	112 ± 98
Hydrocortisone total mg*[2]	7350 ± 2910	2560 ± 1540	4160 ± 3060
Salbutamol IV total mg	162 ± 137	50 ± 55	87 ± 107

* = Statistically significant difference ($p < 0.05$) between groups (Mann Whitney)
[1] = Pancuronium or Vecuronium
[2] = Steroid converted to hydrocortisone equivalent dose
NMBA = neuromuscular blocking agents

A statistically significant difference ($p = 0.01$) was noted between the group with muscle weakness and the group without weakness in relation to the total dose of hydrocortisone (Mann-Whitney test) and in the number of days paralysed ($p = 0.03$). There was a trend towards significance for total dose of neuromuscular blocking agents ($p = 0.09$) and total dose of intravenous salbutamol ($p = 0.06$). These findings should be interpreted with caution in view of the small number of patients involved.

DISCUSSION

The occurrence of muscle weakness in patients with status asthmaticus treated with neuromuscular blockade is not unusual in our experience. The increasing number of reports of this complication in the literature is an indication of an increased awareness or result of an increased incidence of this problem.

The weakness is predominantly secondary to an acute myopathy as demonstrated by the 3 cases reported here. There were minor reflex and nerve conduction changes consistent with a mild neuropathic component. The EMG pattern showed spontaneous activity and myopathic units. This pattern is similar to that reported in a recent abstract[16].

It is of interest that the muscle biopsy appearance in the 3 cases presented was different in each case. Review of other case reports also shows a diverse range of pathological changes ranging from widespread necrosis to type II fibre atrophy. This may reflect variability in severity of the myopathy or in the timing of the biopsy. Another possibility is that the myopathy is not a homogeneous entity but is due to a number of different causative factors. There are reports of cytoplasmic dark staining material[11,15], similar to that described in case 1. A number of authors[11,14] reported a selective loss of thick myosin filaments on immunohistochemical stains and electron microscopy. Selective loss of myosin has been reported in rats with a combination of denervation and use of high dose steroids[17]. Disuse of skeletal muscle is also associated with an increase in the number of glucocorticoid receptors[18]. Therefore there is evidence that a combination of neuromuscular blocking agents and high dose steroid may cause myopathy.

The role of neuromuscular blocking agents in causing neuromuscular complications is becoming increasingly recognised[19]. Apart from the myopathy which occurs in combination with steroid therapy, there is prolongation of neuromuscular blockade (up to 7 days) in patients with renal failure who receive vecuronium[20].

Other agents used in the management of asthma which may contribute to neuromuscular problems include aminoglycosides, inhalation anaesthesia[21], and beta agonist[22] therapy. Non-pharmacological factors may also have a role, as yet undetermined, in the development of muscle weakness in asthmatics[15].

It is important to recognize that a myopathy may complicate status asthmaticus. There are other conditions which may cause weakness in intensive care patients. The critical illness neuropathy, Guillain-Barré neuropathy and post-asthmatic pseudo-polio in children[23] may all require specific treatment or affect other treatments or the prognosis. The myopathy associated with asthma is self limiting, although recovery can take up to several months to occur.

There are a number of recommendations that have been proposed for reducing the potential risk of myopathy[19]. These include using an alternative

neuromuscular blocking agent such as atracurium, limiting continuous dose of neuromuscular blocking agents to a 48 hour period or less, and the frequent use of a peripheral nerve stimulator to minimise the effect of neuromuscular blocking agents. Patients who develop this complication are already being treated for life threatening asthma. This needs to be borne in mind when any alteration in treatment is proposed to reduce the risk of myopathy.

REFERENCES

1. MacFarlane IA, Rosenthal FD. Severe myopathy after status asthmaticus. Lancet 1977; 2:615.
2. Van Marle W, Woods KL. Acute hydrocortisone myopathy. British Medical Journal 1980; 281:271–272.
3. Knox AJ, Mascie-Taylor BH, Muers MF. Acute hydrocortisone myopathy in acute severe asthma. Thorax 1986; 41:411–412.
4. Williams TJ, O'Hehir RE, Czarny D, Horne M, Bowes G. Acute myopathy in severe asthma treated with intravenously administered corticosteroids. American Review of Respiratory Disease 1988; 137:460–463.
5. Hoad NA. Letter. Respiratory Medicine 1990; 84:50.
6. Sury MRJ, Russell GN, Heaf DP. Hydrocortisone myopathy. Lancet 1988; 2:515.
7. Shee CD. Risk factors for hydrocortisone myopathy in acute severe asthma. Respiratory Medicine 1990; 84:229–233.
8. Douglass JA, Tuxen DV, Horne M *et al.* Myopathy in severe asthma. American Review of Respiratory Disease 1992; 146:517–519.
9. Sitwell LD, Weinshenker BG, Monpetit V, Reid D. Complete ophthalmoplegia as a complication of acute corticosteroid and pancuronium associated myopathy. Neurology 1991; 41:921–922.
10. Lacomis D, Samuels MA. Adverse neurological effects of glucucorticosteroids. Journal of General Internal Medicine 1991; 6:376–377.
11. Danon MJ, Carpenter S. Myopathy with thick filament (myosin) loss following prolonged paralysis with vecuronium during steroid treatment. Muscle and Nerve 1991; 14:1131–1139.
12. Apte-Kakade S. Rehabilitation of patients with quadriparesis after treatment of status asthmaticus with neuromuscular blocking agents and high-dose corticosteroids. Archives of Physical Medicine and Rehabilitation 1991; 72:1024–1028.
13. Griffin D, Fairman N, Coursin D, Rawsthorne L, Grossman JE. Acute myopathy during treatment of status asthmaticus with corticosteroids and steroidal muscle relaxants. Chest 1992; 102:510–514.
14. Hirand M, Ott BR, Raps EC *et al.* Acute quadriplegic myopathy: a complication of treatment with steroids, nondepolarising blocking agents, or both. Neurology 1992; 42:2082–2087.
15. Lacomis D, Smith TW, Chad DA. Acute myopathy and neuropathy in status asthmaticus: case report and literature review. Muscle and Nerve 1993; 16:84–90.
16. Mackie G, Road J, Stewart H, Eisen A. Reversible paralysis with status asthmaticus,

steroids and pancuronium: clinical electrophysiological correlates. Neurology 1993; 42 (supplement 2):A166–167.
17. Rouleau G, Karpati G, Carpenter S, Soza M, Prescott S, Holland P. Glucocorticoid excess induces preferential depletion of myosin in denervated skeletal muscle fibers. Muscle and Nerve 1987; 10:428–438.
18. DuBois DC, Almon RR. Disuse atrophy of skeletal muscle is associated with an increase in number of glucocorticoid receptors. Endocrinology 1980; 107:1649–1651.
19. Hansen-Flaschen J, Cowen J, Raps EC. Neuromuscular blockade in the Intensive Care Unit. American Review of Respiratory Disease 1993; 147:234–236.
20. Segredo V, Caldwell JE, Matthay MA, Sharma ML, Gruenke LD, Miller RD. Persistent paralysis in critically ill patients after long-term administration of vecuronium. New England Journal of Medicine 1992; 327:524–528.
21. Tanigaki T, Kondo T, Ohta Y, Yamabayashi H. Transient neuromuscular impairment resulting from prolonged inhalation of halothane and enflurane. Chest 1990; 98:1012–1013.
22. Sykes AP, Lawson N, Finnegan JA, Ayres JG. Creatine kinase activity in patients with brittle asthma treated with long term subcutaneous terbutaline. Thorax 1991; 46:580–583.
23. Editorial. Post-asthmatic pseudo-polio in children. Lancet 1980; 1:860.

WATERSHED CEREBRAL INFARCTION ASSOCIATED WITH PERIOPERATIVE HYPOTENSION

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SUMMARY

The pathogenesis of perioperative stroke is not clear from the literature. To explore the influence of various risk factors we examined the clinical, Duplex ultrasound and computerised tomography findings of all cases suffering cerebral infarction within 24 hours of surgery in a prospective series of 358 coronary or peripheral vascular reconstructive operations. Four patients (1.1%) had cerebral infarcts within 24 hours of surgery, all associated with perioperative systolic blood pressures of less than 90 mmHg. The other significant risk factor was previous cerebral ischaemic symptoms. Haemodynamic cerebral ischaemia occurred immediately after operation in 2 of 10 cases with severe symptomatic carotid stenosis or occlusion (stroke risk 20%; 95% confidence interval 2.52%–55.61%). Two cases with mild carotid disease had cerebral infarcts in previously asymptomatic hemispheres following coronary artery bypass graft surgery. One of these had clinical and computerised tomographic evidence of cortical watershed infarction. We conclude that cerebral haemodynamics are important in perioperative stroke and that symptomatic patients with severe carotid disease may be at high risk of perioperative watershed infarction.

The risk of stroke around the time of elective surgery is small and the pathogenesis of perioperative stroke is likely to be multifactorial[1,2]. One case-control study has shown a slight increase in perioperative stroke risk for patients with carotid bruits[3] but prospective Duplex ultrasound studies record a low risk for those with asymptomatic carotid stenosis[4,5]. However, the presence of severe carotid stenosis in patients requiring major surgery is a concern to physicians and surgeons who justifiably fear that hypotension will lead to watershed infarction in the distal cerebral hemisphere. Such infarcts occur and have been the subject of an autopsy series of patients dying following severe intraoperative hypotension[6], and also the subject of computerised tomography (CT) studies of postoperative strokes in coronary artery graft surgery cases[7,8].

Carotid occlusive disease either was not present or not discussed in those cases. The topography of cerebral infarcts in patients with carotid stenosis and occlusion who survive severe hypotension during surgery has not been determined prospectively. The prevalence of carotid disease is highest in patients with peripheral vascular disease but here also the perioperative stroke risk is low[4,5].

To determine the risk factors for perioperative stroke we examined the blood pressure, carotid ultrasound, clinical and computerised tomography (CT) findings of all perioperative strokes in a prospective series of vascular and coronary surgery candidates[4].

METHODS

The patients studied were 358 consecutive persons undergoing coronary or major vascular surgery. All patients had Duplex ultrasound studies of both carotid arteries before operation. Hypotension was defined as a systolic blood pressure <90 mmHg. Other details of the patient and imaging protocols have been reported previously[5]. In the present report consideration has been limited to the first 24 hours after operation, which therefore excludes from the perioperative stroke cases one patient who, 3 days after operation, had a cardiac arrest and had a cerebral infarct ipsilateral to a previously symptomatic carotid occlusion.

RESULTS

Four patients (1.1%) had strokes within 24 hours of surgery. Two patients had immediate post-operative hypotension and fluctuations in blood pressure which correlated with variation in their neurological deficits. Both had undergone vascular surgical procedures and had previously symptomatic severe carotid disease. One of these (Case 1) had a previously symptomatic carotid occlusion and suffered an ipsilateral watershed infarct (Fig 1) on the day of abdominal aortic aneurysm repair, despite prophylactic endarterectomy of a contralateral asymptomatic 80% carotid stenosis (performed before the commencement of the study). He made a good recovery from a right hemiparesis and posterior dysphasia. The other patient (Case 2), some of whose details have been reported previously[5], had bilateral 80% carotid stenoses and left hemisphere transient ischaemic attacks (TIAs), and developed right hemiparesis and aphasia during hypotension in the recovery room following femoro-popliteal bypass. Although she improved with intravenous fluids, a further period of prolonged hypotension overnight resulted in a permanent severe deficit with CT showing a large left hemisphere infarct sparing an island of tissue in the region of the Sylvian fissure (Fig 2). The left internal carotid artery was not occluded at a further Duplex ultrasound 3 weeks later.

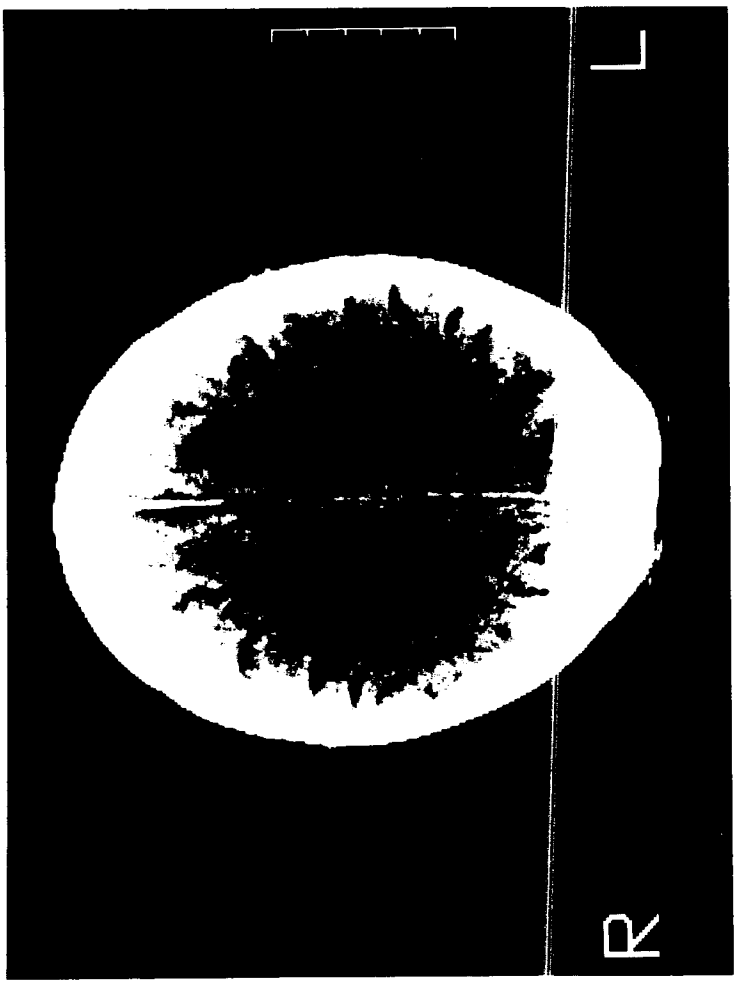

Fig 1 Left hemisphere cortical watershed infarction, Case 1.

Fig 2 Large left hemisphere infarction, Case 2.

Two coronary artery graft patients had intraoperative hypotension and had more subtle neurological deficits detectable on the first post-operative day. One (Case 3) had a posterior aphasia and right arm weakness. Four days later the CT showed a small subcortical infarct on the left, and one cortical infarct in the frontal region on the right (Fig 3). The other (Case 4) complained of disturbed vision. Examination showed that he had difficulty recognising objects in the right visual field. The CT showed a thin wedge-shaped cortically based infarct in the parieto-occipital region on the left side (Fig 4).

DISCUSSION

In a large surgical series the 4 patients with strokes which occurred within 24 hours of operation experienced significant drops in systolic blood pressure. Three of the 4 had clinical and radiological evidence of watershed infarction, and in 2 the infarcts occurred in the territories of previously symptomatic severe carotid occlusive disease. The CTs of Case 1 and Case 4 show typical watershed infarcts. Case 2 had an unusually large infarct but the onset of symptoms with a fall in blood pressure and the initial response to intravenous filling as well as the sparing of an area of brain in the region of the Sylvian fissure are all suggestive of a haemodynamic event. The CT of Case 3 (Fig 3) shows bilateral infarcts. Embolism would be a likely explanation in the context of cardiac surgery, but a haemodynamic mechanism may account for the left hemisphere infarct, where watershed infarcts have been described[7,9,10].

The presence of previous cerebral ischaemic symptoms was a significant risk factor for perioperative stroke, as has been found by others[3]. Hypotension was also a significant risk factor but in asymptomatic patients, even with carotid stenosis, the risk associated with hypotension may be very low[5]. Although we have previously reported a low perioperative stroke risk for asymptomatic carotid stenosis[5] a haemodynamic mechanism does account for a small proportion of strokes and TIAs and patients with previously symptomatic untreated carotid stenosis or carotid occlusion may be at high risk of stroke around the time of major surgery.

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We thank the vascular and cardiac surgeons of St Vincent's Hospital for their participation in this study, and Professor E. Byrne for helpful suggestions. RPG held a John L. Connell Fellowship.

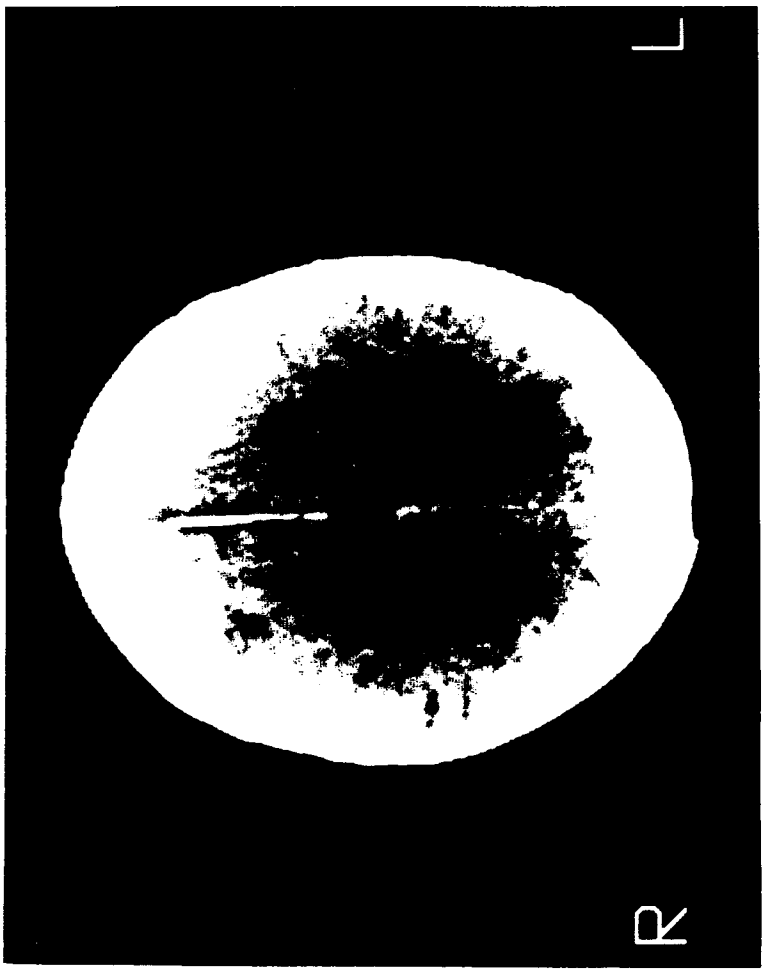

Fig 3 Left parietal subcortical infarction and right frontal cortical infarction, Case 3.

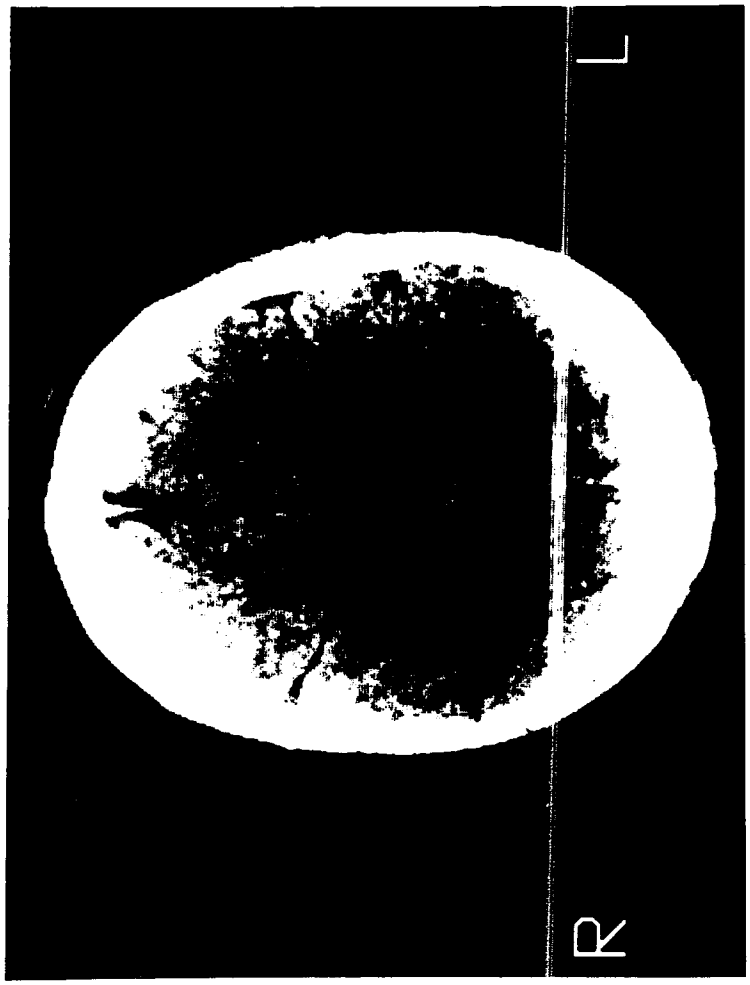

Fig 4 Left parieto-occipital watershed infarction, Case 4.

REFERENCES

1. Ropper AH, Wechsler LR, Wilson LS. Carotid bruit and the risk of stroke in elective surgery. New England Journal of Medicine 1982; 307:1388–1390.
2. Breuer AC, Furlan AJ, Hanson MR *et al.* Central nervous system complications of coronary artery bypass graft surgery: prospective analysis of 421 patients. Stroke 1983; 14:682–687.
3. Reed GL, Singer DE, Picard EH, DeSanctis RW. Stroke following coronary-artery bypass surgery: a case-control estimate of the risk from carotid bruits. New England Journal of Medicine 1988; 319: 1246–1250.
4. Barnes RW, Liebman PR, Marszalek PB, Kirk CL, Goldman MH. The natural history of asymptomatic carotid disease in patients undergoing cardiovascular surgery. Surgery 1981; 90:1075–1083.
5. Gerraty RP, Gates PC, Doyle JC. Carotid stenosis and perioperative stroke risk in symptomatic and asymptomatic patients undergoing vascular or coronary surgery. Stroke 1993; 24:1115–1118.
6. Adams JH, Brierley JB, Connor RCR, Treip CS. The effects of systemic hypotension upon the human brain; clinical and neuropathological observations in 11 cases. Brain 1966; 89:235–268.
7. Howard R, Trend P, Ross Russell RW. Clinical features of ischemia in cerebral arterial border zones after periods of reduced cerebral blood flow. Archives of Neurology 1987; 44:934–940.
8. Ross Russell RW, Bharucha N. Recognition and prevention of border zone cerebral ischaemia during cardiac surgery. Quarterly Journal of Medicine 1978; 47: 303–323.
9. Bladin C, Chambers BR. Watershed infarction. In: Donnan GA, Berkovic SF, Vajda FJE (eds). Stroke. Proceedings of workshop held at the Austin Hospital, John Lindell Lecture Theatre, Melbourne, Australia, October, 1988.
10. Zülch K-J, Hossmann V. Patterns of cerebral infarctions. In: Vinken PJ, Bruyn GW, Klawans HL (eds). Handbook of Clinical Neurology, Vol 53, Part I. Elsevier. Amsterdam. 1988. 175–198.

REGIONAL CEREBRAL BLOOD FLOW DURING MEMORY RECOGNITION AND NEUROPSYCHOLOGICAL PERFORMANCE IN PATIENTS REFERRED FOR INVESTIGATION OF DEMENTIA

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SUMMARY

Regional cerebral blood flow was studied at rest and during a memory recognition activation task in a preliminary investigation carried out in patients with mild to moderate dementia of Alzheimer type and in 2 control groups of subjects. There were differences in the sites of activation-increased blood flows between the normal controls and the controls with major depression, while the Alzheimer's disease subjects showed more variable patterns of flow response, which differed overall from those present in the 2 control groups.

It has now been established that measurable physiological changes antedate the symptomatology of Alzheimer's disease (Barclay *et al*, 1984, Risberg, 1985, Prohovnik *et al*, 1988). The progressive impairment of memory, adaptive behaviour, language and praxis characterising primary degenerative dementia of Alzheimer's type (DAT) has been shown to reflect pathophysiological changes most prominent in the hippocampus, temporo-parietal and frontal association cortices. Clinicians diagnosing DAT, however, have traditionally relied on a constellation of clinical features, neuropsychological findings and tests of exclusion. Whilst such techniques are sufficient to enable the diagnosis of DAT in its more advanced stages, early detection and differentiation of the condition remains problematical

Since neurophysiological changes antedate the symptomatology of Alzheimer's dementia, an increasing number of studies have begun to examine the changes in brain function that underlie this disorder. Most of these studies have examined brain function at rest.

It is possible that specific cognitive activation tasks will engage preferentially those networks underlying the processes of interest, and will reflect more specific patterns of dysfunction than measures of brain function at rest. In addition few previous studies have examined the specificity of changes in brain function - particularly the concomitant changes in depression and those differentiating pseudodementia from Alzheimer's disease.

The aims of the study here described were to examine the sensitivity and specifity of regional cerebral blood flow (RCBF) at rest and during a memory-recognition activation task. We also assessed the correspondence between the neuropsychological profile of psychometric tests and RCBF at rest and during the memory-activation task. We here report preliminary, mainly group findings, and critically analyse some of the formidable vagaries inherent to simultaneous measures of brain function that are undertaken during cognitive activation tasks – which is our particular area of interest.

METHOD

Subjects

Potential subjects were referred to the Departments of Neurology or Psychiatry at Westmead Hospital for investigation of possible dementia. Subjects were screened for a history of significant medical, neurological or psychiatric illness, previous substance abuse or cardiac, respiratory or visual problems. If any of these factors was present subjects were excluded. Fourteen patients were assessed by a neurologist as having DAT (dementia of the Alzheimer type) of mild or moderate severity according to NINCDS-ARDRA criteria. Patients with severe DAT were excluded. All underwent psychiatric appraisal to exclude depression, neurological examination and CT scanning. Resting RCBF was measured in all of these subjects. Measurement of RCBF during activation was not possible in 5 subjects due to inability to comprehend task instructions, English not being the subject's first language, or an inability to tolerate more than one measurement of RCBF. Activation data were therefore available for 9 subjects.

Neuropsychological assessment comprised the following tests:

1. Wechsler Adult Intelligence Scale - Revised (WAIS-R) (Wechsler, 1981): Digit Span, Vocabulary, Similarities, Picture Completion, Block Design and Symbol Digit subtests.
2. Russell's version of the Wechsler Memory Scale (RWMS) (Russell, 1987).
3. Wechsler Memory Scale (Wechsler, 1945): Mental Control and Associate Learning subtests.
4. Controlled Oral Word Association Test (COWAT) (Benton, 1973).
5. Trail Making Test (Lezak, 1983).
6. Colour Form Sorting Test (Goldstein and Scheerer, 1941).

A psychiatric control group comprised 6 elderly (over 60 years) patients who were diagnosed by a psychiatrist according to DSM-IIIR criteria as having major depression. All of these subjects underwent RCBF measurement during rest and activation. In addition, 20 normal controls over the age of 60 were examined.

RCBF Procedure

RCBF was measured using the Xenon-133 intravenous technique. A 32 channel cerebrograph monitored the uptake and clearance of the Xenon-133. Sixteen detectors per hemisphere were arranged in parallel at right angles to the lateral surface of the head. Surface markings for positioning of the detectors were the external auditory meatus and the nasion. A standard template was used to ensure accurate detector positioning.

Two measurements of RCBF were taken: (i) during a resting (eyes closed) baseline condition, and (ii) during a task of recognition memory. A 30 second background count was taken prior to the first measurement of the RCBF. A 5 minute remaining activity count was taken prior to the second measurement of RCBF. For those subjects in whom both measurements were taken, the activation task preceded the resting measurement. The Initial Slope Index (ISI) was chosen as the index of perfusion as it is the most reliable parameter for the measurement of RCBF in pathological conditions where the blood-brain barrier may be disturbed and the partition coefficient for Xenon-133 is not known. ISI values were corrected to pCO_2 (40mm Hg). An initial 5 minute 'dummy-run' was administered to acclimatise each subject to the experimental situation. Conditions were equivalent to those at rest except that cold saline was administered instead of Xenon-133.

The memory recognition task consisted of the recognition of 36 pre-learned words randomly intermixed with 89 distractor words of equal frequency. A button press response (using the middle finger of each hand) was required when the subject recognized a target word.

Impairment of RCBF was defined as an ISI value of at least two standard deviations above or below the range of normal RCBF (as defined in a study of 100 normals) for that individual's age group. Values for each of the 6 detectors in the frontal and the 3 detectors in the temporal lobes were examined. To compare the data, ratings of the degree of RCBF impairment were calculated. We examined the correspondence between 5 of the neuropsychological ratings (scores that are suggestive of frontal and temporal dysfunction) and the RCBF at rest and during activation.

RESULTS

Initial analysis was with statistical probability mapping (SPM) of 't'-scores which were obtained from imaging the distribution of 't'-tests between the groups, at each recording site - thus showing the areas of significant difference between the groups.

Initial analysis of the data revealed the following patterns. In the normal and depressed patients increased flows consistently occurred with activation. In the normal subjects, the activation was most marked in the left frontal region; in the depressed group activation was marked posteriorly, less so in the right hemisphere. In the DAT group, responses were more variable than in the other groups and included subjects who failed to activate and other subjects with extremely high increases in right hemisphere (compared with left hemisphere) activation. As a group, significant differences were evident in right hemisphere activation compared with normal controls. The pattern of distribution of the group data is reflected in Figs 1 and 2.

Analysis of the correspondence between neuropsychological ratings of regional dysfunction and those based on RCBF revealed most overlap when activation data were analysed and frontal lobe function examined. The likelihood of frontal and temporal dysfunction was rated on the basis of performance on the 6 neuropsychological measures of frontal lobe functioning and the 5 neuropsychological measures of recent memory. Neuropsychological ratings were derived as follows:

Number of Tests Impaired	Rating
<1	Unlikely
2	Possible
3	Probable
>4	Definite

The correspondence between neuropsychological ratings and those based on RCBF at rest and during activation was then compared (Table 1, a and b).

Table 1a Correspondence between neuropsychological ratings and those based on RCBF at rest

	% Same	% + 1 Category
Temporal lobe:	30	56
Frontal lobe (Rt):	43	52
Frontal lobe (Lt):	54	65

Table 1b Correspondence between neuropsychological ratings and those based on RCBF during activation

	% Same	% + 1 Category
Temporal lobe:	46	62
Frontal lobe (Rt):	38	85
Frontal lobe (Lt):	54	85

DISCUSSION

The normal control group showed a pattern of RCBF activation of the left frontal lobe (compared with RCBF at rest) that was consistent with expectation, using the verbal recognition-memory task in this study. The Alzheimer disease group did not show this RCBF pattern, and demonstrated significantly greater activation in the right frontal lobe and diminished flow in the left frontal lobe. There are a number of possibilities for this finding that warrant further exploration. One possibility concerns compensatory activation due to failure of activation of the left frontal region and associated neural networks. Another possibility is that anxiety mediated the relationship between RCBF and performance, such that when arousal was high or low, performance deteriorated and perfusion was reduced, and vice versa. This hypothesis is testable and will be explored further.

These preliminary results support the possibility that studies of RCBF during activation may add complementary information of overall measures of brain function to measures of brain function at rest, in the early stages of Alzheimer's disease. This is further supported by associations between psychometric test results and RCBF. The results showed a poor correspondence between judgements made on the basis of RCBF at rest and those derived from the performance on neuropsychological testing. Less than 50% of the patients were classified in the same manner using these 2 techniques. Even when the correspondence between RCBF and neuropsychological results was examined in terms of classification deviating by only one category, at best only 65% of the ratings corresponded.

Correlation of neuropsychological ratings of dysfunction and those derived from examination of RCBF during activation were higher. The greatest correspondence between neuropsychological and activation RCBF results was for ratings of frontal lobe dysfunction. Although only 50% were rated in exactly the same manner, classification deviated by at the most only one category for 85%

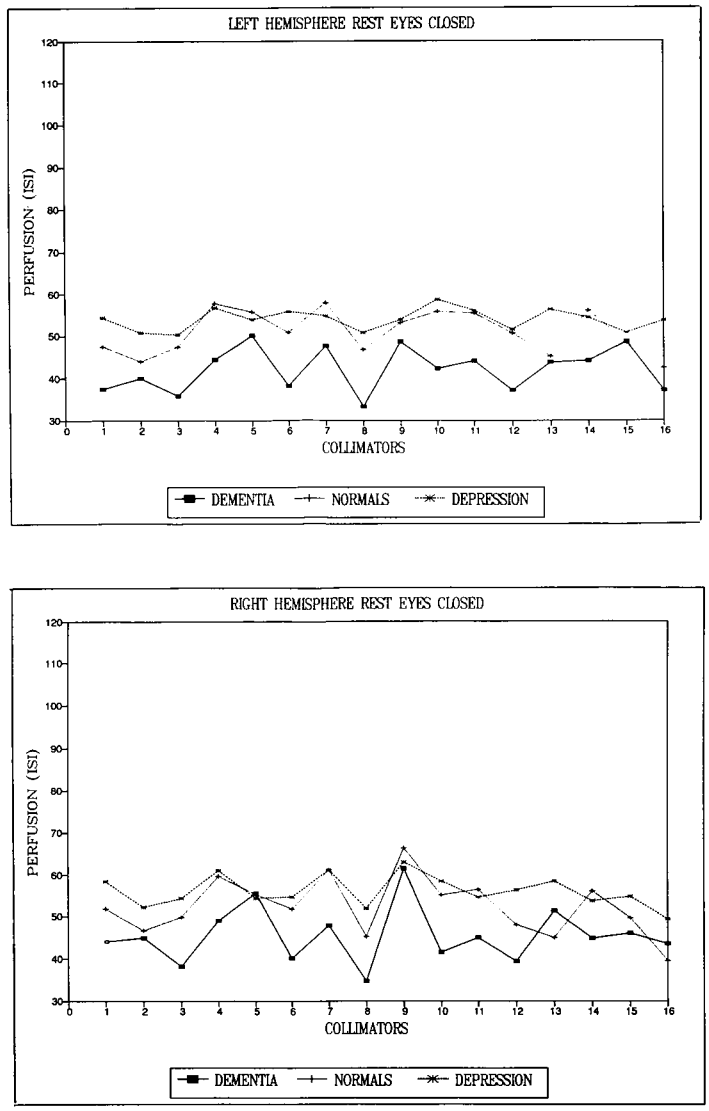

Fig 1 The mean hemispheric distribution of RCBF (ISI) during the condition of rest (eyes closed) for each subject group. Note the reduced cerebral perfusion in the dementia patients. RCBF distribution for the depression group and normal subjects was relatively equivalent during the baseline condition.

Note: Collimators 1 to 6 correspond with the frontal lobes; 7 and 8 with the central lobes; 9 to 11 with the temporal lobes; 12 to 14 with the parietal lobes and 15 and 16 with the occipital lobes.

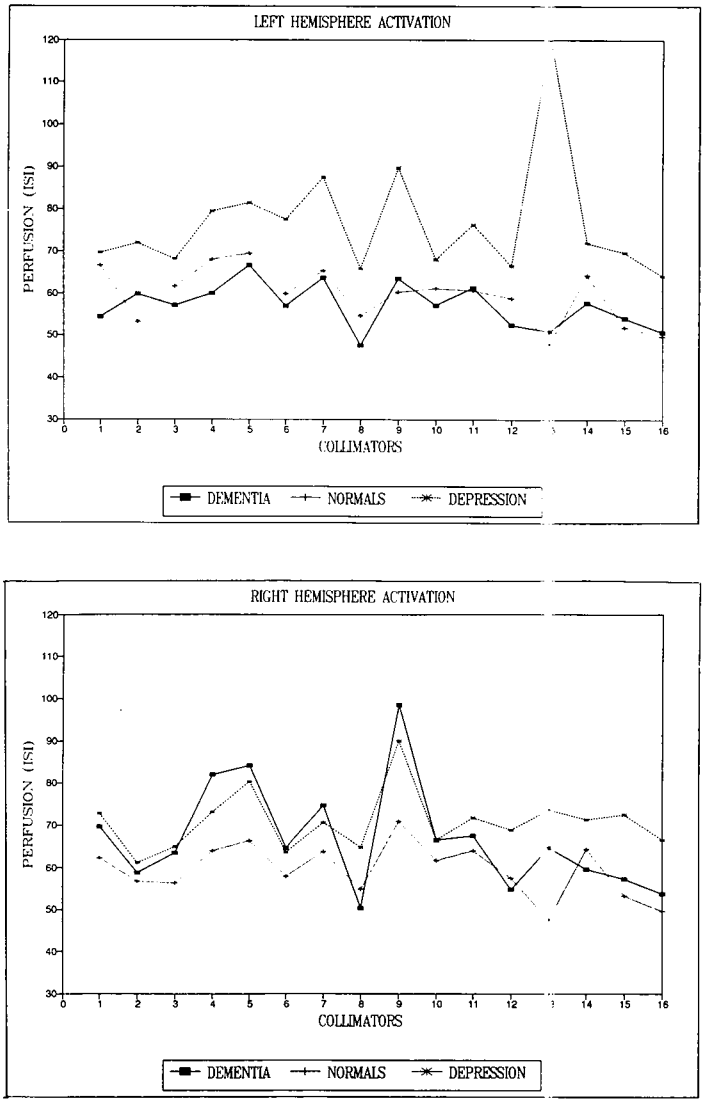

Fig 2 The mean hemispheric distribution of RCBF (ISI) during the verbal recognition memory task for each subject group. In all groups there is increased global perfusion. The dementia patients exhibit marked right hemisphere perfusion, especially in the frontal region, compared to the other subject groups. The depressed patients have greater posterior RCBF than the normals and the dementia subjects. Note: Collimators 1 to 6 correspond with the frontal lobes; 7 and 8 with the central lobes; 9 to 11 with the temporal lobes; 12 to 14 with the parietal lobes and 15 and 16 with the occipital lobes.

of the patients. In essence, there was some consistent correspondence between neuropsychological ratings of regional dysfunction on neuropsychological testings and the findings of regional dysfunction during activation.

With regard to the critical analysis of the RCBF measures of brain function, this is still preliminary, since realistic assessment of any index of cognitive brain function requires examination of the interactions among anxiety, performance and brain function. A consistent association has been found between anxiety and performance – namely that moderate levels of anxiety are associated with better performance than high or low anxiety. Nevertheless integrated analysis of the interrelationships among brain function – psychophysiology – performance has been undertaken only tentatively since the 1960's, and it is our opinion that the heuristic value of previous efforts examining the interrelationships of these measures is minimal – primarily due to a lack of integration of the separate focuses with researchers from the different camps. Despite this, our preliminary group data show significant regional effects. Preliminary assessment of individual patients also suggest that the majority of patients with Alzheimer's dementia either entirely failed to activate or had significantly lower blood flow at rest and significantly higher activity during activation – particularly in the right fronto-temporal regions in this verbal recognition – memory task. Only one of the 10 Alzheimer disease patients had a pattern similar to those of the normal and the depressed groups.

Activation of brain function seems to be a future direction in this field, but it is still in its earliest phases. Some consistent findings are emerging, but the essential methodological point is not to underestimate the difficulty in trying to distinguish independent from dependent variables in brain function – activation task paradigms.

REFERENCES

1. Barclay L, Zemcov A, Blass JP *et al.* Rates of decrease of cerebral blood flow in progressive dementias. Neurology 1984; 34:1555–60.
2. Risberg J. Application of non-traumatic Xenon 133 method in Neuropsychiatry. In: cerebral blood flow and metabolism measurement. Springer-Verlag, Berlin 1985; 72–79.
3. Prohovnik I, Mayoeux R, Sackeim HA, Smith G, Stern Y, Alderson PO. Cerebral perfusion as a diagnostic marker of early Alzheimer's disease. Neurology 1988; 38:931–937.
4. Wechsler D, Wechsler Adult Intelligence Scale - Revised Manual. New York: Psychological Corporation. 1981.
5. Russell EW. Renorming Russell's version of the Wechsler Memory Scale. Journal of Clinical & Experimental Neuropsychology, 1988; 10:235–249.
6. Wechsler D. A standard memory scale for clinical use. Journal of Psychology 1945; 19:87–95.
7. Lezak MD. Neuropsychological assessment. 2nd ed. New York: Oxford University Press, 1983.

EEG MONITORING DURING ANGIOGRAPHIC BALLOON TEST CAROTID OCCLUSION: EXPERIENCE IN SIXTEEN CASES

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SUMMARY

Ipsilateral hemispheric ischaemia related to permanent or temporary arterial occlusion at the time of operation is a potential risk of surgery upon some aneurysms or tumours which involve the internal carotid artery. Presurgical evaluation of the risks of temporary or permanent internal carotid artery occlusion may help predict patients in these circumstances at risk of stroke. Balloon test occlusion studies involve the elective preoperative occlusion of the internal carotid artery by a deflatable balloon inserted into the cerebral circulation under angiographic control. We have performed 16 balloon test occlusion studies; 2 subjects developed clinical and electroencephalographic changes when the carotid artery was temporarily occluded, and these changes reverted to normal when the balloon was deflated. The results of the test occlusion studies helped in planning the surgical management of all the subjects involved.

The surgical management of patients with tumours or aneurysms involving the upper cervical, petrous, or intracavernous carotid artery and skull base is limited by the potential for precipitating cerebral infarction during temporary or permanent occlusion of the internal carotid artery (ICA) at the time of operation[1-4]. Presurgical evaluation of the risks of temporary or permanent ICA occlusion is an important step in the therapeutic decision-making process. Balloon test occlusion studies involve the elective preoperative occlusion of the internal carotid artery by a deflatable balloon inserted into the cerebral circulation under angiographic control. This technique may help predict which patients are at risk of stroke if the artery needs to be permanently ligated at operation. A number of direct and indirect techniques allow assessment of cerebral perfusion during the time of balloon inflation. These include stable xenon/CT[1,3], xenon external probe, SPECT[2], EEG monitoring[5], stump and retinal artery pressure measurement, clinical examination and transcranial Doppler[6]. We report our experience of 16 consecutive cases of elective balloon test occlusions of the ICA

monitored by EEG and clinical examination.

METHODS

Sixteen consecutive subjects being considered for surgery which may have involved ligation or occlusion of the ICA were studied from May 1992 to March 1993. Preoperative evaluation included baseline neurological evaluation and CT scanning to determine the extent of the tumour or vascular anomaly, often supplemented by MRI studies. A pre-angiogram EEG, with a minimum of 20 minutes recording using standard bipolar and referential montages was performed in all cases. The conditions for which surgery was considered included ICA dissections (4 cases), tumours involving the cavernous sinus (3 cases), arterio-venous malformations (1 case), aneurysms (5 cases) and other pathology (3 cases) (Table 1).

Four vessel angiography was performed, usually in the week prior to the proposed surgery, to evaluate the anatomy of the extra and intracranial vasculature and to determine the potential for collateral flow around the circle of Willis. A 2.0F deflatable balloon catheter was inserted into the common carotid artery, systemic anticoagulation with intravenous heparin begun, and the balloon then advanced into the ICA to the level of the 1st or 2nd cervical vertebral body. Arterial blood pressure was monitored every 2 minutes by an inflatable automated cuff, and oxygen saturation by a finger probe.

The balloon was then inflated. During the period of balloon inflation, the patient was examined clinically using a modified Wada test[7,8] performed at least every 3 minutes. The EEG was recorded continuously during the angiogram and balloon occlusion studies using a Siemens 21 channel machine and standard bipolar montages. The paper speed was 15 mm per second, with the 50 Hz notch filter applied. Filter settings were 0.5 and 30 Hz.

If any persistent neurological or EEG changes were noted, the balloon was immediately deflated, and the patient observed until the deficits cleared and the EEG returned to normal. If no neurological deterioration occurred, the balloon was kept inflated for 20 minutes.

RESULTS

There were 11 female and 5 male subjects. The mean age was 50.8 years, with a range of 17 to 73 years. Table 1 lists the demographic data and the clinical indication for the balloon test occlusion studies. Two patients were anaesthetized and ventilated (maintained with inhalational anaesthetics) during the balloon test occlusion studies; these subjects underwent EEG monitoring alone. Other patients were given intravenous midazolam as required.

In 14 cases, including both ventilated patients, there was no change in the

clinical and/or EEG appearance during the 20 minutes of ICA occlusion. In 2 patients, persistent EEG changes were noted, consisting of an initial reduction of beta activity, followed by persistent low amplitude polymorphic delta activity, and finally reduction in amplitude ipsilateral to the occlusion. The EEG changes appeared at 9 minutes (Case 10) and 17 minutes (Case 15) from the time of balloon inflation. In both cases clinical changes were also noted, but the EEG alterations preceded the clinical changes by 2.0 and 2.3 minutes respectively. The balloon was immediately deflated, and the EEG and clinical signs reverted to normal within 3 minutes, in both cases.

Twelve patients subsequently underwent operative procedures (Table 1). There were no postoperative strokes. Patient 15, who failed her balloon occlusion test, did not undergo an operation and has been managed medically. Subject 10, who failed the balloon occlusion at 9 minutes, underwent a Dolenc procedure with elective saphenous vein bypass using thiopentone-induced EEG-monitored intraoperative burst suppression coma, and made a full clinical recovery.

DISCUSSION

The development of interventional balloon catheter techniques[3,9,10,11] has expanded the therapeutic choices available to treat intracavernous and giant carotid aneurysms and tumours of the skull base. Surgical approaches now include clipping the vessel directly, cavernous sinus trapping with saphenous-vein bypass grafting, balloon occlusion of the ICA proximal to the aneurysm and balloon embolization of the aneurysm lumen[3,9,10,12]. As part of the process for determining the optimal approach for each patient, the balloon occlusion test is performed routinely in some centres[2,4]. The clinical balloon occlusion test is a version of the Matas test[13]. A number of direct and indirect techniques are available that allow assessment of cerebral perfusion in association with the balloon occlusion. In cerebral test occlusion studies, the methodology must be sensitive enough to detect those who will have inadequate collateral circulation, and thus spare them from hemiplegia. At the same time, the method must not be too sensitive, or those who might benefit from carotid occlusion may be unnecessarily rejected for surgery[6].

Techniques used to gain an indication of cerebral blood flow (CBF) include stable xenon/CT[1,3], SPECT[2,6], transcranial Doppler (TCD)[6], the measurement of stump pressures, somatosensory evoked potential recording[3], compressed spectral array[14] and EEG[5]. The latter 3 methods identify patients whose CBF falls below 17 to 20 ml/100 gm/min with carotid occlusion[3]. Quantitative studies, and

experience with monitoring of carotid endarterectomy indicate that there is a high risk of cerebral infarction if cerebral flow is reduced below 20ml/100 gm/minute[15].

Xenon enhanced tomographic (Xe/CT) CBF provides a quantitative measure of flow, and has been used extensively in some centres to identify the subset of patients whose CBF falls to between 20 and 30 ml/100 gm/minute. This subgroup comprised 15% of over 300 patients studied and its members were felt by Linskey *et al*[3] to be at moderate risk of stroke if the ICA was permanently ligated, but at low risk if the occlusion was temporary. While the measurement of CBF with xenon-CT has some theoretical advantages, it is technically demanding, costly and carries a 4% risk of complications (including arterial dissection)[6]. HMPAO-SPECT, while more readily available, does not provide a quantitative measure of cerebral blood flow, and its value has been questioned[1,16].

EEG monitoring is readily available, and experience gained in carotid endarterectomy monitoring can be applied to balloon test occlusions. In Case 10, in whom EEG and clinical changes occurred during the preoperative test occlusion, the subsequent operative technique was modified. A bypass procedure with thiopentone induced burst-suppression coma was used electively. Case 15 was managed medically.

Pretreatment evaluation of patients considered for surgical treatment of aneurysms or tumours of the ICA or skull base has become an important part of the therapeutic decision making process at our, and other institutions[3,6]. EEG monitoring during elective balloon test occlusion studies appears to identify a group thought to be at high risk of stroke if the ipsilateral ICA is occluded.

Table 1 Details of patients studied

Case Number	Age years	Sex	Artery Occluded	EEG	Reason for Test Occlusion
1	67	M	L.ICA	Stable	Dural AV fistula
2	17	M	R.ICA	Stable	Pituitary tumour
3	58	M	R.ICA	Stable	R. cavernous sinus aneurysm
4	55	F	L.ICA	Stable	Giant carotid aneurysm
5	40	F	L.ICA	Stable	Tear in ICA, ventilated
6	62	F	L.ICA	Stable	L. ICA aneurysm (N.O)
7	40	F	L.ICA	Stable	L. ICA dissection
8	46	F	R.ICA	Stable	Traumatic dissection (N.O)
9	46	F	L.ICA	Stable	Bilateral opth. A. aneurysm
10	63	M	R.ICA	Delta, Clinical	Cavernous sinus meningioma
11	33	F	L.ICA	Stable	L. ICA dissection
12	52	M	L.ICA	Stable	Bilateral intracavernous meningiomas
13	64	F	L.ICA	Stable	Intracavernous aneurysm repair
14	44	F	R.ICA	Stable	R. ICA dissection
15	73	F	R.ICA	Delta, Clinical	Cavernous meningioma (N.O)
16	53	F	R.ICA	Stable	Giant aneurysm (N.O)

N.O = not operated upon, R. = right, L. = left.

REFERENCES

1. Yonas H, Linskey M, Johnson DW *et al.* Internal carotid balloon test occlusion does require quantitative CBF. American Journal of Neuroradiology 1992; 13:1147–1148.
2. Moody EB, Dawson RC, Sandler MP. Tc-HMPAO SPECT imaging in interventional neuroradiology: validation of balloon test occlusion. American Journal of Neuroradiology 1991; 12:1043–1044.
3. Linskey ME, Sekhar LN, Horton JA, Hirsch WL, Yonas H. Aneurysms of the intracavernous carotid artery: a multidisciplinary approach to treatment. Journal of Neurosurgery 1991; 75:525–534.
4. Steed DL, Webster MW, De Vries EJ *et al.* Clinical observations on the effect of carotid artery occlusion on cerebral blood flow mapped by xenon computed tomography and its correlation with carotid artery back pressure. Journal of Vascular Surgery 1990; 11:38–44.
5. Andrews JC, Valavanis A, Fisch U. Management of the internal carotid artery in surgery of the skull base. Laryngoscope 1989; 99:1224–1229.
6. Monsein L. Assessment of collateral cerebral circulation during test occlusion of the carotid artery. American Journal of Neuroradiology 1992; 13:1148–1149.
7. Wada J. A new method for the determination of the side of cerebral speech dominance: a preliminary report on the intracarotid injection of sodium amytal in man. Igaku to Seibutsugaku 1949; 14:221–222.
8. Peterson RC, Sharbrough FW, Jack CR. Intracarotid amobarbital testing. In: Wyllie E (ed). The treatment of the epilepsies: principles and practice. Philadelphia: Lea & Febinger, 1993; 1051–1061.
9. Berenstein A, Ransohoff J, Kupersmith M *et al.* Transvascular treatment of giant aneurysms of the cavernous carotid and vertebral arteries. Functional investigation and embolization. Surgical Neurology 1984; 21:3–12.
10. Higashida RT, Halbach VV, Dowd C *et al.* Endovascular detachable balloon embolization therapy of cavernous carotid artery aneurysms: results in 87 cases. Journal of Neurosurgery 1990; 72:857–863.
11. Debrun G, Fox A, Drake C *et al.* Giant unclipped aneurysms: treatment by detachable balloons. American Journal of Neuroradiology 1981; 2:167–173.
12. Fox AJ, Vinuela F, Pelz DM *et al.* Use of detachable balloons for proximal artery occlusion in the treatment of unclippable cerebral aneurysms. Journal of Neurosurgery 1987; 66:40–46.
13. Matas R. Testing the efficiency of the collateral circulation as a preliminary to the occlusion of the great surgical arteries. Journal of the American Medical Association 1914; 63:1441–1447.
14. Morioka M, Matsushima T, Fujii K *et al.* Balloon test occlusion of the internal carotid artery with monitoring of compressed spectral arrays (CSAs) of electroencephalogram. Acta Neurochirugica 1989; 101:29–34.
15. Daube JR, Harper CM, Litchey WJ, Sharbrough FW. Intraoperative monitoring. In: Daly DD, Pedley TA (eds). Current practice of electroencephalography. New York: Raven, 1990; 741–776.
16. Frackowiak R. Forum. American Journal of Neuroradiology 1992; 13:1151–1152.

BASILAR ARTERY OCCLUSION FOLLOWING YOGA EXERCISE : A CASE REPORT

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SUMMARY

Basilar artery occlusion developed in a 34 year old woman 2 months after adopting unusual neck postures during yoga practice. On angiography, her basilar artery was filled with intraluminal clot while the vertebral arteries were normal. We postulate that a severe reduction in blood flow and possibly an intimal tear triggered thrombosis of the vertebral artery and that the final stroke mechanism was artery-to-artery embolism.

Posterior circulation stroke following neck rotation or injury is well documented[1]. Most cases have occurred after chiropractic manipulation. However 2 have developed vertebral artery occlusion following yoga exercises, both being young patients (aged 25 and 28 years, respectively) with confirmed left vertebral artery occlusion on angiography[2,3]. The vertebral artery, lying in close proximity to the atlas, axis and atlanto-occipital membrane, may be liable to injury during neck rotation in certain yoga exercises. We now report another case of such a complication associated with yoga, the clinical picture and pathogenesis being distinctive.

CASE REPORT

A 34-year old female clerk without any previous history of migraine, hypertension, diabetes, smoking or use of oral contraceptives was admitted to hospital because of sudden left-sided weakness. Four months prior to admission, she started to practise yoga exercise for self-relaxation. Two months later, after standing on her head for 5 minutes, she developed a sudden sharp pain in the neck and then numbness of the right hand. Cervical spondylosis was diagnosed by an orthopaedic surgeon but her symptoms did not improve after physiotherapy and neck traction. She then experienced frequent spontaneous vertiginous attacks associated with nausea, occurring up to several times a day; the attacks became more severe on the day of admission. On examination she was alert but disorientated with a severe left hemiplegia and hemianaesthesia. There was marked horizontal and vertical nystagmus but her visual fields and speech were intact. The clinical diagnosis of a posterior fossa stroke was made.

Computed tomography (CT) of the brain performed on the 1st day and 3rd day yielded normal findings. Magnetic resonance imaging (MRI) of the brain on the 5th day revealed normal signals on T_1 weighted images but a well defined area of increased intensity in the right pons, right thalamus and in a small part of the right occipital lobe on T_2 weighted images, consistent with multiple infarcts (Fig 1). Vertebral angiography showed that both vertebral arteries were patent with normal outlines in the cervical portion and without significant asymmetry between the two sides. However, the terminal basilar artery was occluded and both posterior cerebral arteries (PCA) were not opacified (Fig 2). The right carotid angiogram was normal and there was adequate filling of the right PCA. Penetrating blood vessels to the pons were too small to be visualised. A left carotid angiogram was not performed.

The following investigations for risk factors for stroke produced normal or negative results: routine blood picture and serum biochemistry, ESR, fasting blood glucose, cholesterol and triglycerides concentrations, VDRL, serum protein electrophoresis, immune marker screening, anticardiolipin antibody, lupus anticoagulant, coagulation profile including protein C, protein S and antithrombin III, radiographs of chest and cervical spine, electrocardiogram and 2-D echocardiogram.

The patient's conscious level deteriorated after admission and a heparin infusion was given for 6 days, after which ticlopidine was introduced for secondary stroke prophylaxis. With intensive physiotherapy, her left upper and lower limbs gradually recovered to full strength. Apart from mild clumsiness of her left hand, she was normal 1 year after the ictus.

DISCUSSION

More than 50 patients with posterior circulation stroke have been described following neck rotation or neck injury[1]. Most were young patients without pre-existing vascular disease, cervical fractures or dislocations. The clinical syndromes usually occurred within 48 hours of the neck injury and included pure motor stroke[4], Wallenberg's syndrome[5], fatal brain stem infarction[6], `locked in' and occipital lobe syndromes[7]. Vertebral artery dissection is an important pathogenetic mechanism causing such stroke and most likely to occur at C_1–C_2 level, where there is maximal stress following mechanical stretch. Brain stem ischaemia frequently follows if there is subsequent stenosis or occlusion of the vertebral artery, intraluminal thrombosis or artery-to-artery embolism. However, thrombus formation without concomitant dissection has also been reported following intimal tears in association with abrupt changes in head position[8-10]. At post-mortem or on angiogram, intraluminal clot has been found in the basilar artery or PCA, without vertebral artery involvement. The present case further illustrates such a possibility and in addition highlights several atypical features. The long delay (2 months) between the presumed injury and the stroke, with

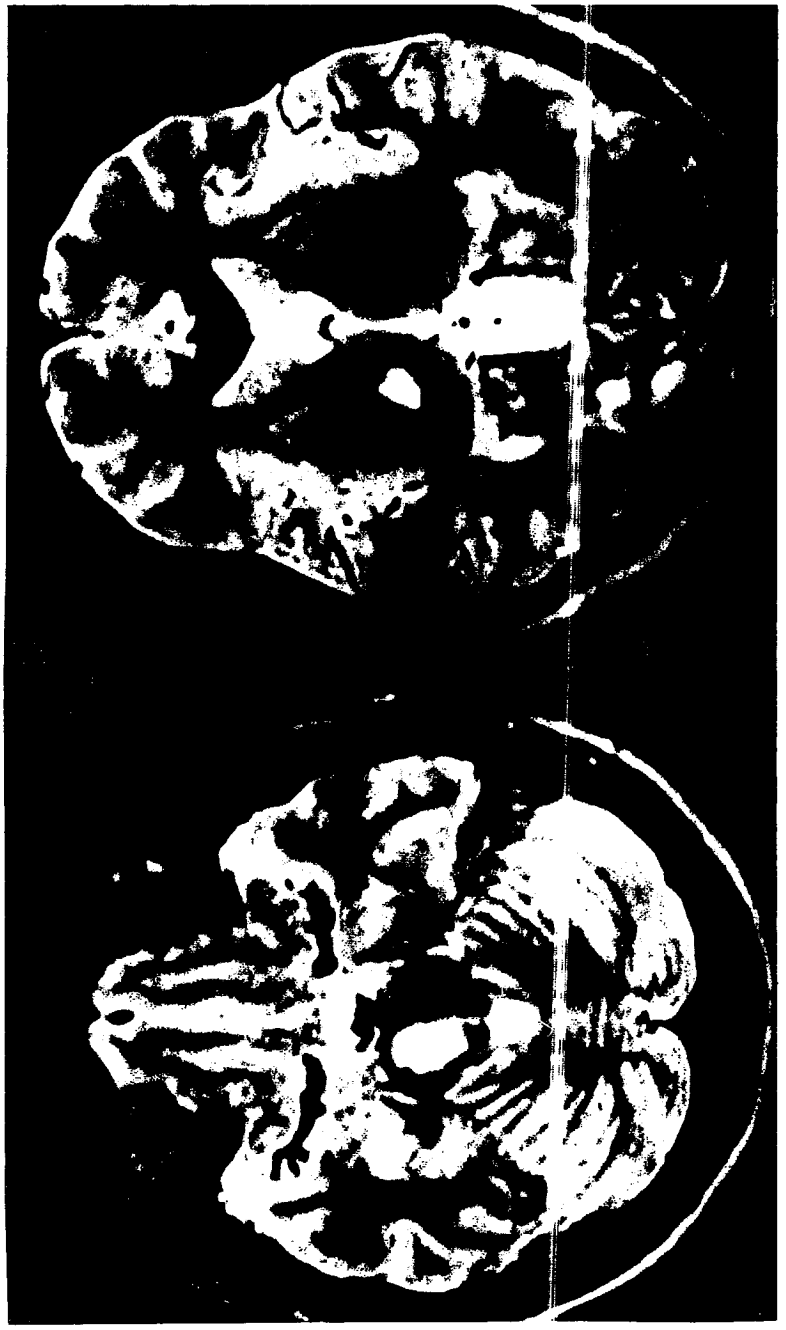

Fig 1 Spin-echo T2 weighted 3 mm axial and cuts. Note the marked hyperintensity of the right side of the pons, the right lateral thalamus and the medial aprt of right occipital lobe.

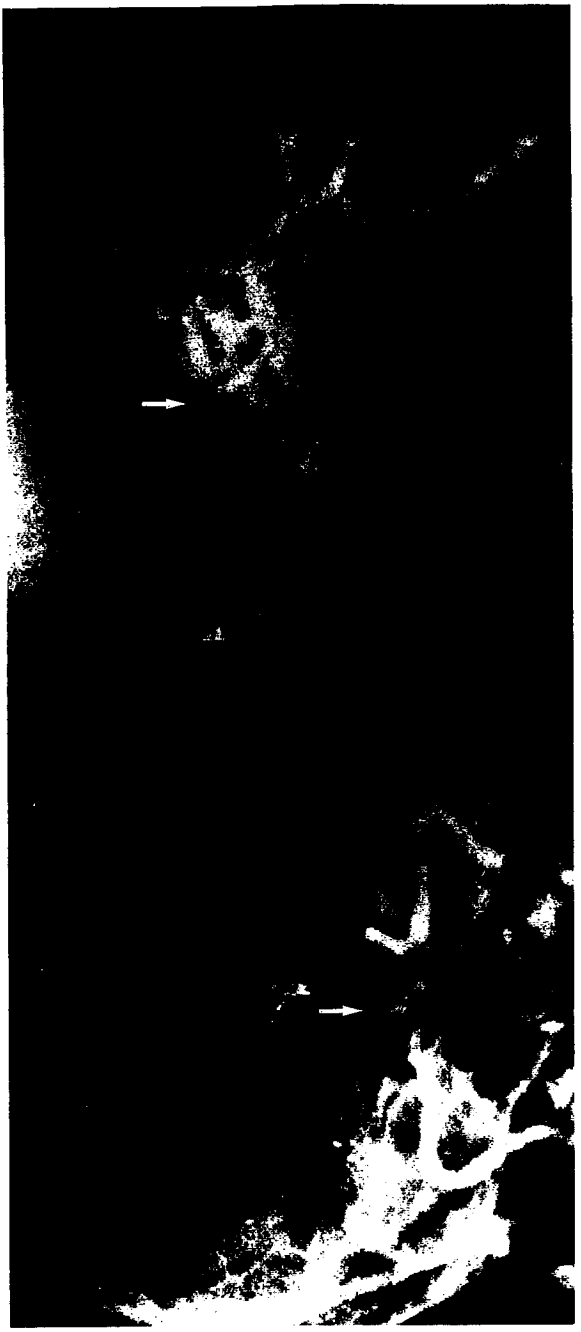

Fig 2 Right vertebral angiogram (a) anteroposterior and (b) lateral views. Note the absence of terminal branching of the basilar artery (arrows).

arteries being seen on angiogram, seem to make a casual relationship difficult to establish, but the unusual neck movement related to the onset of neck pain is the only identifiable factor in the pathogenesis. This is further supported by the absence of any history of transient ischaemic attacks (TIA) before commencing yoga, the presence of a persistent TIA (numbness of the right hand) upon neck rotation, the continuation of TIAs for 2 months after yoga, and the absence of any other risk factors for stroke, including any predisposing osseous or arterial lesion. Although we cannot exclude the possibility of occult cardiogenic embolism, this would be very unlikely as a coincidental event to her yoga practice. The onset of her symptoms at the time of inverted standing might raise the possibility of a paradoxical embolus through a patent foramen ovale in view of the increased probability of a right to left shunt in these circumstances. However this is very unlikely in the absence of any evidence of pulmonary embolism or deep vein thrombosis and it fails to explain the recurrent TIAs which occurred after yoga exercise had been stopped.

Neck rotations in the physiological range have been shown by various studies to significantly reduce the blood flow in the vertebrobasilar system; the mechanisms have been reviewed by Sherman[10]. We postulate the pathogenesis of the stroke in our patient as follows. The unusual neck posture may have produced a dramatic reduction of blood flow to the posterior circulation and possibly caused an intimal lesion to one of the vertebral arteries, which may have become the site of local thrombosis. The concomitant severe neck pain is suggestive of vertebral artery dissection. However, this can neither be confirmed nor excluded by the normal angiographic appearance 2 months later, since this exceptionally long interval may have allowed sufficient time for spontaneous healing. The frequent attacks of vertigo preceding the ictus were likely to have been multiple TIAs which may have been the result of clot propagation, progressive vascular stenosis or minute embolisation. The final stroke event was due to clot disintegration with embolism and subsequent occlusion at the top of the basilar artery and some of its terminal branches. This pathogenetic mechanism is consistent with the clinical and radiological picture. Anatomically, infarction of the right medial pons and anterolateral thalamus can be produced by branch occlusion of the basilar artery (thalamopolar and paramedian branches). Similarly, medial occipital infarction may arise after occlusion of the internal occipital branch of the PCA. This type of artery-to-artery embolism has been documented in both radiological and pathological studies[11,12]. Recognition of this mechanism and its differentiation from lacunar infarction is important in determining the correct management.

Another unusual feature in the present case is the absence of any tegmental

signs and 3rd cranial nerve palsy which frequently follow occlusion of the PCA or the `top of the basilar' syndrome. A possible explanation would be an adequate blood supply via the carotid system which probably limited the thrombosis of the right PCA. Such a collateral circulation is likely to have developed, given the long delay from the neck insult to the ictal onset, and it may have been an important contributing factor to her satisfactory outcome.

Although yoga has a beneficial role in decreasing blood coagulability and stress related disorders[13], its unsupervised practice may be potentially dangerous or even lethal, especially if the yoga manoeuvres involve the neck.

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REFERENCES

1. Caplan LR. Vertebrobasilar occlusive disease. In: Barnett HJM, Mohr JP, Stein BM, Yatsu FM (eds): Stroke: pathophysiology, diagnosis and management. New York, Churchill Livingstone, pp 560–561.
2. Hanus SH, Homer TD, Hater DH. Vertebral artery occlusion complicating yoga exercise. Archives of Neurology 1977; 34:574–575.
3. Nagler W. Vertebral artery obstruction by hyperextension of the neck: report of three cases. Archives of Physical and Medical Rehabilitation 1973; 54:237–240.
4. Phillips SJ, Maloney WJ, Gray J. Pure motor stroke due to vertebral artery dissection. Canadian Journal of Neurological Science 1989; 16:348–351.
5. Frumkin LR, Baloh RW. Wallenberg's syndrome following neck manipulation. Neurology 1990; 40:611–615.
6. Dunne JW, Conacher GN, Khangure M *et al.* Dissecting aneurysms of the vertebral arteries following cervical manipulation: a case report. Journal of Neurology, Neurosurgery and Psychiatry 1987; 50:349–353.
7. Frisoni GB, Anzola GP. Neck manipulation and stroke (letter). Neurology 1990; 40:1910.
8. Pratt-Thomas HR, Berger KE. Cerebellar and spinal injuries after chiropractic manipulation. Journal of American Medical Association 1947; 133:600–603.
9. Levine SR, Quint DJ, Pessin MS, Boulos RS, and Welch KMA. Intraluminal clot in the verterbrobasilar circulation: clinical and radiographic features. Neurology 1989; 39:515–522.
10. Sherman DG, Hart RG, Easton JD. Abrupt change in head position and cerebral infarction. Stroke 1981; 12:2–6.
11. Koroshetz WJ, Ropper AH. Artery to artery embolism causing stroke in the posterior circulation. Neurology 1987; 37:292–296.
12. Ferbert A, Bruckmann H, Drummen R. Clinical features of proven basilar artery occlusion. Stroke 1990; 21:1135–1142.
13. Chohan IS, Nayar HS, Thomas P, Geetha NS. Influence of yoga on blood coagulation. Thrombosis Haemostatsis 1984; 51:196–197.

PENTOXIFYLLINE IN THE TREATMENT OF ACUTE ISCHAEMIC STROKE – A REAPPRAISAL IN CHINESE STROKE PATIENTS

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SUMMARY

A double-blind, randomized and placebo-controlled trial was conducted on 110 Chinese patients with ischaemic stroke who were stratified into 2 subtypes (cortical and lacunar infarcts) according to their clinical and CT findings. Treatment was started within 36–48 hours after the stroke onset. Pentoxifylline was administered intravenously in a dose of 600 mg daily for 5 days, together with oral aspirin 150 mg daily. Neurological deficits were scored on admission and at one week. Demographic data were comparable between the treatment and placebo groups. For cortical infarcts, there were significantly more patients in the placebo group who deteriorated and died than in the treatment group ($p<0.02$). As for the lacunar infarcts, there was no difference between groups in the numbers of patients who improved or deteriorated. Our study shows that the positive effect of pentoxifylline can be demonstrated only in patients with cortical infarction. Early deterioration and mortality were significantly decreased in these patients. The clinical course of lacunar infarction was not affected by pentoxifylline. It is not clear whether aspirin may potentiate the antiplatelet function of pentoxifylline and contribute to its temporary clinical efficacy in this way.

Pentoxifylline (PTX) is a hemorheological agent that acts by increasing red cell deformability[1], inhibiting platelet aggregation[2] and reducing plasma fibrinogen concentrations[3]. The effect of PTX is a reduction of the whole blood viscosity and an improvement of microcirculatory flow and tissue oxygenation. In experimental studies, PTX has been shown to be effective in reducing vasogenic oedema[4] and it prevents ischaemic deterioration of cerebral metabolism and death[5]. In clinical studies, PTX has been reported to have temporary beneficial effects with improvement of neurological scores in the first few days of ischaemic stroke[6]. However, its therapeutic effect in the different subtypes of ischaemic stroke is not known although patients with severe deficits at the onset seem to benefit most from PTX treatment[6]. There is also no information in the literature concerning the clinical efficacy of PTX in Chinese patients who are

known to have a different ischaemic stroke pattern to Western patients[7,8].

The aim of the present study was to examine the effect of PTX on the different subtypes of acute ischaemic stroke in Hong Kong Chinese patients.

METHODS

A total of 110 Chinese stroke patients who fulfilled the following selection criteria was recruited for the study: no previous history of stroke or other neurological diseases; an onset of symptoms of stroke less than 48 hours previously; no clinical evidence of a transient ischaemic attack; exclusion of cerebral haemorrhage by CT, and no significant systemic diseases present. The patients were divided into 2 groups according to their clinical and CT findings: there were 41 patients with cortical infarcts and 69 patients with lacunar infarcts; they were then randomized into PTX and placebo treatment groups in a double-blind fashion.

Treatment was started within 36 to 48 hours after the onset of the stroke. Patients in the PTX group, which consisted of 22 patients with cortical infarcts and 36 patients with lacunar infarcts, received a continuous intravenous infusion of 600 mg PTX daily for 5 days together with oral aspirin 150 mg daily. Patients in the placebo group, which consisted of 19 patients with cortical infarcts and 33 patients with lacunar infarcts, received isotonic saline and matching placebo injections over same period of time, together with oral aspirin 150 mg daily.

The neurological deficits of each patient were scored in relation to the level of consciousness, higher cortical function, motor function and cranial nerve function, the maximum score achievable being 58. The scoring system was adopted from the Scandinavian Stroke Study Group with modification, viz. combining the prognostic and long term scores into one scoring system[9] (Table 1). The effect of treatment was assessed by the same investigator (YWC) by comparing the neurological deficit score on entry and at one week. Changes in score at one week as compared with entry scores allowed patients to be labelled as 'improved', 'unchanged' or 'deteriorated'. Chi-square testing with Yates' correction was used to compare the numbers of patients who improved, deteriorated or died at one week between the PTX and the placebo (i.e. aspirin alone) groups.

RESULTS

Demographic data, initial neurological deficit scores and sites of lesions were comparable between the PTX and placebo groups for patients with cortical (Table 2) and lacunar infarcts (Table 3).

Table 1 **Neurological deficit scoring (modified from Scandinavia Stroke Study Group) 1986**

	Score
Consciousness	
fully conscious	6
somnolent, can be awakened to full consciousness	4
reacts to verbal command, but is not fully conscious	2
Eye movements	
no gaze palsy	4
gaze palsy present	2
conjugate eye deviation	0
Arm, motor power	
raises arm with normal strength	6
raises arm with reduced strength	5
raises arm with flexion in elbow	4
can move, but not against gravity	2
paralysis	0
Hand, motor power	
normal strength	6
reduced strength in full range	4
some moevement, fingertips do not reach palm	2
paralysis	0
Leg, motor power	
normal strength	6
raises straight leg with reduced strength	5
raises leg with flexion of knee	4
can move, but not against gravity	2
paralysis	0
Orientation	
correct for time, place and person	6
2 of these	4
1 of these	2
completely disorientated	0
Speech	
no aphasia	10
limited vocabulary or incoherent speech	6
more than yes/no, but not longer sentences	3
only yes/no, or less	0
Facial palsy	
none/dubious	2
present	0
Gait	
walks 5 m without aids	12
walks with aids	9
walks with help of another person	6
sits without support	3
bedridden/wheelchair	0
Maximal score	58

Table 2 Demographic and clinical characteristics of 41 patients with cortical infarction

Characteristics	PTX group (n = 22)	Placebo group (n = 19)
Age (yrs)		
range	61–86	52–81
mean	70.7	69.1
Male: Female	15:7	14:5
Deficit score (initial)	21 ± 6	19 ± 8
Deficit score (1 week)	35 ± 7	30 ± 5*
Site of lesions		
Parietal	20	17
Frontal	1	1
Temporal	1	1
Occipital	0	0

PTX = pentoxifylline *Deficit score at 1 week for survivors only

Table 3 Demographic and clinical characteristics of 69 patients with lacunar infarction

Characteristics	PTX group (n = 36)	Placebo group (n = 33)
Age (yrs)		
range	50–88	52–81
mean	68.1	66.9
Male: Female	23:13	21:12
Deficit score (initial)	48 ± 3	44 ± 6
Deficit score (1 week)	51 ± 4	49 ± 5
Site of lesions		
Capsular	21	14
Corona radiata	5	3
Thalamic	2	2
No CT lesion	8	14

PTX = pentoxifylline

The effect of PTX on the clinical outcome at one week is showed in Table 4. For cortical infarcts, there was no difference between groups in the numbers of patients who improved, but there were significantly more patients in the placebo group who deteriorated and died ($p < 0.02$). Death was considered to be neurological in nature for all these 8 cases, with cerebral oedema and extension of the infarct being confirmed by CT scan in 6 and by postmortem studies in 2. The initial neurological scores in these 8 patients ranged from 15 to 22. None had electrocardiographic evidence of myocardial infarction before death. For lacunar infarcts, there was no difference between groups in the numbers of patients who improved or deteriorated.

Table 4 Outcome of 110 stroke patients at one week

Characteristics	PTX group (n=58)	Placebo group (n=52)
Cortical infarct		
Improved	12 (54.5%)	10 (52.6%)
Unchanged	7 (31.8%)	1 (5.4%)
Deteriorated	3 (13.7%)	8 (42.0%)*
Lacunar infarct		
Improved	32 (89.0%)	31 (93.9%)
Unchanged	2 (5.5%)	2 (6.1%)
Deteriorated	2 (5.5%)	0

*$P < 0.02$ (All 8 patients died).

DISCUSSION

The treatment of acute ischaemic stroke has been unsatisfactory so far, though new therapies such as haemodilution, thrombolysis and calcium channel blockers are being pursued vigorously. Numerous experimental[1-5] and clinical studies[6,10,11] have been performed to try to establish the efficacy of PTX in the treatment of acute ischaemic stroke. The largest and most convincing study is the one reported by the Pentoxifylline Study Group[6] which concluded that PTX improved neurological deficit scores during the first few days of infusion therapy (3 days), especially in a subset of patients with severe deficits at admission. However, this initial benefit was not maintained in the subsequent oral treatment period (day 4 to day 28). This has been attributed to reduced PTX plasma levels following oral administration of the drug[11].

In the present study, PTX (600 mg daily) was infused continuously for 5 days rather than 3 days, and the neurological deficits were separately scored for the 2 ischaemic subtypes, viz., cortical and lacunar infarction, in patients. The importance of analysing the treatment effect according to the brain ischaemia subtypes lies in the fact that lacunar infarction usually runs a more favourable course than cortical infarction, and the therapeutic effect may be diluted if both subtypes are analysed together. Another more important implication is that lacunar infarction is more prevalent in Chinese stroke patients than in Caucasian stroke patients; lacunar infarction was present in 30% of ischaemic strokes in a local study[7] in contrast to 12.2% in a representative US study[13]. This is related to a racial difference in the distribution of occulsive cerebovascular lesions, with the Chinese having more intracranial lesions and the whites more extracranial[8]. It follows that one should be cautious in interpreting studies on stroke patients of different racial origins.

Our study confirms the early beneficial effect of PTX on acute ischaemic stroke, but only in that early clinical deterioration and death are significantly decreased in patients with cortical infarcts. The clinical course of patients with lacunar infarcts, which as a whole have a better neurological outcome, is not altered by PTX treatment. Our main concern is to avoid delay in the initiation of therapy which may undermine the beneficial effect of PTX. The late entry of our patient into the treatment protocol was an inevitable consequence of the delayed hospital admission of most of our stroke patients, especially the lacunar stroke patients who tend to seek advice late because of their mild degree of disability. Nevertheless, we believe that any benefits determined by the present study should be genuine, given that the therapy was not administered within the optimum periods. Our other concern depends on the relatively small numbers of patients recruited to the study. We therefore made no efforts to compare the absolute value of the deficit, but took mortality as the end-point and compared the numbers of deaths between the placebo and treatment groups.

A recent study has observed a lack of efficacy of hemodilution in acute ischaemic stroke[14] and has raised doubts about the therapeutic potential of haemorheological agents in ischaemic stroke. Being a methylxanthine derivative, PTX has in addition to its haemorheologic effects, some mild vasodilator, platelet inhibitory, and central nervous stimulant properties. Aspirin was used together with PTX in our study, and it is not clear whether this may potentiate the antiplatelet function of PTX and thus contribute to the temporary clinical efficacy.

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REFERENCES

1. Ehrly AM. The effect of pentoxifylline on the deformability of erythrocytes and on the muscular oxygen pressure in patients with chronic arterial disease. Journal of Medicine 1979; 10:331–338.
2. Weithmann KU. Reduced platelet aggregation by pentoxifylline stimulated prostacyclin release. Vasa 1981; 10:249–252.
3. Smud R, Sermukslis B, Kartin D. Changes in blood viscosity induced by pentoxifylline. Pharmatherapeutica 1976; 1:229–233.
4. Ganser V, Boksay I. Effect of pentoxifylline on cerebral oedema in cats. Neurology 1974; 24:487–493.
5. Hartmann JF, Becker RA, Cohen MM. Effect of pentoxifylline on cerebral ultrastructure of normal and ischemic gerbils. Neurology 1977; 27:77–84.
6. Hsu CY, Norris JW, Hogan EL *et al.* Pentoxifylline in acute nonhemorrhagic stroke – a randomized, placebo-controlled, double-blind trial. Stroke 1988; 19:716–722.
7. Huang CY, Chan EL, Yu FL, Woo E, Chin D. Cerebrovascular disease in Hong Kong Chinese. Stroke 1990; 21:230–235.
8. Feldmann E, Daneault N, Kwan E *et al.* Chinese-white differences in the distribution of occlusive cerebrovascular disease. Neurology 1990; 40:1541–1545.
9. Scandinavian Stroke Study Group. Multicenter trial of hemodilution in ischemic stroke – background and study protocol. Stroke 1985; 16:885–890.
10. Ott E, Lechner H, Fazekas F. Hemorheological effects of pentoxifylline on disturbed flow behavior of blood in patients with cerebrovascular insufficiency. European Neurology 1983; 22(suppl 1):105–107.
11. Herskovits E, Vazquez A, Famulari A *et al.* Randomized trial of pentoxifylline versus acetylsalicylic acid plus dipyridamole in preventing transient ischaemic attacks. Lancet 1981; 1:966–968.
12. Hsu CY, Savitsky JP. Pentoxifylline alters the natural course of acute nonhemorrhagic stroke. Stroke 1990; 21:152.
13. Kunitz SC, Gross CR, Heyman A *et al.* The Pilot Stroke Data Bank: definition, design, and data. Stroke 1984; 15:740–746.
14. Scandinavian Stroke Study Group: Multicenter trial of hemodilution in acute ischaemic stroke. I. Results in total patient population. Stroke 1987; 18:691–699.

SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY IN INTRACTABLE INFANTILE SEIZURES

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SUMMARY

We aimed to determine the site of ictal foci and the pathogenesis of seizures in 4 infants with intractable seizures. The patients were studied using simultaneous video and electroencephalographic (EEG) monitoring, structural studies and ictal and interictal single photon emission computed tomography (SPECT). Ictal neurophysiology showed multifocal seizure propagation in Patients 1 and 2 and generalised abnormal electrical patterns in Patients 2, 3 and 4. Magnetic resonance imaging (MRI) demonstrated a focal abnormality in Patient 4. SPECT studies showed focal or multifocal increased uptake in 3 subjects (Patients 1,3,4) and increased uptake in the thalamic and basal ganglia regions of 2 subjects (Patients 2,3). SPECT studies contributed to an understanding of the pathogenesis of seizure initiation and propagation in the 4 patients studied.

Intractable infantile seizures are frequently difficult to classify and their pathogenesis is often unclear. Single photon emission computed tomography (SPECT) has been shown to be an invaluable tool in the localisation of epileptic foci in adult patients with complex partial seizures (CPS) originating in the temporal lobe[1,2] and a good correlation with other modalities has been shown[3]. There is limited work in other seizure disorders, particularly those involving children[4,5]. We studied 4 patients with intractable seizures in infancy using SPECT, seeking to localise active epileptogenic foci. We correlated these data with those gained from structural studies and simultaneous video electro-encephalographic (EEG) monitoring.

METHODOLOGY

The patient cohort was 4 infants with intractable seizures. Each was extensively investigated (Table 1). Polygraphic EEGs and video images were recorded simultaneously on a La Mont Video Patient Monitoring System (Medical Systems International) with the positioning of electrodes based on a modification of the 10–20 system[6]. A detailed clinical description of each seizure was documented and the seizures were classified with reference to Volpe[7], Mizrahi and Kellaway[8] and the revised classification of Epilepsies and Epileptic Syndromes[9]. The simultaneous electrical pattern was analysed on paper printout noting, particularly, the localisation of the seizure onset and the evolution of additional foci.

SPECT studies

Two studies were performed on each patient. An interictal scan was performed when the infant was not having a clinical seizure, though simultaneous EEG monitoring was not carried out. Freeze dried hexamethyl propylene amine oxime (Exametazime, Amersham International) was reconstituted with technetium – 99m in 5 mls of saline and injected into a peripheral vein. The patient was then scanned.

The seizure for which the ictal scan was carried out was clinically identical to that recorded previously on video EEG monitoring in patients 1, 2 and 3. Patient 4 had video EEG monitoring carried out simultaneously to the ictal SPECT scan. Patients 1 and 3 were injected during the clinical seizure and patients 2 and 4 during a series of infantile spasms. Intravenous diazepam was used to control seizures at the time of scanning in patients 1 and 3. SPECT imaging was performed within 2 hours of injection using a General Electric 400 AC Starcam.

To display the data, a mid-sagittal image was identified and transaxial slices were reconstructed in the plane of a line drawn from the inferior surface of the frontal lobe to the most posterior aspect of the occipital pole. Coronal images were then reconstructed perpendicular to this plane.

RESULTS

The diagnosis, structural findings, interictal EEGs and seizure types recorded on telemetry are shown in Table 1.

Detailed descriptions of the clinical and electric seizures recorded on simultaneous video EEG monitoring are presented below for each patient, in addition to further clinical details.

Table 1 Patient data

Patient	Diagnosis	Structural Study	Interictal EEG	Seizure type	Age – SPECT study (months)
1	Cerebral malformation (Cause unknown)	Diffuse neuronal migration disorder**	Burst-suppression	Tonic	1
2	Chromosome 2 duplication 46,XX, tan dup (2) (q32.1–>q33.3) E I E E[9]	Normal***	Burst-suppression	Tonic, Infantile spasms: Multifocal myoclonus	2
3	Unknown	Mild cerebral atrophy**	Multifocal epileptiform patterns	Multifocal myoclonus	4
4	Cerebral malformation (Cause unknown)	Neuronal migration disorder R hemisphere: pachygyria L temporal cyst**	Multifocal epileptiform patterns R > L	Infantile spasms	12

* Early infantile encephalopathy with burst suppression
** Magnetic resonance imaging
*** Computed tomography

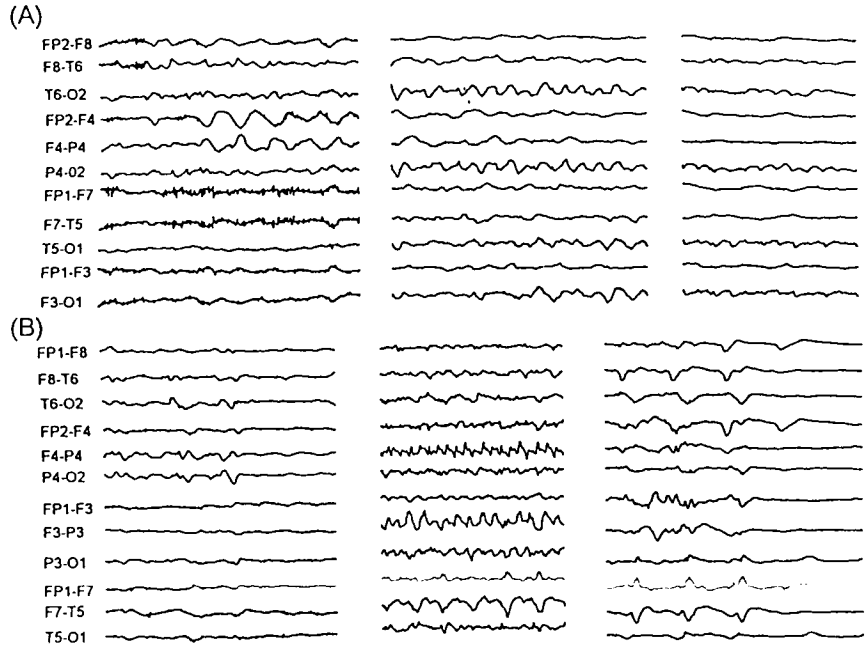

Fig 1 Ictal EEG studies. The figure shows the onset, evolution and termination of seizures.

Patient 1(A) Onset: Right (R) frontal seizure. Middle: Simultaneous independent posterior temporal seizures: R frontal seizure decreasing in amplitude. Termination: Bilateral temporal seizures decreasing in amplitude.

Patient 2(B) Onset: Decrement of left (L) hemisphere activity followed by generalized decrement. Middle: Simultaneous independent R frontoparietal, L frontoparietal and L posterior temporal seizures. Termination: Independent seizures end; generalized decrement.

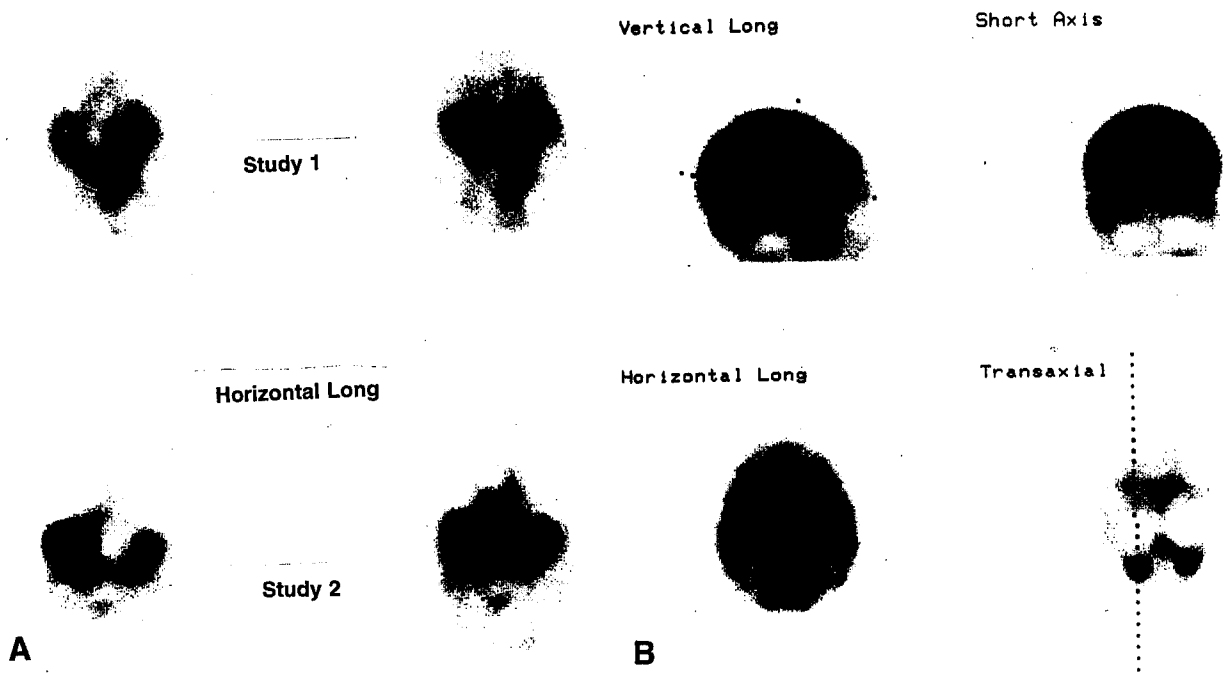

Fig 2 Ictal SPECT findings:

Patient 1 (A) top: Increased uptake R. temporal lobe.
(A) bottom: Increased uptake L. temporal lobe
Patient 2 (B): Increased uptake thalamic nuclei and basal ganglia.
Interictal study: similar to ictal study.

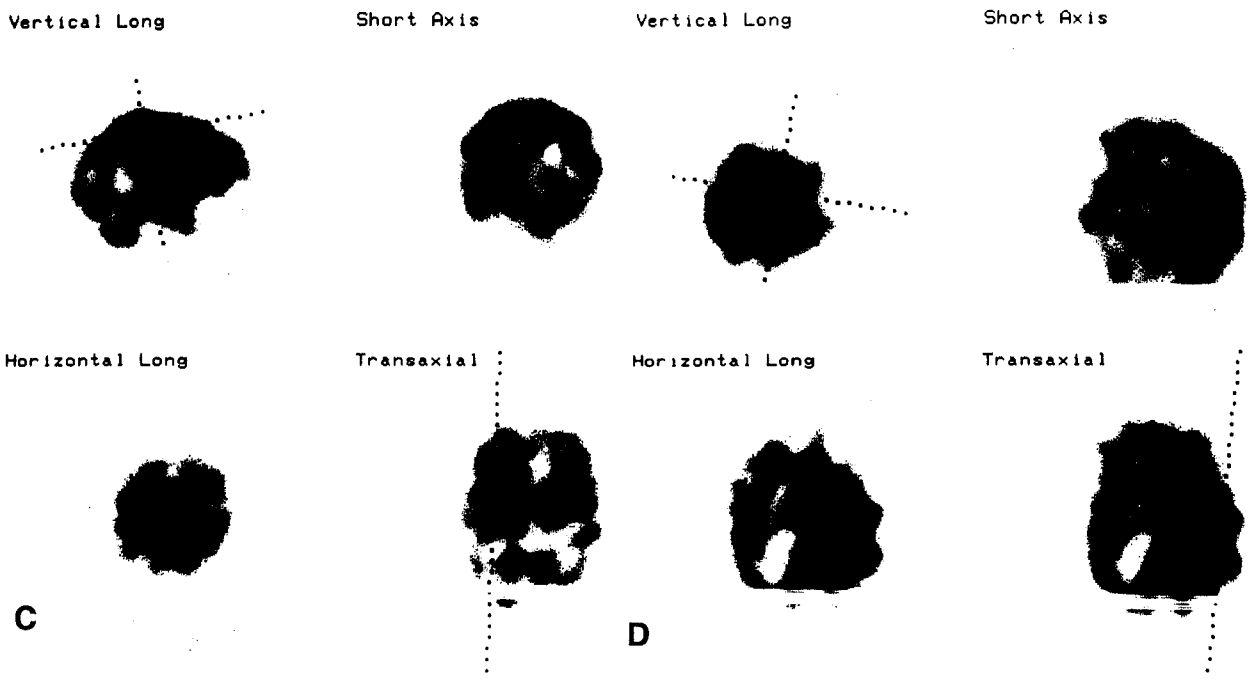

Fig 3 Ictal SPECT findings: Patient 3: (C): Increased uptake R posterior central region.
Interictal study: Symmetrical increased activity in basal ganglia.
Patient 4: (D): Increased uptake R frontal, L frontoparietal, L temporal regions.
Ictal and intericatal showed decreased uptake in R. hemisphere.
Interictal studies not shown in Fig.

Patient 1

Seizures commenced on day 1. The seizures were stereotyped with their major clinical features comprising tonic deviation of the head and eyes, mouthing movements and independent cycling motions of both legs. The seizure pattern at the onset, middle and termination is seen in Fig 1a. This seizure type was investigated in the ictal SPECT study.

The infant made no developmental progress and the seizures were intractable despite use of phenobarbitone, phenytoin, clonazepam, steroids and sodium valproate. He died at 7 weeks of age. A post mortem was not performed.

Patient 2

This patient presented at 2 weeks of age with seizures. A diagnosis of early infantile encephalopathy with suppression bursts was made. She had multiple seizure types – infantile spasms, atypical absences, myoclonic jerks and partial seizures, all refractory to anticonvulsant medication. The clinical sequence of 4 stereotyped events recorded was as follows: tonic posturing of the left leg, tonic posturing of the torso and of the lower limbs, clonic movement of the left leg, a distressing cry, a motionless stare and finally vomiting. The electrical seizure at its onset, middle and termination is seen in Fig 1b. Infantile spasms were also recorded and were associated with a generalized decrement of electrical activity or a decrement of the activity of either hemisphere. Myoclonic jerks were documented, sometimes associated with an electrical decrement and sometimes without a change in the EEG. The ictal SPECT study was carried out during a series of infantile spasms.

The patient continued to have intractable seizures and did not make developmental progress. She died at 3 months of age. At post mortem the brain was macroscopically normal. Astrocytes resembling Alzheimer type 2 cells were seen in the left posterior globus pallidus and right cerebellar hemisphere.

Patient 3

This patient presented with seizures on day 1. These seizures were intractable throughout life despite use of multiple anticonvulsant medications. One seizure of 80 minutes duration was recorded. The infant was unresponsive and movements could not be stimulated or restrained. The initial features of the seizures were myoclonic jerks of the left hand and tonic posturing of the left arm, followed by a complex sequence with myoclonic movements of all limbs, eye deviation, asymmetrical cycling movements of

lower limbs, tonic posturing of the lower limbs and an episode of desaturation. The most prominent clinical feature was multifocal myoclonus. The EEG showed a reduction in voltage and slowing of the electrical pattern. This seizure type was studied with ictal SPECT.

The patient was severely delayed developmentally at the time of his death at 10 months of age. A post mortem was not carried out.

Patient 4

This subject presented with left sided simple partial motor seizures at 2 months of age, complex partial seizures with dystonic posturing of the left arm evolved and at 3 months, infantile spasms appeared. The latter seizure type was studied with SPECT and simultaneous video EEG telemetry. The ictal electrical pattern recorded at the time of SPECT showed a generalised attenuation, with greater suppression over the left hemisphere.

The patient remained severely delayed developmentally with a left hemiplegia and intractable infantile spasms at 12 months of age, despite use of multiple anticonvulsant medications.

The ictal SPECT findings are shown in Fig 2 and the interictal findings are recorded in the legend. The timing of the SPECT study is recorded in Table 1. As Patients 1 and 2 were having multiple seizures, interictal studies may have occurred at the time of electrical or subtle clinically unrecognised seizures.

DISCUSSION

All 4 patients had intractable seizures. Multifocal seizure foci were recorded on video EEG monitoring in Patients 1 and 2, a generalised change in Patient 3 and a generalised but asymmetrical change in Patient 4. SPECT demonstrated focal or multifocal cortical increased uptake in Patients 1, 3 and 4. No focus was identified in Patient 2.

EEG monitoring in Patient 1 demonstrated the evolution of independent electrical foci involving both temporal lobes (Fig 1). It is of interest that both non-ictal and ictal SPECT studies showed increased uptake in a temporal lobe. Electrical monitoring at the time of a SPECT study, particularly in a patient having many seizures hourly and with multiple seizure foci, could give information on the electrical stage of the seizure as seen in Fig 1a, and

whether an intended interictal study really occurred at the time of an electrical but sub-clinical seizure. In this patient with a diffuse neuronal migration disorder, multiple regions of the cortex were important in seizure propagation, as seen in both neurophysiological and SPECT studies.

The findings of Patient 2, who was studied during a series of infantile spasms, were similar to the proton emission tomography (PET) results of Chugani[10] who found that 32 of 44 infants with infantile spasms demonstrated increased local cerebral metabolic rates for glucose in the lenticular nuclei. This was not associated with any specific EEG abnormality during the PET studies. Chugani concluded that the lenticular nuclei may contribute to the pathophysiological state predisposing to infantile spasms. The neurophysiology of Patient 2 (Fig 1b) suggested that multiple regions of the cortex were important in seizure propagation of the one seizure type. SPECT study of this seizure type may have given a different result.

The ictal SPECT study in Patient 3 demonstrated increased uptake in the right posterior central region. This finding did not correlate with the neurophysiology or the magnetic resonancing imaging result.

Patient 4 first presented with simple partial motor seizures (left sided), but complex partial seizures with dystonic posturing of the left arm and infantile spasms developed. In view of Chugani's work, the question was whether he was a candidate for surgery[11,12]. The findings of bilateral abnormalities on MRI, multifocal epileptogenic activity on the interictal EEG and a diffuse, though asymmetrical, change on EEG monitoring at the time of the spasm suggested that surgery was not feasible. The SPECT study supported this decision, showing multiple areas of increased cortical uptake during a series of spasms.

Little is known of the pathogenesis of intractable seizures in infancy. SPECT studies showed focal or multifocal cortical increased uptake in 3 of the patients reported in this paper and increased uptake in the thalamic and basal ganglia region of 2 patients. In these cases there was little correlation between the SPECT studies and the neurophysiological ones. Video EEG monitoring during the ictal SPECT studies of each major seizure type present is important and may show a better correlation between the results of the two techniques. Further studies in this age group are needed as the sample size here reported is small and the patients had heterogeneous disorders. A better understanding of the relative importance of the cortex and the deeper cerebral structures in seizure propagation may influence the choice of anti epileptic medication in the future.

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REFERENCES

1. Rowe CR, Berkovic SF, Sia STB, Austin M, McKay WJ, Kalnins RM, Bladin P. Localisation of epileptic foci with postictal single photon emission computed tomography. Annals of Neurology 1989; 26:660–668.
2. Rowe CC, Berkovic SF, Austin MC, McKay WJ, Bladin PF. Patterns of postictal cerebral blood flow in temporal lobe epilepsy: qualitative and quantitative analysis. Neurology 1991; 41:1096–1103.
3. Duncan R, Patterson J, Hadley DM *et al.* CT, MR and SPECT imaging in temporal lobe epilepsy. Journal of Neurology, Neurosurgery, Psychiatry, 1990; 53:11–15.
4. Uvebrant P, Bjure J, Hedström A, Ekholm S. Brain single photon emission computed tomography (SPECT) in neuropediatrics. Neuropediatrics 1991; 22:3–9.
5. Chiron C, Raynaud I, Jambaque O, Dulac S, Ricard M, Bourguignon A, Syrota A. 133Xe brain SPECT imaging in cryptogenic West syndrome: correlations between regional cerebral blood flow defects and neuropsychological disorder. Journal of Nuclear Medicine 1987; 28:592.
6. Jasper HH. The Ten Twenty electrode system of the international federation. Electroencephalography and Clinical Neurophysiology 1958; 10:371–375.
7. Volpe JJ. Neonatal seizures: Current concepts and revised classification. Pediatrics 1989; 84:422–428.
8. Mizrahi EM, Kellaway P. Characterization and classification of neonatal seizures. Neurology 1987; 37:1837–1844.
9. Commission on Classification and Terminology of the International League against Epilepsy. Proposal for revised classification of epilepsies and epileptic syndromes. Epilepsia 1989; 30:389–399.
10. Chugani HT, Shewmon A, Sankar R, Chen BC, Phelps ME. Infantile spasms: II. Lenticular nuclei and brain stem activation on positron emission tomography. Annals of Neurology 1992; 31:212–219.
11. Chugani HT, Shields D, Shewmon A, Olson DM, Phelps ME, Peacock WJ. Infantile spasms: I. PET identifies focal cortical dysgenesis in cryptogenic cases for surgical treatment. Annals of Neurology 1990; 27:406–413.
12. Chugani HT, Shewmon DA, Peacock WJ, Shields WD, Mazziotta JC, Phelps ME. Surgical treatment of intractable neonatal-onset seizures: the role of positron emission tomography. Neurology 1988; 38:1178–1188.

PLASMA VIGABATRIN ENANTIOMER RATIOS IN ADULTS AND CHILDREN

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SUMMARY

The new anticonvulsant vigabatrin (γ-vinyl-γ-aminobutyric acid) is normally supplied as a racemate, but its anticonvulsant effect is thought to reside in its [S]-enantiomer only. The plasma concentration ratio of the [R] to [S] enantiomers appears to remain constant across the vigabatrin dosage interval in adult volunteers, and in the present study this has also proved to be the case in 12 chronically treated adult epileptic patients. However, in 8 epileptic children chronically treated with other anticonvulsants and given add-on vigabatrin therapy because of failure to control seizures, plasma [R]:[S]-vigabatrin ratios changed across the drug dosage interval, the [R]-vigabatrin levels tending to be relatively higher soon after intake, and to fall more rapidly than the [S]-vigabatrin concentrations over the next few hours (mean half-lives 2.52 ± SD 0.49 and 6.53 ± SD 6.62 hours). The reason for the shorter half-life of [R]-vigabatrin in children remains to be elucidated, but it appears that measurement of racemic vigabatrin plasma concentrations in children, though not in adults, may lead to somewhat misleading conclusions as regards the amount of the circulating anticonvulsant [S]-vigabatrin.

Vigabatrin (γ-vinyl-γ-aminobutyric acid) is a new anticonvulsant agent which is now coming into use worldwide. However, at the time of writing it has not been registered for marketing in Australia, though it has been available for compassionate and clinical trial purposes. The drug is provided for clinical use as a racemate, and it has been shown that its presumed mechanism of anticonvulsant action, irreversible inhibition of the enzyme GABA transaminase which results in raised brain concentrations of the inhibitory neurotransmitter γ-aminobutyric acid (GABA), resides in its [S] enantiomer only[1]. The [R] enantiomer is presumed to be biologically inactive. The enantiomers do not interconvert in humans, and the only published assay capable of measuring the enantiomers specifically at biologically relevant concentrations involves gas chromatography mass spectrometry, a technique that is both expensive and not

widely available. Relatively little importance has been attached to monitoring the plasma concentrations of racemic vigabatrin, let alone those of its [S] enantiomer, because of the assay difficulties and because the anticonvulsant effects of the drug would not be expected to necessarily correlate with the concurrent plasma concentration of the [S] enantiomer. This is the case because the inhibition of GABA transaminase produced is irreversible, so that the highest plasma [S]-vigabatrin concentration present during the past few days determines the drug's effect (so long as all the enzyme is not inhibited at lower plasma concentrations). Such considerations have probably retarded study of the clinical pharmacokinetics of the individual vigabatrin enantiomers, though Haegele and Schechter[2] have defined their kinetic parameters in healthy volunteers following single oral doses of the drug.

We have developed a relative convenient and inexpensive enantio-specific gas chromatographic assay suitable for routine plasma level monitoring and clinical pharmacokinetic studies of [R] and [S]-vigabatrin in humans[3]. During use of this assay we have noted differences in the time courses of the plasma concentrations of [R]-vigabatrin and [S]-vigabatrin in children, but not in adults. Whilst this phenomenon has been reported previously in young children only[4], our experience suggests that it probably applies throughout childhood and it therefore seems worthy of further description.

MATERIALS AND METHODS

PATIENTS

The subjects studied fell into two age groups, viz. adults and children. The study was not designed specifically to investigate plasma [R]-vigabatrin:[S]-vigabatrin ratios in these two age groups. Rather, the adults, all with epilepsy resistant to other anticonvulsants, had their plasma vigabatrin enantiomer levels measured as a element of a study directed at assessing the effects of the drug on certain tests of psychological function[5]. Their plasma vigabatrin enantiomer levels were measured during the psychological tests, and these took place under steady-state conditions at variable times after administration of oral vigabatrin doses. Therefore the study provided an opportunity to investigate plasma [R]:[S]-vigabatrin ratios at different times across the dosage interval. The children studied all had therapeutically refractory epilepsy, and received add-on vigabatrin under a compassionate use protocol with the plasma sampling being approved by the Royal Children's Hospital Ethics Committee, Brisbane. The approval extended to taking between 2 and 8 venous blood samples during the same dosage interval for measurement of plasma [R] and [S]-vigabatrin concentrations. Details of the adult and child patients, including details of their epilepsy and its therapy at the times of study, are set down in Tables 1a and 1b, respectively.

Table 1a Details of adults studied

Subject	Age (yrs)	Sex	Wt (kg)	Epilepsy	VGT Dose	Delay dose-measurement (hrs)	Other anticonvulsants taken
1	48	F	56	CPE, PEG	3000	1.67	VPA
2	25	M	82	CPE, PEG	2000	2.67, 5.75	PHT, PB, CZP
3	17	M	60	SPE, CPE	3000	9.5, 9.5	PHT, CZP
4	43	F	51	CPE	3000	3, 4.67	PB, VPA
5	42	M	85	CPE, PEG	2000	5.5, 2.2	PHT, PMD, CBZ, CZP
6	46	M	86	SPE, CPE, PEG	3000	9, 9.5	PHT, PMD, CBZ, CZP
7	44	F	88	CPE	2000	3, 8.2	PB, CBZ, VPA, CZP
8	20	F	59	SPE, CPE	3000	8.75, 8.25	CBZ, CZP
9	17	F	56	SPE, CPE, PEG	2000	9.5, 9.5	PMD, CBZ
10	38	M	89	CPE, PEG	3000	3, 3.5	PHT, PMD, SUL
11	39	M	81	CPE	2000	2.5, 4.6	PHT, MPB. CBZ, SUL
12	29	M	81	CPE	2000	4	PHT, CBZ

Abbreviations: SPE = simple partial seizures: CPE = complex partial seizures: PEG = partial seizures becoming secondarily generalised: CBZ = carbamazepine: CZP = clonazepam: MPB = methylphenobarbitone: PB = phenobarbitone: PMD = primidone: PHT = phenytoin: SUL = sulthiame: VPA = valproate

Table 1b Details of children studied

Subject	Age (yrs)	Sex	Wt (kg)	Epilepsy	VGT Dose	Delay dose-measurement (hrs)	Other anticonvulsants
1	11	M	36	CPE, PEG	500	3, 7	CBZ
2	11	M	27	CPE	1000	3, 8	CBZ
3	1	M	10	GE	500	2, 4	CBZ, PHT, PB
4	4	F	9.5	GE	500	5.75, 8, 9.7	PB, VPA, CLOB
5	4	F	12.2	PEG	500	2, 4, 7	PHT, CLOB
6	5	M	17	GE	500	2, 5, 7.5, 8.25	PHT, VPA, ETHO
7	7	F	31	CPE, PEG	750	2, 4, 7, 9, 10, 11	CBZ, MSX
8	10	F	20.6	GE	250	1, 2, 4, 7, 9, 10, 11, 12	CLOB, ACET

Abbreviations: GE = seizures of generalised epilepsy: CPE = complex partial seizures: PEG = partial seizures becoming secondarily generalised: ACET = acetazolamide: CBZ = carbamazepine: CLOB = clobazam: ETHO = ethosuximide: MSX = methsuximide: PB = phenobarbitone: PHT = phenytoin: VPA = valproate

VIGABATRIN ENANTIOMER ASSAYS

Simultaneous plasma concentrations of [R]-vigabatrin and [S]-vigabatrin were measured by the gas chromatographic assay of Schramm *et al*[3]. The assay is sensitive enough to measure [R]-vigabatrin concentrations of 1 mg/litre and [S]-vigabatrin concentrations of 0.5 mg/litre in 250 µlitres of plasma.

DATA ANALYSIS

In the children, in whom there were available at least two concentration values at different times during the descending phase of the plasma concentration-time curves for each enantiomer, a one compartment linear pharmacokinetic model described by the equation

$$C_t = C_0 e^{-kt}$$

where C_t = plasma concentration at time t
C_0 = plasma concentration at time zero, if the whole dose had absorbed instantly
k = the elimination rate constant,

was fitted to the data using an iterative curve fitting program (Stemfit). Half lives were calculated as $t_{1/2} = .693/k$.

Regressions were calculated and illustrated with the aid of the program AXUM, and statistical significances of differences were tested by Confidence Interval Analysis[6].

RESULTS

Plasma [R]-vigabatrin concentrations were plotted against simultaneous plasma [S]-vigabatrin levels in the adults and the children (Fig 1). The correlation between the two sets of concentrations was much closer in the case of the adults ($r^2 = 0.97$) than of the children ($r^2 = 0.85$). The reason for the weaker correlation in the children is made apparent by Fig 2, which illustrates the time course of the behaviour of the simultaneous plasma [R]-vigabatrin and [S]-vigabatrin concentrations across a dosage interval in one child. The [R] enantiomer achieved substantially higher peak concentrations than the [S] enantiomer, but its concentrations also fell more rapidly so that, by the expiry of some 6 hours from intake, plasma concentrations of the [R] enantiomer were below those of the [S] enantiomer.

The elimination half-lives and C_0 concentrations for each enantiomer in the 8 children are shown in Table 2. The mean half-life of the [R] enantiomer was 2.52 ± SD 0.49 hours, and that of the [S] enantiomer was 6.53 ± SD 6.62 hours. After log transformation of the data to compensate for the very unequal

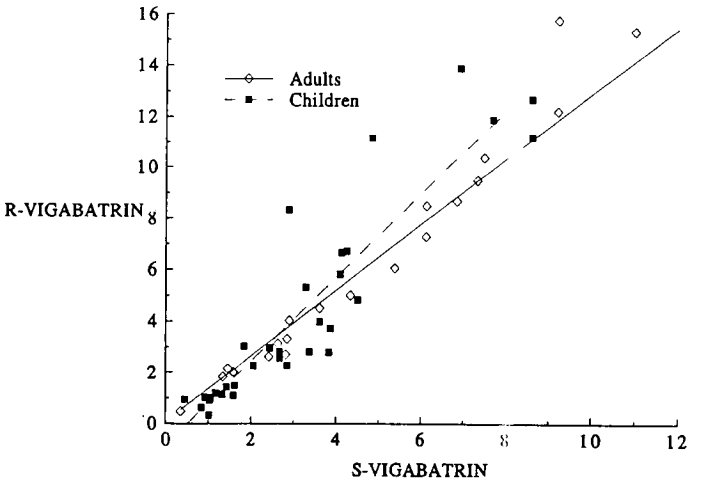

Fig 1 Plasma [R]-vigabatrin concentrations plotted against simultaneous plasma [S]-vigabatrin concentrations in adults and children. There is a greater scatter of points about the regression line in the case of children.

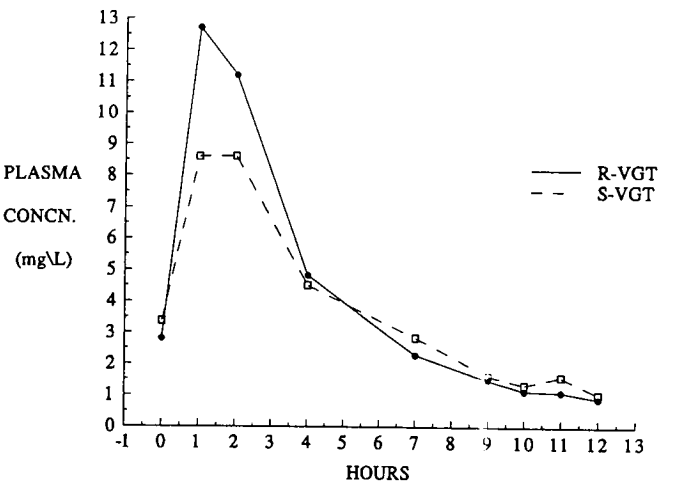

Fig 2 Time-courses of plasma [R]- and [S]-vigabatrin concentrations across a dosage interval in a single subject.

variances, the mean difference (0.724) had a 99% confidence interval of 0.242 to 0.972, the [R] enantiomer having a shorter half-life in all 8 children. The mean C_0 value for the [R] enantiomer was 17.43 ± SD 5.84 mg/l, and that of the [S] enantiomer 6.72 ± 3.10 mg/l, the mean difference of 10.7 mg/l having a 99% confidence interval of 4.61 to 16.8 mg/l. Thus the [R] enantiomer tended to achieve statistically significantly higher initial plasma levels than its fellow, and to be eliminated distinctly more quickly. The half life values of both enantiomers were compared for the 4 children younger than 6 years, and for the 4 older children. The mean values for the younger children were: [R]-vigabatrin 2.27 ± SD 0.54 hours, [S]-vigabatrin 8.72 ± SD 9.39 hours, and for the older children: [R]-vigabatrin 2.77 ± 3.12 hours, [S]-vigabatrin 4.33 ± SD 1.05 hours. The half lives for the [R] enantiomer in the 2 age groups were not statistically significantly different (difference = 0.495; 95 % confidence interval = –0.269 to 1.26), but if the [S] enantiomer half lives were log transformed (to compensate for the unequal variances) the difference in the means (0.372) had a 95% confidence interval of 0.225 to 2.11).

Table 2 Half-lives and C_0 values for the vigabatrin enantiomers in the children

Subject	Half-life		C_0value	
	[R]-VGT	[S]-VGT	[R]-VGT	[S]-VGT
1	2.56	3.79	14.95	6.95
2	3.15	5.83	5.83	2.61
3	1.86	4.94	23.50	6.23
4	3.04	22.77	20.69	4.47
5	2.26	3.40	15.54	6.75
6	1.93	3.76	17.13	4.42
7	2.89	4.26	24.26	11.11
8	2.47	3.45	17,53	11.24

The nature of the data available for the adults did not permit calculation of elimination parameters for the enantiomers. However, it was possible to determine simultaneous [R]:[S]-vigabatrin concentration ratios at different times after drug intake for both the adults and the children. The regressions for the [R]:[S] ratios on time after vigabatrin intake for the adults and the children are

shown in Fig 3. The regression for the adults (ratio = 1.376 – 0.013 hours) was not statistically significant i.e. the [R]:[S] ratio was not time-dependent. However, in the children the regression (ratio = 1.800 – 0.089 hours) was significant at the P = 0.00013 level, indicating that the [R]:[S] ratio fell with time across the dosage interval. The regressions for the adults and the children differed in slope (–0.076, 95% confidence interval = –0.135 to –0.0174), but not in vertical difference between the regression lines (0.0316, with a 95% confidence interval of –0.158 to 0.221). Thus the enantiomer ratios behaved differently in the two age groups as the hours passed following drug intake.

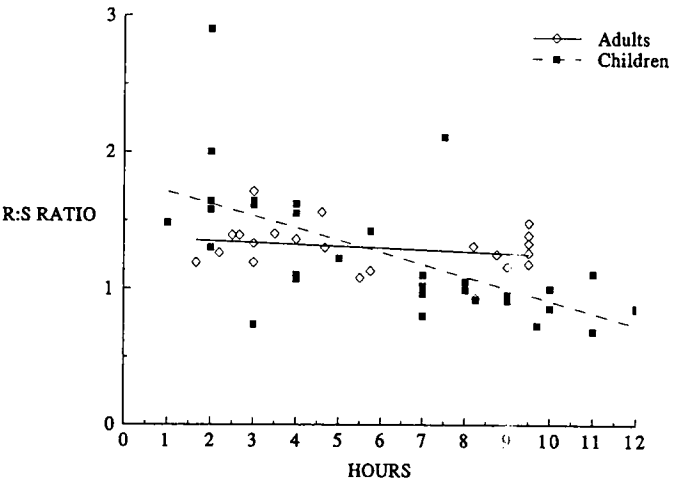

Fig 3 Regressions for plasma [R]:[S]-vigabatrin concentrations across the dosage interval.

DISCUSSION

The present findings did not emanate from a study specifically designed to demonstrate a difference in the behaviour of simultaneous plasma [R] and [S]-vigabatrin concentrations in adults and children. Nonetheless, statistically significant differences between the behaviour of the [R]:[S] enantiomer ratios in the two age groups emerged from the analysis of data collected for quite different purposes. At the time the study was planned there was no reason for anticipating that the [R]:[S] ratios would alter across the vigabatrin dosage interval. The only studies of the time courses of the plasma enantiomer concentrations then available[2] showed them to remain parallel over the dosage interval in adult

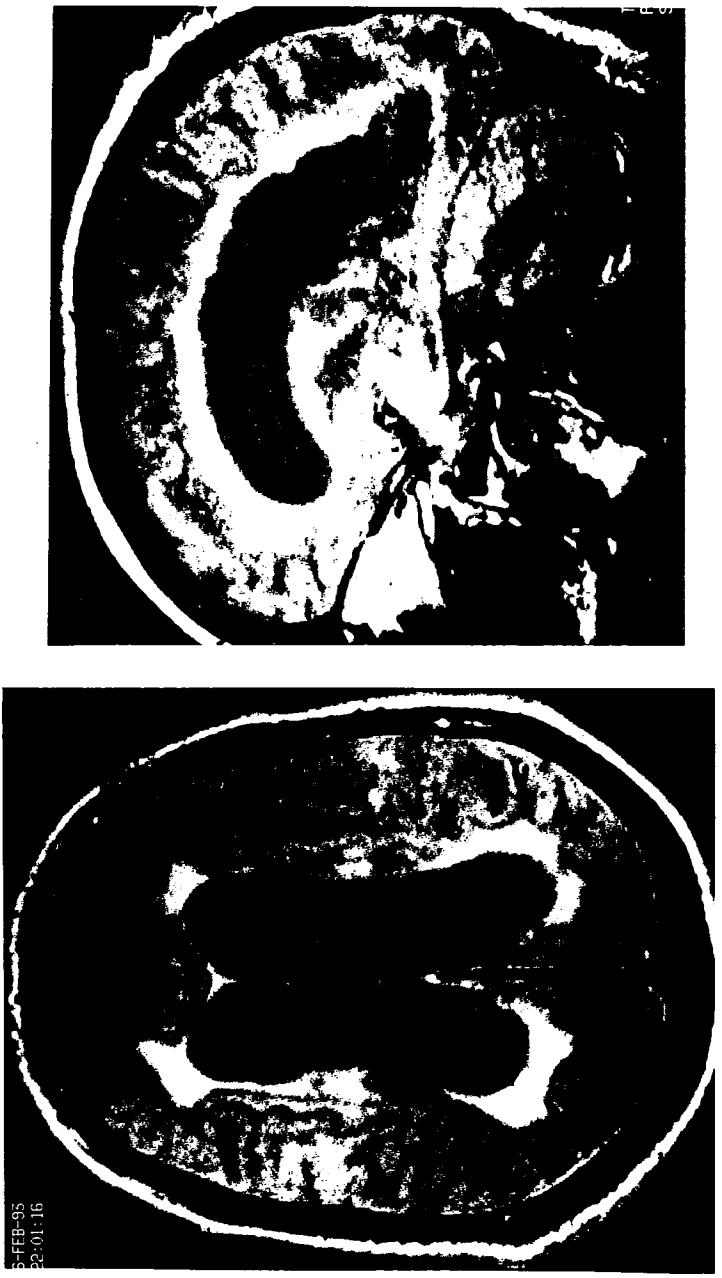

Fig 2 MRI brain scans of Case B showing marked generalised ventricular dilatation and periventricular high signal without significant cerebral atrophy.

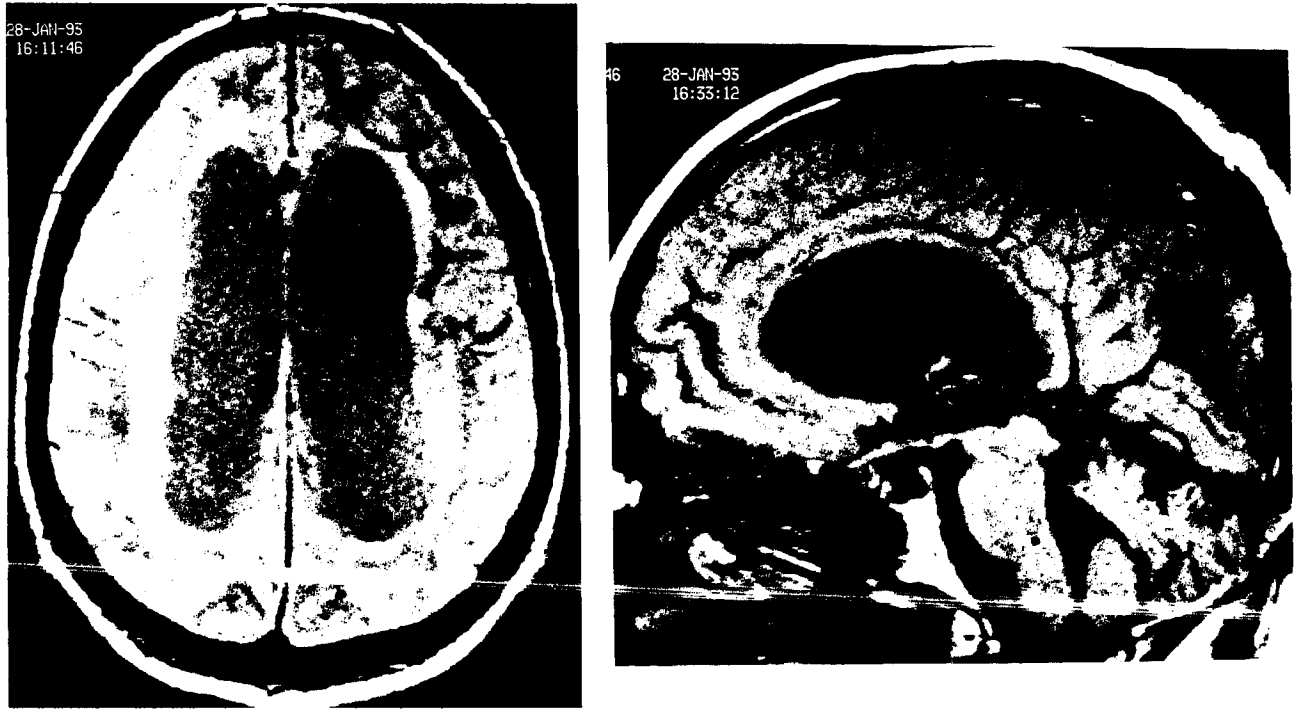

Fig 1 MRI brain scans of Case A showing marked generalised ventricular dilatation and periventricular high signal without significant cerebral atrophy.

In 1990 he was admitted with several years of progressive gait ataxia, deteriorating cognitive state and urinary incontinence. CT head scan showed marked dilatation of all ventricles with periventricular lucency and without evidence of cortical atrophy. CSF flow studies were performed demonstrating a normal opening pressure of 16 cm H_2O. However the flow rate was markedly abnormal suggesting an absorptive defect. Radionuclide images demonstrated ventricular filling with poor distribution over the hemispheres. CSF analysis revealed oligoclonal banding. The right visual and somatosensory evoked responses were prolonged. Neuropsychological testing revealed marked short term memory impairment, reduced speed of information processing and mild inefficiency of intellectual processes. A right VP shunt was then performed with a significant post-operative improvement in his gait, cognitive state and incontinence over several months.

His condition remained relatively stable until February 1993 when he was admitted with several months of progressive gait disturbance, deterioration in cognitive state and urinary incontinence. On examination he was disorientated to time and his short term memory was very poor. He was unable to walk due to severe gait ataxia. There was bilateral optic atrophy and gaze evoked nystagmus with a mild right internuclear ophthalmoplegia. Tone was increased with mild pyramidal weakness in the legs. Reflexes were brisk throughout and plantar responses were extensor. An MRI brain scan showed marked dilatation of all ventricles with periventricular high signal intensity which was consistent with communicating hydrocephalus (Fig 1). Neuropsychological assessment revealed that there had been a generalized decrease in his cognitive and memory function since 1990, but the pattern remained similar.

He subsequently underwent a revision of his ventriculo-peritoneal shunt. Pre- and post-surgery neuropsychological and physiotherapy assessments revealed no immediate change in his condition. On follow up at 4 months, however, there had been a marked improvement in his mental state, gait and level of functioning.

Case B

A previously well 52 year old man had an episode of numbness in his right leg lasting for several months in 1973. In 1983 he had an attack of left optic neuritis with resultant permanent severe loss of visual acuity. In 1985 he presented with several months of gait ataxia with a moderate spastic paraparesis and as well had evidence of a personality change with aggressiveness and odd behaviour. Investigations revealed bilaterally prolonged visual evoked responses and mild ventricular dilatation on CT. A diagnosis of multiple sclerosis was made. Following diagnosis he developed progressive impairment in his cognitive abilities and gait. Neuropsychological testing in 1989 demonstrated marked global cognitive impairment especially affecting memory, new learning, and planning and organisation. He had a number of acute exacerbations which were treated with courses of steroids with some improvement. His last exacerbation was in 1991 but his mental state, gait and urinary incontinence continued to deteriorate steadily.

APPARENT HYDROCEPHALUS AND CHRONIC MULTIPLE SCLEROSIS: A REPORT OF TWO CASES

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SUMMARY

Generalised ventricular dilatation with or without cerebral atrophy is common in longstanding multiple sclerosis. This has been widely assumed to be due to periventricular white matter atrophy rather than true communicating hydrocephalus although it can be difficult to distinguish between these on radiologicial grounds. Here we report 2 chronic MS patients who had progressive dementia, gait disturbance and urinary incontinence and in whom neuroimaging, and in one case CSF infusion studies, suggested hydrocephalus. Both significantly improved following shunting procedures. We suggest that further study is required to investigate whether a significant proportion of patients with chronic MS and dilated ventricles have shunt-responsive hydrocephalus.

Obstructive hydrocephalus in association with the mass effect of an acute demyelinating periaqueductal plaque in a patient with multiple sclerosis (MS) has previously been reported[1]. However generalized ventricular dilatation is commonly observed in chronic MS but has generally been believed to be secondary to periventricular white matter atrophy[2]. We report 2 cases of apparent hydrocephalus in patients with chronic progressive MS who improved following ventriculo-peritoneal (VP) shunting.

CASE HISTORIES

Case A

A 45 year old man had been diagnosed as suffering from multiple sclerosis in 1985 when he had presented with right leg parasthesia and weakness as well as some mild cognitive and memory deficits. Over the next few years he developed progressive right sided clumsiness and loss of vision in the right eye. An MRI brain scan was performed in 1988 and showed plaques consistent with MS.

ACKNOWLEDGEMENT

Dr. L. Nagarajan was supported by a Royal Children's Hospital Clinical Research Fellowship throughout the study.

REFERENCES

1. Meldrum BS, Murugaiah K. Anticonvulsant action in mice with sound-induced seizures of the optical isomers of gamma-vinyl-GABA. European Journal of Pharmacology 1983; 89:149–152.
2. Haegele KD, Schechter PJ. Kinetics of the enantiomers of vigabatrin after an oral dose of the racemate or the active S-enantiomer. Clinical Pharmacology and Therapeutics 1986; 40:581–586.
3. Schramm TM, Mc Kinnon GE, Eadie MJ. Gas chromatographic assay of vigabatrin enantiomers in plasma. Journal of Chromatography (Biomedical Applications) 1993; 616:39–44.
4. Rey E, Pons G, Richard MO, Vauzelle F, D'Athis Ph, Chiron C, Dulac O, Beaumont D, Olive G. Pharmacokinetics of the individual enantiomers of vigabatrin (γ-vinyl GABA) in epileptic children. British Journal of Clinical Pharmacology 1990; 30:253–257.
5. Sheean G, Schramm T, Anderson D, Eadie MJ. Vigabatrin - plasma enantiomer concentrations and clinical effects. Clinical and Experimental Neurology 1992; 29:107–116.
6. Gardner MJ, Altman DG. Statistics with confidence. London: British Medical Journal, 1989.
7. Rey E, Pons G, Olive G. Vigabatrin. Clinical pharmacokinetics. Clinical Pharmacokinetics 1992; 23:267–278.

volunteers, with the [R]-vigabatrin concentrations higher than the [S] ones throughout. Other adult data subsequently available have shown essentially similar results, as did our findings in chronically treated adult epileptic patients. The differences in the enantiomer concentration ratios was initially ascribed to different volumes of distribution of the enantiomers, though it was subsequently suggested that the [S] enantiomer might be less efficiently absorbed from the alimentary tract than its fellow through the specific aminoacid uptake mechanism. However, there are published data consistent with food intake not altering the pharmacokinetics of the drug[7].

There has been a precedent for our findings in children in the work of Rey *et al*[4], who found in children under 2 years of age that the half-life of [R]-vigabatrin was shorter than in that 4 to 14 year olds, whereas the half-life of the [S] enantiomer did not differ between the two age groups. This is the only indication we have traced in the literature to suggest that the kinetics of the enantiomers may be age-dependent. In contrast, our data showed a shorter [R]-vigabatrin half-life as compared with the [S]-vigabatrin half life in children of all ages, not merely in very young ones. Further, the [S]-vigabatrin half life appeared longer in the younger children, but this finding may have been unduly influenced by one atypical subject and it may be unwise to put undue weight on it. The [R] enantiomer half-life mean value in our children appeared shorter than the corresponding literature values for adults, though that for the [S] enantiomer did not. The basis for the faster elimination of the vigabatrin [R] enantiomer in childhood remains to be explained. It is believed that the drug is eliminated mainly by virtue of renal excretion without prior metabolism. If this is the case in children, they may have a greater renal capacity to excrete the [R] enantiomer, but this needs to be established by measurements. However, it should be remembered that the children investigated in the present study, and in that of Rey *et al*, received other anticonvulsants which might have induced their hepatic microsomal oxidative mechanisms. If part of the [R]-vigabatrin in the child's body is eliminated by virtue of oxidative metabolism, the shortened half-life of this enantiomer in such children might be explained, but as yet we have not seen experimental evidence to support this possibility. The mechanisms involved in the unexpected behaviour of [R]-vigabatrin in epileptic children is a scientific issue which should be investigated in its own right. From a more pragmatic point of view, if plasma concentrations of racemic vigabatrin come to be measured as a guide to the potential adequacy of anticonvulsant therapy with the drug, the outcome may be misleading in children, though less so in adults, because of the time-dependent change in the [R] to [S]-vigabatrin concentration ratio in the young.

In February 1993 he was admitted to a country base hospital, having deteriorated to the level that his wife was unable to care for him. His CT head scan was reported as showing gross communicating hydrocephalus with only very minimal cortical atrophy and he was transferred to our institution for consideration of shunting.

On examination he was disorientated to time and with very poor short term memory. He was unable to walk due to severe gait ataxia. There was a dense left central scotoma with a relative afferent pupillary defect and bilaterally pale optic discs. There was moderate spasticity in the legs with a slight spastic catch in the arms. Reflexes were generally brisk with extensor plantar responses. The day following transfer he underwent a right shunt with no obvious improvement in his neurological state. CSF taken at the time of surgery showed oligoclonal banding but was otherwise normal. An MRI brain scan performed a few days later demonstrated marked enlargement of the ventricular system consistent with communicating hydrocephalus, as well as a marked atrophy of the corpus callosum and a periventricular high signal (see Fig 2). All other investigations were normal. Neurobehavioural examination revealed severe cognitive dysfunction and memory impairment and there was no obvious improvement over the week post shunting.

He was transferred back to his referral centre for rehabilitation. There was steady improvement in his cognitive state and gait. Upon discharge home 4 months later he was functioning at his best level in over 2 years.

DISCUSSION

Both of our patients fulfilled the criteria for clinically definite and laboratory-supported definite MS[3]. Review of the literature on pathological[6,11] and radiological[2,9,12] series suggests that ventricular dilatation, with or without cortical atrophy, commonly occurs in chronic MS. Whilst earlier reports have labelled this appearance hydrocephalus, subsequent reviews have attributed the ventricular dilatation to periventricular white matter atrophy. There can be, however, significant difficulty differentiating on radiological grounds between chronic MS and communicating hydrocephalus as periventricular white matter hyperintensity is seen in both conditions and is therefore not a useful discriminating feature[10].

In one study correlating neuroimaging with clinical features in patients with chronic MS, 5 of 11 patients with ventricular dilatation had dementia and gait disturbance as prominent features[9]. To our knowledge, however, there have not been any reports of CSF flow studies or of the results of shunting in these patients.

In our 2 patients we believe that true normal pressure hydrocephalus (NPH), not just white mater atrophy, was present. They both exhibited the classic triad of symptoms of NPH ie. gait disorder, mental deterioration and urinary incontinence.[4,5] Whilst MS alone could account for the clinical features, the abnormal CSF flow studies in case A and the dramatic response to CSF shunting in both patients argue against this. The common finding of generalized thickening of the leptomeninges with collagenous tissue in pathological studies of chronic MS[6] suggests impaired CSF absorption as a possible mechanism for the development of NPH. Other possible mechanisms include: obstruction to the fourth ventricular outflow by brainstem oedema, obstruction to the cerebral aqueduct by gliosis, and oversecretion of CSF[6].

Although chance association cannot be excluded, the frequent finding of ventricular dilatation in chronic MS patients raises the prospect that a number of these patients may have significant NPH that may benefit from a CSF shunting procedure. Clearly further studies in this area are required, either using CSF flow studies (infusion[7,13] or MRI[8]) or continuous intracranial pressure monitoring.

REFERENCES

1. Butler EG, Gilligan BS. Obstructive hydrocephalus caused by multiple sclerosis. Clinical and Experimental Neurology 1989; 26:219–223.
2. Jacobs L, Kinkel WR, Polachini I, Kinkel RP. Correlations of nuclear magnetic resonance imaging, computerized tomography, and clinical profiles in multiple sclerosis. Neurology 1986; 36:27–34.
3. Poser CM, Paty DW, Scheinberg L, *et al.* New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Annals of Neurology 1983; 13:227–231.
4. Adams RD, Fisher CM, Hakim S, Ojemann RG, Sweet WH. Symptomatic occult hydrocephalus with 'normal' cerebrospinal fluid pressure. A treatable syndrome. New England Journal of Medicine 1965; 273:117–226.
5. Hakim S, Adams RD. The special clinical problem of symptomatic hydrocephalus with normal cerebrospinal pressure. Observations on cerebrospinal fluid hydrodynamics. Journal of the Neurological Sciences 1965; 2:307–327.
6. Barnard RO, Triggs M. Corpus callosum in multiple sclerosis. Journal of Neurology, Neurosurgery and Psychiatry 1974; 37:1259–1264.
7. Borgesen SE, Gjerris F. The predictive value of conductance to outflow of CSF in normal pressure hydrocephalus. Brain 1982; 105:65–86.
8. Bradley WG Jr, Whittemore AR, Watanabe AS, Davis SJ, Teresi LM, Homyak M. Association of deep white matter infarction with chronic communicating hydrocephalus: implications regarding possible origins of normal-pressure hydrocephalus. American Journal of Neuroradiology 1991; 12:31–39.
9. Jacobs L, Kinkel WR. Computerized axial transverse tomography in multiple sclerosis. Neurology 1976; April supp. BT 11.

10. Zimmerman RD, Fleming CA, Lee BCP, Saint-Louis LA, Deck MDF. Periventricular hyperintensity as seen by magnetic resonance: prevalence and significance. American Journal of Roentgenology 1986; 146:443–450.
11. Brownell B, Hughes JT. The distribution of plaques in the cerebrum in multiple sclerosis. Journal of Neurology, Neurosurgery and Psychiatry 1962; 25:315–320.
12. Hershey LA, Gado MH, Trotter JL. Computerised tomography in the diagnostic evaluation of multiple sclerosis. Annals of Neurology 1979; 5:32–39.
13. Lundar T, Nornes H. Determination of ventricular fluid outflow resistance in patients with ventriculomaly. Journal of Neurology, Neurosurgery and Psychiatry 1990; 53:896–898.

BOOK REVIEWS

A Textbook of Epilepsy, edited by J. Laidlaw, A. Richens, D. Chadwick. 4th edn. 1993 Churchill-Livingstone. 754 pages.

This is the 4th edition of a multiauthor work which first appeared 17 years ago under the editorship of Laidlaw and Richens. The book has matured into a comprehensive and up-to-date coverage of the medical, psychological, social and legal aspects of seizure disorders. Other books may deal in greater detail with particular aspects of epilepsy e.g. seizure syndromes, epilepsy in childhood, antiepileptic drugs, medical and surgical treatment, but this volume would be more than adequate to provide the general neurologist with all the information he or she is likely to require about the disorder. It is generally well written, well produced and well referenced, and can be recommended without reservation.

M.J. Eadie

Stroke. Pathophysiology, Diagnosis, and Management, edited by H.J.M. Barnett, J.P. Mohr, B.M. Stein and F.M. Yatsu. 2nd edn. 1992 Churchill-Livingstone. 1270 pages

The 2nd edition of this book appears 6 years after the first. Its authorship is international, though drawn mainly from the United States and Canada. None of the 70 or more authors comes from Britain or Australia. The range of topics discussed in its 51 chapters is comprehensive, though there is no account of the history of stroke. The book is well produced, splendidly illustrated and contains a vast amount of information. Its bulk would probably defeat any but the most dedicated enthusiast who attempted to read it from cover to cover, but it will be a most valuable source of reference for all who are involved in the investigation and treatment of cerebral vascular disease.

M.J. Eadie

Instructions to Authors

Manuscript Preparation: Articles should be submitted in English in double spaced typing, preferable on A4 (206mm x 294mm) paper. Two copies of all text, tables and illustrations are required.

1. If the typewritten text is accompanied by a 5¼ inch or 3½ inch disc running under MS-DOS and containing the full text in final form in WordPerfect format (5.0 or later versions), or as an ASCII file, it will be published without cost to the authors, if judged suitable.

2. If only typewritten text is submitted, an attempt will be made to convert this to a WordPerfect compatible file by optical scanning, at cost to the author. Should this attempt fail, the text will be converted to WordPerfect format manually, at a higher cost to the author.

Directions for the maximum size of illustrations will apply, as indicated later in these Instructions.

Submission Dates: Articles may be submitted to the Editors at any time, but the date of absolute closure for receipt of articles to be considered for any year's issue will be the last day of the month in which the Annual Scientific Meeting of the Australian Association of Neurologists is held. All material submitted which has not been presented at the Meeting will be sent to referees, and if the article is judged acceptable, it will be necessary to have the final text incorporating alterations suggested by referees in the Editor's hands by the above closing date, or it will be held over to the following year.

Subdivision of Articles: Manuscript should be prepared and paginated in the following manner.

1) Title page
2) Text pages
   - Introduction
   - Methods
   - Results
   - Discussion
3) Summary
4) Acknowledgements
5) List of references
6) Tables

7) Figures and captions
8) Footnotes

Title Page: There should be a separate title page with title, authors and institutions where the work was done, indicating city and country, and a condensed running title of not more than 50 letters including spaces. The name and address of the author to whom correspondence should be addressed should appear separately as the second page.

Summary: The summary should not exceed 150 words. It should be factual, not descriptive, and should present the reason for the study, the main findings (give specific data if possible), and the principal conclusions.

House Style: Papers reporting clinical studies or experimental work lend themselves to the sectional style of presentation and review articles also can be improved by a more limited use of this approach.

Method: Description of the experimental method should be succinct, but of sufficient detail to allow experiment to be repeated by others.

Results and Discussion: Conclusions and theoretical considerations must not appear in the results section, nor is a recapitulation of the results acceptable for the discussion section. Where relevant, a concise statement of the implications of the experimental results, should appear as a separate section.

References: References should be numbered consecutively in the order in which they are first mentioned in the text. References cited *only* in tables or in legends to figures should be numbered in accordance with a sequence established by the first identification in the text of the particular table or illustration.

Full, not abbreviated, titles of all journals should be given.

Examples of correct forms of references are given below.

Journals

Standard journal article - (List all authors when six or less; when seven or more, list only first three and add *et al.*).
You CH, Lee KY, Chey WY, Menguy R. Electrogastrographic study of patients with unexplained nausea, bloating and vomiting. Gastroen-terology 1980; 79:311-314.

Books and Other Monographs

Personal author(s)
Eisen HN. Immunology: an introduction to molecular and cellular principles of the immune response. 5th edn. New York: Harper and Row, 1974; 406.

Chapter in a book
Weinstein L, Swartz MN. Pathogenic properties of invading micro-organisms. In: Sodeman WA Jr, Sodeman WA (eds). Pathologic physiology: mechanisms of disease. Philadelphia: W.B. Saunders, 1974; 457-472.

Tables: Type double spaced on a separate sheet, number with Arabic numerals (1,2 etc) and provide a legend for each. Tables should be comprehensible without reference to the text. Data given in tables should in general not be duplicated in the text or figures. Any necessary descriptions should appear as numbered footnotes at the bottom of the table.

Illustrations: If they are to be mounted across the width of the page illustrations should be no wider than 110mm, and no taller than 140mm. Illustrations larger than this, up to 165 x 90mm, can be mounted along the length of a page. Illustrations larger than these dimensions will be reduced in size photographically, at the author's expense.

Illustrations are referred to in the text by Arabic numerals (1,2 etc). Legends for illustrations should be typed on a separate sheet, numbered correspondingly and should make the illustration understandable independently of the text. If no specific mention of it is made in the text the approximate position of each illustration should be marked in the margin.

For line drawings, good-quality glossy prints or black ink drawings are requested. Symbols, abbreviations and spelling should be consistent with the text. Figures should be professionally drawn and photographed, if possible.

Lettering and symbols on figures should be large enough to be easily readable, bearing in mind the maximum size of illustrations set out 3 paragraphs above.

The author's name, the figure number and top of the figure must be indicated (lightly) on the back of each figure.

If illustrations from previous articles or books are to be used in papers submitted, the written permission of author(s) and publisher must accompany each illustration.

Abbreviations and Symbols: Use recognised abbreviations SI symbols for units. The first time an uncommon abbreviation appears, it should be preceded by the full name for which it stands. In general abbreviations which are not currently familiar to all neurologists, though in use within particular specialised areas, are better avoided.

Drug names: Generic names should always be used, but if not available, brand names which take an initial capital can be used. In original articles, the maker of the study drug must be given.