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SECOND ASIAN AND OCEANIAN CONGRESS OF NEUROLOGY

The Second Congress of the Asian and Oceanian Association of Neurologists will be held in Melbourne from 1st to 5th May, 1967. The following symposia will be included, together with free papers.

The application of recent neurophysiology and neurochemistry to clinical neurology.

Mental defects and disorders of behaviour in children.

Neurological causes of blindness.

Clinical and physiological lessons from stereotactic procedures.

Regional neurology.

Malignancy and the nervous system.

The neurological congress will be preceded by the second congress of the Asian and Australasian Neurosurgeons in Sydney.

Overseas neurologists, and offers of papers, will be welcomed.

Further information may be obtained from the Honorary Secretary:

Dr. John Game,
61 Collins Street.
Melbourne.

The congresses will provide an excellent opportunity to meet Asian colleagues, to evaluate the state of neurology in Australia, and to see the country.

SUBSCRIPTIONS

to the

PROCEEDINGS OF THE AUSTRALIAN ASSOCIATION OF NEUROLOGISTS

The yearly volumes of the Proceedings of the Australian Association of Neurologists may be purchased for $4 Australian. The next volume will present some of the papers given to the Second Asian and Oceanian Congress of Neurology.

Supplementary congress numbers will present the remainder of the contributions, and will each cost $4. Copies of the supplementary numbers may be ordered, and paid for when their number is known.

Order and cheques or bank drafts for Volume 5, and orders for the supplementary numbers to Volume 5, should be sent to

The Secretary,
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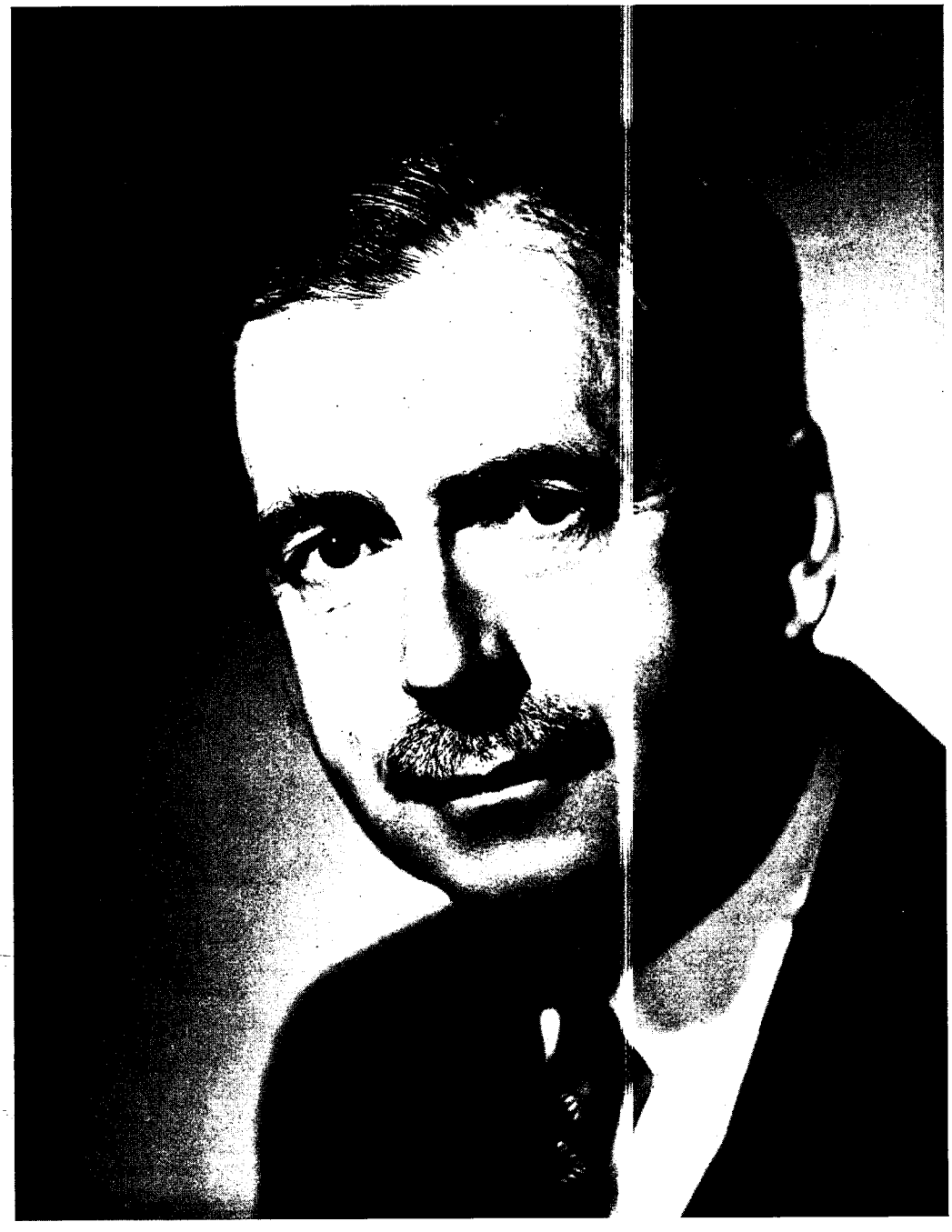

"A wise man is strong:
Yea, a man of knowledge increaseth strength."

PROVERBS XXIV, 5

It is the unanimous wish of the members of the Australian Association of Neurologists that this number of our Proceedings should honour our recently retired President, Dr. E. Graeme Robertson.

A national characteristic of Australia has been said to be that we tend to decry any attempt to acclaim outstanding men. Some responsible and otherwise not unsympathetic critics have also levelled the taunt of mediocrity at Australia.

A young nation, seeking to contribute to the cultural history of mankind, cannot ignore these strictures.

Whatever may in general be their truth, or otherwise, it has been the good fortune of our Association that mediocrity has not been a trait of either our first President, Dr. Leonard Cox, nor of our second, Dr. Graeme Robertson. These two men established neurology in Australia in its own right, and what standards and values we have achieved as an Association we owe largely to them as Founders.

Cox's contribution was primarily to establish neuropathology in this country in relation to clinical neurology. Robertson's contributions to neurology were to bring the virtuosity of the clinical neurology of Queen Square to Australia, and the refinement of pneumo-encephalography to the degree of perfection. He is known for this work throughout the world.

His contributions to the Australian Association of Neurologists are best known to our individual members, but they are exemplified in this journal of our Proceedings.

He has set the standards of presentation at our meetings, and has insisted upon excellence in the preparation of papers for publication in this journal, which is his personal creation and bears the hallmark of his own standards. These standards have become the example for the Association.

Of the time and work he gave to the Association during his years of Presidency, I have probably seen more than anyone, and if I did not speak of them they would probably remain untold—for he would never tell.

JOHN A. GAME,
President

10th November, 1966

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POSTTRAUMATIC EPILEPSY*

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INTRODUCTION

It is common knowledge that convulsions may occur some time after a head injury. For this reason any episodic posttraumatic neurological incident arouses the fear that it may be an indication of the falling sickness. Fortunately, most paroxysmal disturbances which befall the head injured person are not of that nature, but stem from vasomotor instability. Many are definitely related to a change from the lying to the sitting or standing position. They are often described as blackouts, fainting or dizziness and last only a few seconds. Rarely do they cause an individual to fall to the floor. Often they develop when the patient gets up for the first time, but occasionally they occur later in the convalescence. They may or may not be associated with headache. Although some epileptic attacks may resemble such spells, the latter's association with changes in posture, and their subsidence in a few weeks usually permits an accurate differential diagnosis.

The characteristics of a genuine epileptic attack are usually so typical that even a lay person has no trouble recognizing its nature. In less than 10 per cent of cases, the manifestations are atypical and bizarre. Spells described as a dizzy episode, a paroxysmal light-headedness, a faintness or a blackout without loss of consciousness or a fall, may be fragments of an epileptic attack. However, their nature must remain conjectural unless the episode, at times, progresses to a convulsive seizure, or fortuitously occurring while an electroencephalogram is being run is seen to be associated with focal or generalized discharges. A few cases, in any large series of head injuries must remain unclassified, be listed as posttraumatic epilepsy suspect, or as borderline attacks. Even in the first few months after a head injury their number is small; later it diminishes almost to a vanishing point as the true nature of the condition becomes apparent.

* Presented to the Australian Association of Neurologists, 16 May, 1966, and to the Neurosurgical Society of Australia, 22 May, 1966, while the author was the Eliza Savage Fellow, 1966.

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FACTORS RELATED TO THE DEVELOPMENT OF POSTTRAUMATIC EPILEPSY

Not all individuals who sustain head injuries become epileptic; in fact, only a small percentage of those who suffer even severe damage to the brain will ever have a seizure. A number of reasons for this susceptibility of certain individuals have been proposed. Some relate to the subject (intrinsic) and some to the wounding (extrinsic).

Intrinsic or genetic factors. Both direct epileptic tendencies and indirect propensities such as might be reflected by hereditary nervous or mental disorders have been suggested as playing a role in posttraumatic epilepsy. However, a careful genealogical inquiry has not revealed a statistically significant difference in the incidence of epilepsy in the families of men who did not and who did develop seizures as a result of a head injury. Some predisposing conditions such as a head injury in early childhood or trauma at the time of birth has been proposed as a factor. It has been suggested that the first-born, who is more likely to have trauma in the primiparous pelvic outlet than later children, might be more susceptible to seizures. However, these hypotheses have not been borne out in testing. Nor is there proof that individuals having childhood diseases associated with convulsions are more likely to have posttraumatic epilepsy than children who did not develop seizures. Hence, although an intrinsic factor is suspected, its nature has not been determined.

Extrinsic factors. Unquestionably, many parameters of the head injury influence the probability of seizures developing at some future time. Some of these, such as depth of wounding, have been well documented; others, such as the presence of intracerebral foreign bodies, are conjectural and not proved. These factors may be divided into those relating to the impact, the site of injury and the wound.

The impact. The parameters of impact are related to the physical characteristics of the agent—its mass, shape, density, velocity and its mode of contact. In clinical parlance these factors are inherent in the concepts of missile and non-missile injuries, blunt and penetrating head wounds and so forth. There has developed the idea that there is a basic difference in wounds categorized in this fashion. If such is the case, it would be logical to discuss the sequelae of these different forms of injury as distinct entities, and not as events along a continuum. Yet these divisions are often arbitrary, and some have vague borderlines between them. The closed head injury with a basal fracture in one classification may be called an open head injury in another.

Because accurate assessment of the physical forces of impact are not obtainable in most cases, the above categorizations are useful in that they indicate general classes or degrees of impact forces which affect the head. In this respect, they give an index of the severity and nature of the stresses to which the brain is subjected.

The wounding. There is general agreement that factors related to the severity of wounding in all its aspects—stresses at impact, vascular reaction including oedema, iatrogenic complications, reaction to foreign bodies, infection, healing and so forth—are important determinants of the probability of epilepsy. These factors may be assessed in terms of the physical parameters of anatomical involvement, the degree of physiological impairment, and the rate and amount of anatomical and physiological restitution. In clinical terms, the physical parameters of anatomical involvement include the extent of wounding (scalp, skull, meninges, brain) and the dimensions of the wound. The physiological impairment relates to such clinical phenomena as duration of unconsciousness, periods of amnesia and degree of neurological deficit. Restitution includes the factors of healing—cerebral edema, infection, reaction to foreign bodies and blood, type of wound healing, primary or delayed, and so forth. Each of these factors plays a role of varying importance in determining the likelihood of an epilepsy developing. For example, only 15 per cent of patients with large scalp lacerations develop seizures, whereas 41 per cent of those with brain wounds are so afflicted (Table 1).

TABLE 1

Relation of Epilepsy to Depth of Wound

Depth of Wound	*Epilepsy (percentage)*		
	Ashcroft (1) $N = 229$	*Caveness* (2) $N = 356$	*Walker and Jablon* (11) $N = 207$
Scalp	24		15
Dura mater	23	19·5	17
Brain	45	50	41

Location of injury. Another important extrinsic factor relates to the location of the brain wound. Recent experimental work has shown that various parts of the brain are to different degrees capable of epileptogenicity. Motor cortex, hippocampus and amygdala much more readily develop epileptoid activity than the polar regions of the cerebral cortex and the subcortical ganglia. Hence, the part of the brain damaged becomes an important con-

TABLE 2

Relation of Location of Missile Wounds with Dural Penetration and Epilepsy.

Region	*Russell and Whitty* (9)		*Walker and Jablon* (11)	
	No.	%	*No.*	%
Prefontal	160	39	153	28·8
Frontal	127	55		
Parietal	170	65	213	40·1
Temporal	71	38	79	14·9
Occipital	73	38	86	16·2

sideration in the likelihood of a posttraumatic epilepsy (Table 2).

Attempts to correlate the chances of a posttraumatic epilepsy with one or two highly sensitive factors have not been wholly successful. Ritchie-Russell and Smith examined a hundred factors and concluded that the severity of brain damage most closely related to duration of unconsciousness and the period of amnesia. In closed head injuries the period of unconsciousness is roughly proportional to the severity of brain damage and the likelihood of epilepsy. But in open head injuries, this factor is not so sensitive for patients with large losses of brain tissue may have had no impairment of consciousness at the time of the injury and yet be subject to a high risk of epilepsy (Table 3).

TABLE 3

Relationship of Period of Unconsciousness to Epilepsy.

	Closed head injuries and scalp lacerations without neurologic deficit		*Open head injuries with hemiparesis*	
	Number	% epilepsy	Number	% epilepsy
No unconsciousness	2/40*	= 5·0%	16/26*	= 61·5%
Unconsciousness up to two hours	5/53*	= 9·4%	12/25*	= 48%
Unconsciousness more than two hours	4/18*	= 22·2%	23/35*	= 65·6%

* This fraction represents the number of individuals in this category with epilepsy over the total number of individuals in the category.

THE TYPE OF SEIZURE

Almost all varieties of attacks are encountered in posttraumatic epilepsy, although there is a somewhat higher percentage of focal seizures than in the "idiopathic" epilepsies. The focal manifestations, in general, correspond to the functional representation of the damaged brain, but there are discrepancies due to contracoup and secondary cerebral wounds. Although the convulsions presumably originate in a traumatized cerebral focus, at least one-fourth of posttraumatic epileptics have no conscious warning of their generalized seizure. Motor phenomena such as twitching or jerking of a limb, sometimes associated with paresthesias, are an equally common mode of onset of the seizure. Somewhat less frequent are sensory auras with or without comcomitant or subsequent twitching or jerking. Other manifestations of cerebral dysfunction, such as visual, auditory or psychic disturbances less commonly herald the onset of the attack. The so-called psychomotor attacks are not frequently seen. It is extremely rare, if ever, that true petit mal epilepsy with a 3/sec. spike and dome electroencephalographic pattern occurs as the result of a head injury. Although a few isolated cases have been reported, their validity has been doubted by some authors.

With the passage of time the type of attack may change, usually from a generalized to a focal seizure or in some cases, the pattern of the seizure alters so that attacks which initially began with one aura may be ushered in by a different warning after a few years (Table 4).

TABLE 4

Relationship of Type to Change in Character of Seizure Over a Ten Year Period

	Total	*Number Changing*	*Percentage*
Focal attacks only	22	5	22·4
Focal, some becoming generalized	77	13	16·9
Focal, all becoming generalized	48	7	14·6
Generalized only	32	5	15·6
Psychomotor and other	4	0	—

A detailed examination of those cases in which the character of the attacks changed suggests that patients with motor, sensory, adversive or other auras had attacks in later years beginning with phenomena usually ascribed to a focus in the temporal lobe, namely gustatory, olfactory, epigastric aura. In fact, this type of metamorphosis was present in some 12 of 30 cases having a change in the character of the

attack. The electrographic abnormalities in some cases also showed a shift from a frontal to a temporal focus.

The Onset of the Epilepsy

Seizures may occur after a blow to the head at almost any time. In approximately 1 per cent of individuals sustaining a head injury, a convulsive seizure results from the immediate trauma to the head. These seizures may not be of serious prognostic omen, if other factors of wounding are minor. However, the prognosis is grave for stuporous patients who have generalized seizures some hours after a severe head injury. Focal seizures in the first days are less serious. They are usually considered to be due to cerebral lacerations or blood in the spaces about the brain; in fact, some surgeons consider that their occurrence is an indication for an exploratory burr hole. In the first few weeks after a head injury, focal seizures, presumably due to the acute inflammatory reaction to brain damage, are not uncommon. Their prognostic significance for later seizures is not agreed upon; some authors consider them so benign that they do not classify them as posttraumatic epilepsy. However, about half of these patients will have later attacks.

Although few patients with closed head injuries will develop seizures after the first month or two, those with open head wounds have a considerable risk of seizures occurring within the first two years. In the latter group approximately 50 per cent of those who will develop epilepsy have had their first attack by 6 months after injury, and 80 per cent have had an attack before the end of the second year. Thereafter, patients with closed head injuries who have had no attacks are unlikely to have seizures, but those with open head wounds have about a 1 per cent chance each year of developing attacks until the 10th posttraumatic year (Fig. 1). Occasionally a patient may have his first attack 15 or 20 years after a head injury, but such a late onset should lead to a very careful search for other possible causes.

The Incidence of Posttraumatic Epilepsy

The incidence of posttraumatic epilepsy varies with the type, location and severity of head injury (Table 5). For closed head in-

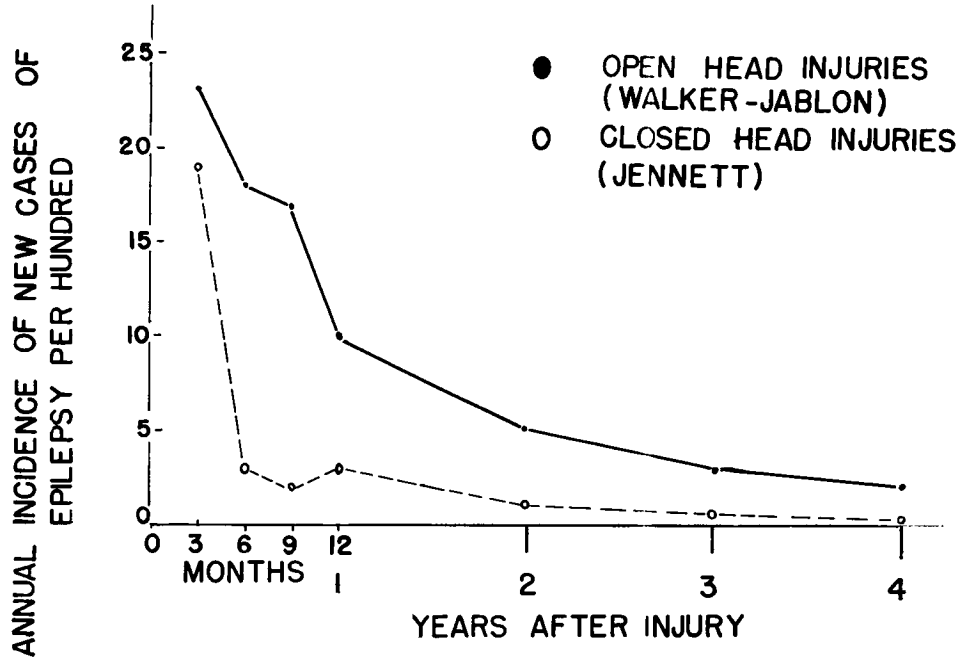

FIG. 1—Graph to show the annual incidence of posttraumatic epilepsy in a series of closed (circles) and open (dots) head injuries.

TABLE 5

Incidence of Epilepsy in Closed Head Injuries* and Dural Penetrating Injuries.

Author	Closed		Open	
	No. cases	*% epilepsy*	*No. cases*	*% epilepsy*
World War I				
Rawling (6)	47	9	226	33
Credner (3)	244	19·7	1,234	49
World War II				
Walker and Jablon (11)	267	14·2	472	36
Wilson (13)			196	17
Civilian				
Row-botham (7)	430	2·5		
Jennett (5)	1,000	8·0**		
Korean				
Caveness (2)	149	10	130	50

* Includes scalp lacerations.
** Deduced from 3·8% early epilepsy plus 5% late epilepsy of which ¼ to ⅓ were early epileptics.

juries, a figure of 1-5 per cent is usually quoted, for open cranial wounds the figures range from 20-50 per cent. If an attempt is made to control the severity and type of wounding, the incidence of seizures is remarkably similar. For example, the overall incidences of posttraumatic epilepsy in missile head-injured men of World War I (1), World War II (12), and of the Korean campaign (2) are practically identical, 33·5 per cent, 33 per cent and 34 per cent respectively. This finding has been advanced as evidence that surgical advances did not affect the frequency of convulsive sequelae. But the optimist might well argue that the improved surgical treatment saved the more seriously wounded men of the later wars, many of whom unquestionably would have succumbed had they been similarly wounded in World War I, so that had the true incidence and other factors, including surgical treatment been the same, there would have been a higher percentage of posttraumatic epilepsy in the later wars.

COURSE OF THE POSTTRAUMATIC EPILEPSY

Sufficient information is now available from the long-term follow-up of relatively large series of patients to describe the natural history of posttraumatic epilepsy. It becomes apparent that some individuals, following a head injury, will have a few convulsions in the first year or so and no subsequent attacks, even though anticonvulsive medication is not maintained. Patients with generalized attacks without aura are a little more apt to have this benign course than individuals with focal seizures that become generalized. However, in this latter group, there is a tendency for the attack to remain focal without spread. Hence, in a series of posttraumatic epileptic patients, the prevalence of major attacks decreases more rapidly than that of minor attacks. In addition, the focal attacks become less severe and decrease in frequency so that occasional paraesthesiae of a hand may be the only remaining fragment of the focal seizure (Fig. 2).

Yet, it must be admitted that occasionally an individual who has been free of attacks for 5 or 6 years may have a seizure often precipitated by an intercurrent infection, traumatic or psychic stress and/or the sudden withdrawal of anticonvulsive medications. In general, these attacks are isolated and upon returning to a normal regime, the patient continues indefinitely without further attacks.

In a series of 230 patients who had had one or more seizures after a head injury and who were followed for 15 years, approximately one-fourth had no attacks of any type from the 5th to the 15th years and almost half had no major convulsions in that period. In other words, 10 to 15 years after injury, 60 per cent of these posttraumatic epileptics no longer have attacks which render them incapable of taking care of themselves. A number of factors point toward a favourable prognosis. The occurrence of only generalized and few attacks within the first month or so of injury, and a tendency for the attacks to decrease in frequency or severity in the first two years, are good prognostic omens. Anticonvulsant medication may be a factor, but it is not essential, for many patients whose attacks ceased are not taking drugs.

THE VALUE OF ELECTROENCEPHALOGRAPHY IN POSTTRAUMATIC EPILEPSY

High hopes have been entertained for the electroencephalogram to provide prognostic information regarding head injuries. Single

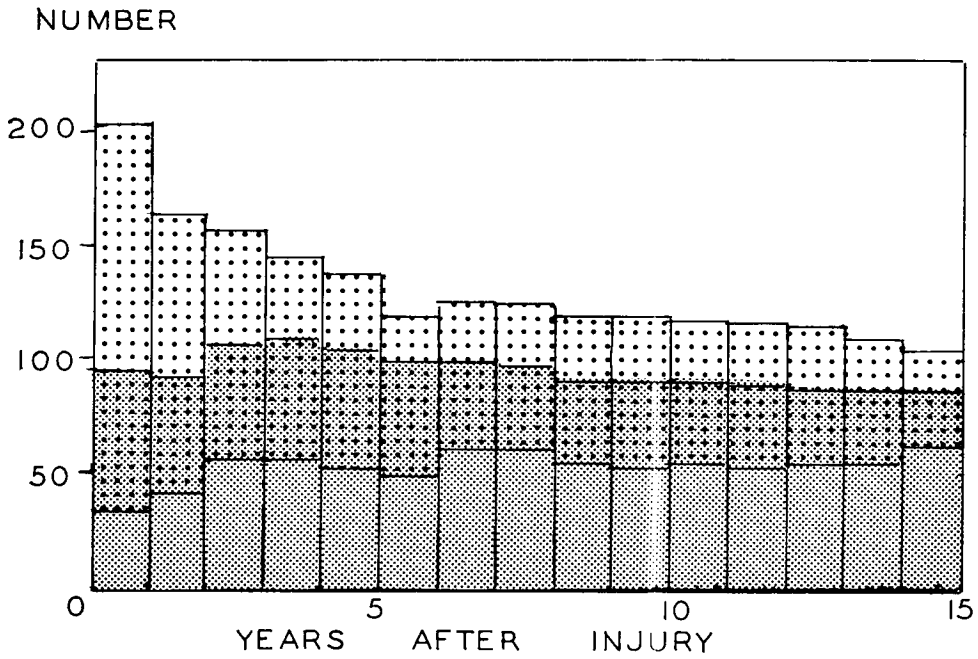

FIG. 2—Graph to show the prevalence of posttraumatic epilepsy in a series of 230 men known to have had one or more seizures following a head injury. The clear areas represent no seizures. The coarse dotted areas represent major attacks and the stippled areas represent minor attacks without loss of consciousness. Note that the number of men having minor attacks remains constant because the number of men ceasing to have minor attacks is balanced by the number of men whose attacks change from major to minor. After the fifth year the attack profile is relatively stable.

TABLE 6

Electroencephalographic Findings as an Index of Prognosis of Posttraumatic Epilepsy.

EEG Characteristics	*State of Epilepsy after 5 Yrs.*			
5-9 Yrs. after injury	*No attacks* No. cases	%	*Cont'd. attacks* No. cases	%
Normal or borderline	27	39	56	34
Generalized abnormality	17	25	32	23
Focal abnormality	25	36	55	33

records are difficult to evaluate because the pretraumatic electroencephalographic pattern is unknown. Serial tracings may give significant information regarding the extent and degree of brain damage, but they do not indicate which patients will eventually develop posttraumatic epilepsy and which will not, nor do they indicate the prognosis of an individual having seizures (Table 6). It is now apparent that no specific electroencephalographic pattern is consistently associated with posttraumatic epilepsy. Even spikes generally considered to have epileptic significance are not much more frequent in the records of patients with posttraumatic epilepsies than those of non-epileptic patients with head injuries. Serial recordings do not enable an accurate prognosis, for although episodic discharges may precede convulsions, they may also resolve without overt seizures.

On the negative side a normal electroencephalogram after a head injury is indicative of little or no brain damage; an abnormal tracing is usually associated with some brain injury. A series of brain waves showing gradual recession of abnormalities over a period of months is

TABLE 7

Relationship of Electroencephalographic Findings 15 Years After Injury to State of Epilepsy

EEG Characteristics	*State of Epilepsy from 5-15 Years after Injury*			
15 Years After Injury	No attacks No. cases	%	Cont'd. attacks No. cases	%
Normal or borderline	14	37	18	25
Generalized abnormalities	11	29	19	26
Focal abnormalities only	13	34	35	49

looked upon as a satisfactory resolution of the injury, whereas the persistence of abnormalities is a cause of apprehension. Perhaps the use of activating techniques will allow more definite conclusions to be drawn regarding the future probabilities of epilepsy.

If a convulsive state has developed, the electroencephalogram still seems to be more sensitive to the degree of brain damage than to the epileptic state. Within the first ten years of injury, electroencephalograms of patients who had had one or more seizures, of patients who were having recurent seizures and of those who had not had a convulsion for at least two years, did not specifically differ in terms of wave, form, frequency or synchrony (Table 6). However, with a longer span of freedom—ten years—the brain waves of the non-epileptic tended to be less abnormal than those of the other groups (Table 7).

THE TREATMENT OF POSTTRAUMATIC EPILEPSY

Theoretically the administration of anticonvulsant medications should decrease the likelihood of an epilepsy developing. However, no well-controlled series has yet been presented to prove this hypothesis. Because all anticonvulsant drugs occasionally have undesirable side-effects, it does not seem advisable to prescribe such medication indiscriminately for patients who have had minor head injuries, and who consequently have very minimal chances of developing seizures. On the other hand, patients with severe open wounds of the head, who have a 50 per cent chance of developing a seizure, may well be advised to take an anticonvulsant drug for one or two years after their injury. If no attacks have occurred by this time, the odds are slight that they will develop later. The effectiveness of anticonvulsant medication in posttraumatic seizures has been questioned by some epileptologists. However, in my experience the attacks are easier to control by anticonvulsive medication than those of other forms of epilepsy. Phenobarbital in doses of 100 mg three times daily, or dilantin 100 mg four times daily, is advisable for a patient who has had one or more attacks following a head injury. If they persist, the medication should be increased and mysoline added. Rarely is it necessary to consider surgical exploration of the wound and resection of an epileptogenic focus.

THE DISABILITY FROM POSTTRAUMATIC EPILEPSY

A few convulsive attacks after a head injury should not be considered a permanent disability, even if they are associated with some neurological handicaps such as a hemiparesis or aphasia. Almost all authors agree that the presence of a posttraumatic epilepsy rarely constitutes a real handicap to social or economic rehabilitation. However, the patient will have a few anxious years, but he should not become depressed nor give up his occupation because of such attacks. A rather extensive experience indicates that if the individual has better than a normal intelligence, the odds are about seven out of eight that he will be able to continue his normal occupation. Even motoring, after a short

TABLE 8

Relationship of Work Status and Intelligence.

Intelligence	*Employed* (*N* = 96) *No.*	*%*	*Non-Employed* (*N* = 117) *No.*	*%*
– 89	11	11	65	55
90 – 109	53	55	46	40
110 +	32	34	6	5

interlude, is not denied him. Unfortunately, individuals with low intelligence do not make as satisfactory adjustments to their handicap as those with normal or superior intelligence (Table 8).

SUMMARY

The factors influencing the development of a posttraumatic epilepsy are many and related to events preceding, concomitant with and subsequent to the injury.

The incidence of epilepsy following head injuries varies from practically nil to over 50 per cent, depending upon the severity of injury and the site of cerebral involvement.

Even if seizures develop, spontaneous regression and cessation may occur 3-4 years after the injury.

The medical treatment consists of anticonvulsant therapy until the patient has become free of attacks for at least two years.

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Symposium on Muscle

SOME HISTORICAL AND CLINICAL NOTES

JOHN A. GAME

Melbourne

INTRODUCTION

As an introduction to a discussion of diseases of muscle it seems reasonable to review the present clinical position and what has led up to the present state of our clinical knowledge.

History shows that everyday practice of medicine lags a full decade or more behind original work, as it does in most other arts and sciences.

MUSCULAR DYSTROPHY

In referring to the early clinical history Walton and Nattrass (1954) state that progressive atrophy of the skeletal muscles was recognized in the early part of the nineteenth century, and in 1850 Aran gave a detailed description of this syndrome. In 1852 Meryon described its familial occurrence in some cases. It was, however, not until 1860 that Luys distinguished another group of patients in whom atrophy was secondary to changes in the grey matter of the spinal cord, and in whom the disease was not familial. Still the distinction between neuronal and primary muscular atrophy was slow, and in 1868 the pseudo-hypertrophic muscular paralysis of children described by Duchenne was believed to be similar to progressive muscular atrophy in adults.

It was not until 1884 that Erb separated the cases in which there was no pathological lesion of the nervous system. So the concept of muscular dystrophy, or myopathy, was born.

The greater part of the next century was occupied by nosological separation of muscular syndromes. In broad terms the climax of this era was reached and interest stimulated in some less well recognized areas by Walton and Nattrass (1954). As a result of painstaking clinical observation and inductive reasoning they reached what appears to be the ultimate clinical classification based upon criteria of genetics and natural history of the various types.

MYOSITIS

From the study of Walton and Nattrass (1954), there emerged a small group of cases, described separately by Nattrass (1954), in his Presidential Address to the Section of Neurology, Royal Society of Medicine, which fell out of line with the natural history by recovering spontaneously. After excluding two cases as benign congenital myopathy and one as dermatomyositis, there were five remaining cases. Except for a rapid initial progress, three of these five cases resembled pseudo-hypertrophic muscular dystrophy in every other respect, including enlargement of the calves. The other two were also typical muscular dystrophies, but without pseudo-hypertrophy, although one of them even had a family history of the Duchenne type. But, in both the onset was unusually acute and in one the course was remittent and there was evidence of fatiguability of muscles.

In these five cases muscle biopsy revealed characteristic features of polymyositis (although Nattrass does not describe these features nor show any illustrations of the sections). It is interesting that all five cases were children.

At about the same time Eaton (1954), at the Mayo Clinic was also collecting a group of cases with "a resemblance to muscular dystrophy except that the illness developed too rapidly and often too late in life". Although

suggesting amyotrophic lateral sclerosis the process was "localized too symmetrically to the proximal muscles and fasciculations (were) usually lacking". Despite a striking resemblance to myasthenia gravis, specially when dysphagia and facial weakness were prominent, the weakness was neither relieved by neostigmine nor exaggerated by curare. He began to wonder whether the muscular troubles of these patients were the same as those of dermatomyositis. His colleague, Lambert, found that there were electromyographic similarities, and so Eaton studied his cases by muscle biopsy and found 25 cases of polymyositis. In contrast to Nattrass's group these were all adults ranging in age from 26 to 70 years.

To me it seems that this essentially clinical paper established polymyositis in clinical neurology in the English-speaking world, and drew with notable incisiveness the outlines of the clinical picture of the condition.

Some of the outstanding features to which Eaton drew attention are worth noting briefly, as a summary of this condition. The weakness is symmetrical in the muscles of the shoulder and pelvic girdles, it is even and not patchy, and becomes moderate or severe within a period of weeks or months. There may be no pain, nor tenderness, nor induration, nor atrophy, nor changes of reflexes—a situation which, it must be admitted, taxes the skill of all but the most experienced clinicians. Dysphagia was present in half the cases (often elicited only in response to enquiry) and the extensor muscles of the neck were affected in about one-third of the cases. He stressed that neither the histological picture nor the electromyographic abnormalities are pathognomonic. It is interesting to read the list of diagnoses which had been given to these patients before referral. They include muscular dystrophy, motor neurone disease, myasthenia gravis, polyneuronitis, psychoneurosis, thyrotoxic myopathy (the basal metabolic rate was raised in many cases), Addison's disease, rheumatoid arthritis, rheumatic fever, Raynaud's disease, trichinosis, sarcoidosis, periarteritis nodosa, and brucellosis.

It is true that at about the same time, and even earlier, similar recognition was occurring in Europe, e.g. Christensen and Levison (1950), and Van Bogaert and Radermecker (1954).

Exemplifying my earlier statement, it must also be said that this general clinical recognition had been preceded by a decade, or much more, of pertinent personal observations, both clinical and pathological. Clinical interest had no doubt been sparked by Shy and McEachern's observations (1951a and 1951b), that their "menopausal muscular dystrophy" was one of the few neurological conditions to gain benefit from cortisone. It was left for Denny-Brown (1952) to recognize that the cases of which they spoke were really polymyositis, and Adams, Denny-Brown and Pearson (1953) gave a clear pathological description of this condition.

As long ago as 1909-1910 Batten had drawn attention to cases of "muscular dystrophy" in the literature which had been reported as recovering. He accepted three cases, one of Marina (1908), one of Erb (1908), and one of Jendrassik (1909). Bramwell (1922) reported a fourth case (earlier seen by Batten), which he accepted as recovering from "muscular dystrophy" and suggested that the recovery be attributed to "incorrect diagnosis due to absence of family history". Thus a difference was recognized, but not polymyositis.

It is interesting that Bramwell's case was really only mentioned in discussion when reporting two cases with wasting confined to the quadriceps "of probable myopathic origin" (Bramwell, 1922). These cases are of historical interest because they were recalled by Denny-Brown (1938-1939), when he reported a similar case. The continuing thread of the story is that Denny-Brown drew attention to the histological similarity of his case with those of Nevin (1936).

Before discussing the latter a digression is necessary. Walton (1964) said that Denny-Brown "concluded that the disorder was probably a form of polymyositis", but reference to the paper shows no evidence of this statement. Denny-Brown in fact said the "muscle shows myopathic changes of the type recorded by Nevin (1936)", and he concluded his description with the sentence, "There is no inflammatory reaction". However, some years later, in retrospect, Denny-Brown obviously regards this particular case, which he had reported in 1938-1939, as one of myositis (Adams, Denny-Brown and Pearson, 1962), and by inference

he held the same view about Bramwell's cases and Nevin's. Walton (1956) himself later described two similar cases in which the pathological changes in one were clearly those of muscular dystrophy; there was no biopsy of the second case. Reference to these papers shows that Walton's cases had pseudo-hypertrophy of the lateral vasti which was not reported in the cases of Bramwell or Denny-Brown, and thus it seems to me dubious whether Walton's cases are in fact comparable with those of Bramwell and Denny-Brown, and by their nature do not belong to the history we are tracing.

The cases of Nevin (1936), to the histology of which Denny-Brown (1938-1939) had likened the histology of his case of quadriceps wasting, were in fact clinically different as they had more widespread involvement of the muscles. Nevin drew a distinction between his two cases and the usual late cases of muscular dystrophy, both clinically and pathologically. He himself also noted the difference from the recognized clinical features of myositis and considered that histologically, too, his cases were different from previously described cases of myositis. He concluded that the histological appearances were more like the changes occasionally seen in cases of dystrophy, although as mentioned, it is clear by inference from Denny-Brown's later writing (Adams, Denny-Brown and Pearson, 1962) that in retrospect he regards these cases as examples of myositis. Thus, as do Bramwell's and Denny-Brown's "quadriceps" cases, they have a place in the emerging story of polymyositis. Now we can see the link between the various early cases of "recovery from muscular dystrophy", the cases of quadriceps wasting, and Shy and McEachern's "menopausal myopathy", which had caught everyone's eye because it responded to cortisone. Denny-Brown (1954) and Adams, Denny-Brown and Pearson (1962) produced reasons why all these cases can be considered as examples of polymyositis.

This brief historical survey has shown that "polymyositis", *without skin involvement*, has become an established clinical entity only in the last ten to twelve years, at least in the English-speaking world. Today it is recognized that polymyositis without skin lesions makes up about half the total cases of myositis (Pearson, 1964), and that polymyositis accounts for about half, or more, of the cases of myopathy in adult life (Walton and Adams, 1958, and Barwick and Walton, 1963). It is clear from reviewing the literature that the main factors which brought polymyositis into general recognition as a clinical entity were better clinical observation, increased use of muscle biopsy and electromyography. Clinically we are mainly indebted to Walton and Nattrass (1954) and Eaton (1954), preceded by isolated but important clinical observations by Batten (1909-1910), Bramwell (1922), and Shy and McEachern (1951a and 1951b), and pathological observations of Denny-Brown (1952), and Adams, Denny-Brown and Pearson (1953 and 1962).

Polymyositis, *with skin involvement*, goes back very much further, and according to Pearson (1964) was first recorded by Wagner (1863), and "Dermatomyositis" was first delineated as a clinical entity by Unverricht (1887).

Myopathy Associated with Malignancy

Overlapping the emergence of polymyositis as an entity, and often not clearly distinguished from polymyositis of unknown aetiology, the general recognition of muscular changes associated with malignancy gradually came to notice.

The association of dermatomyositis and malignancy has been longer recognized, and many references to this are given by Walton and Adams (1958), who suggest that Stertz was the first, in 1916. Also, Arundel, Wilkinson and Haserick (1960) have shown that when dermatomyositis develops in a male over the age of 40 there is more than a 50 per cent chance that he will eventually demonstrate a tumour.

To remain within the confines of our discussion, one also sets aside those patients, seen by most of us as neurologists, who have what appears to be a typical myasthenia gravis brought to light by surgery, particularly thoracic surgery. My impression is that some of these are ordinary cases of myasthenia, unmasked by the use of tubocurarine in anaesthesia. I recall one in particular who had no myasthenic or other symptoms and signs before or after operation, but was abnormally sensitive to

curare. I accepted her as a potential myasthenia gravis. In others the apparently typical myasthenia may or may not be associated with the lung lesion, which in one case of my own was not neoplasm, but torula.

Thus the discussion is narrowed to the group of patients, some perhaps best labelled as Myasthenic-Myopathic Syndrome by Henson (1964), in his section of the book edited by Walton on *Disorders of Voluntary Muscle* (London, 1964). Priority in recognition of this condition is probably again attributable to Denny-Brown (1948).

In his report of two cases of sensory neuropathy associated with carcinoma, he found in the muscles "proliferation of sarcolemmal nuclei and increased cellularity of the connective tissue of a kind seen in chronic myositis, or rapidly progressive myopathy", although "the coexistence of 'myositis' was not suspected during life". This latter fact probably explains why most of us as clinicians overlooked the description of the muscle changes and their possible significance, although the paper was widely read at the time. Further attention was drawn to the matter by Henson (1953), and Henson, Russell and Wilkinson (1954). The latter report included 19 cases of carcinomatous neuropathy. Eight of these cases "suffered an atrophic paresis which was most marked in the limb girdles and proximal parts of the limbs", and it is notable that two suffered weakness of the neck muscles, and two had fatiguability of muscles which "may be reasonably termed myasthenic". The course of the complaint was remittent in six patients. Thus revealed in this paper are several of the features which have since become recognized as typical of the syndrome of polymyositis with malignancy.

Histologically a variable degree of atrophy of muscle-fibres was noted, although slight and of an unspecific character. But the changes which were seen are consistent with later pathological reports of this condition. The absence of associated skin lesions was noted.

Shy (1962) clarified the situation in a clinico-pathological study of 131 patients with proximal muscle weakness occurring sporadically after the age of 30.

In patients under the age of 50 the most commonly associated disorder was one of the collagen diseases. Over the age of 50, cancer was found in 70 per cent of the men and in 24 per cent of the women. When followed for more than three years, all the men over 50 were found to have neoplasm. The primary sites were many, but the prostate was most frequent. Muscle weakness may precede the recognition of the neoplasia by two or three years.

In concluding this brief delineation of the myopathy associated with malignancy, it is worth drawing attention to the interesting feature stressed by Adams, Denny-Brown and Pearson (1962), which is the "out of phase" characteristic: the polymyositis may regress as the tumor continues to grow.

Interstitial (Nodular) Polymyositis

A clinical concept of this has, to me, always been difficult. The aspects brought out by Adams, Denny-Brown and Pearson (1953 and 1962) are therefore of some solace. Their view is that the specificity of interstitial polymyositis is open to question, and they state that multiple perivascular and adventitial nodules of inflammatory cells with destruction of contiguous muscle fibres may occur with remarkable frequency in the so-called connective tissue diseases and rarely in other diseases. This accords with one's own clinical experience. Whilst it is not uncommon to see the most severe degree of muscle wasting with such conditions as rheumatoid arthritis, and such cases are referred to one as probably cases of disease of muscle or neuronal muscular atrophy, there is, as these authors comment, surprising retention of power in the muscles when tested by conventional neurological methods and the deep reflexes are intact.

Yet the same authors state that the condition which they designate as interstitial nodular polymyositis merges imperceptibly with the characteristic muscle lesion of polymyositis. This pathological observation may help to explain our clinical difficulty.

Metabolic and Endocrine Myopathies

The delineation of the endocrine myopathies has been reasonably clearly established, but the metabolic disorders are more complex. Considering the large bulk of skeletal muscle and the complexity of its metabolism, it is surprising that there are not more of these conditions and that they are not more frequently seen.

Paralysis associated with altered serum potassium has been discussed at our meetings not very long ago, by John Allsop (1964).

The conditions associated with glycogen metabolism are very rare, but intriguing, and we shall hear of McArdle's Syndrome (1951), in this symposium today. As this condition may be associated with myoglobinuria in some cases one hopes to learn something of the differentiation from the apparently separate condition known as paroxysmal myoglobinuria.

Clinical Recognition of Muscle Disorders

It may well be as well to admit that the clinician's first difficulty may be to recognize that the patient's complaint reflects a disorder of locomotion at all.

A woman with two black eyes, complaining that she falls for no accountable reason, can raise thoughts of vertebral arterial insufficiency, akinetic epilepsy or third ventricular cyst—and these thoughts may persist if her knee jerks are present and the thighs not significantly wasted. Similarly a complaint of dysphagia, or a skin rash, or difficulty in raising the head from the pillow, do not immediately suggest disorders of skeletal muscles.

However, assuming that weak or wasted muscles are recognized as the major clinical problem, the clinician today may still stumble on the next hurdle of differentiating primary muscular disorders from neuronal atrophy or disorder of the motor end-plate. As has been seen it is only a little over 80 years since myopathies were clearly separated from neuronal muscular atrophy, but today the clinical signs are so well established that their reiteration to an audience such as this would be redundant.

Having incriminated the muscles, the differentiation between muscular dystrophy and other myopathies such as polymyositis may present the next problem, but it is one which demands precise resolution today since the therapeutic outlook is not as black as it was. Walton (1964) lists 10 clinical features of differentiation between polymyositis and progressive muscular dystrophy. I shall not repeat them, but merely emphasize that like most myopathies, polymyositis generally affects proximal muscle groups—for some reason clinically more elusive than the readily apparent distal weakness and wasting with foot drop and wrist drop. But in polymyositis the proximal limb muscles are mostly affected to an equal extent, whereas in muscular dystrophy the pattern of proximal involvement is more selective. It is also of importance to note again that the deep reflexes may be spared in polymyositis relatively longer. When these two features of polymyositis are added to the relatively little muscle wasting and the absence of pain or tenderness in the muscles, in some cases, very careful and systematic examination is necessary to avoid the pitfall of the diagnosis of hysteria.

Beyond these few statements no attempt will be made to cover the full range of differential diagnosis of muscular disorders.

It may be worth recaling from my personal experience, however, that some patients have presented unexpected problems, such as myopathy referred as a mental defect—a grossly overfed child with fat obscuring wasting having spent life lying in bed and denied any exposure to the environmental factors which induce learning; parietal wasting referred as lower motor neurone disease; rheumatoid athritis, not infrequently referred as a wasting disease of muscles; atonic cerebral palsy referred as myopathy; a cauda equina tumor referred as a case of distal myopathy, having no sensory or sphincter disturbance; and a recent case of polymyositis presenting with an almost faultless history of intermittent claudication.

Conclusion

Such is the clinical problem. Diseases of muscle present in many guises, which are unmasked only by a meticulous application of the established clinical neurological discipline. The long list of neurological scientists which may be called to aid in the ultimate diagnosis bears testimony to the very broad front of advancing neurology and the considerable dimensions of personnel and equipment needed to support it. A comparably increased burden and responsibility falls upon the clinical neurologist to select his material accurately so as not to deploy this valuable and expensive force needlessly.

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DUCHENNE-TYPE DYSTROPHY: SELECTIVE ASPECTS OF DIAGNOSIS AND MANAGEMENT

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Sydney

Duchenne-type dystrophy has been distinguished from other genetically determined myopathies by an onset in early childhood, relentless progression, a consistent distribution of weakness, and frequently, selective muscle enlargement and joint contractures. In addition, the genetic transmission is usually as a sex-linked recessive with expression in the male. However, the majority of cases are sporadic (Walton and Nattrass, 1954), and in these, the diagnosis rests almost entirely on the pattern of clinical involvement, an aspect that has received relatively little attention in recent times when compared with the detailed description of, for instance, Erb (1894).

It is the purpose of this communication to review selective aspects of this disorder based on the findings in 25 male patients with an age-range of five to 20 years.

INHERITANCE

Six patients had affected brothers and/or male relatives. Twelve patients had one or more unaffected male siblings. Two patients were adopted and no familial details could be obtained, while the rest had only female siblings.

CLINICAL PRESENTATION

In all cases, attention was drawn to the disorder before the age of seven years by difficulties in walking and arising from a sitting position. Nineteen children were recognized by their parents as abnormal between the ages of two and four years. Enlargement of the calves was noted in 17 children.

The initial progress of the disorder was slow in the majority, and the transition period during which the arms also became affected, usually passed unnoticed. Most of the children became chair-bound within 10 years of the onset, and only five patients were still ambulant when first examined.

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DISTRIBUTION OF MUSCLE WEAKNESS

All patients in this study were old enough to co-operate actively in muscle testing. Initially, the M.R.C. (1943) muscle strength ratings were used, but these were found to be unsatisfactory in advanced cases because of the non-linearity of the gradings, which allowed significant differences in performance to pass unrecognized. A modified scale (Fig. 1) was therefore adopted and found to yield consistent results on repeated testing, which correlated more satisfactorily with the overall functional status of the patient.

MUSCLE STRENGTH RATING

8: Normal power
7: Slight weakness
6: Moderate weakness
5: Severe weakness
4: Movement complete against gravity
3: Movement complete with gravity eliminated
2: Movement incomplete with gravity eliminated
1: Flicker of movement
0: No visible movement

FIG. 1—Expanded M.R.C. (1943) scale of muscle strength.

The distribution and severity of muscle weakness is summarized in Fig. 2. The ocular and bulbar muscles were always normal. Difficulty in burying the eye lashes completely was frequently a feature, but this was the only clinical evidence to suggest facial involvement. The extensors of the neck and plantar flexors of the feet were normal, or nearly normal, even in the most advanced cases. Generally, the flexors of

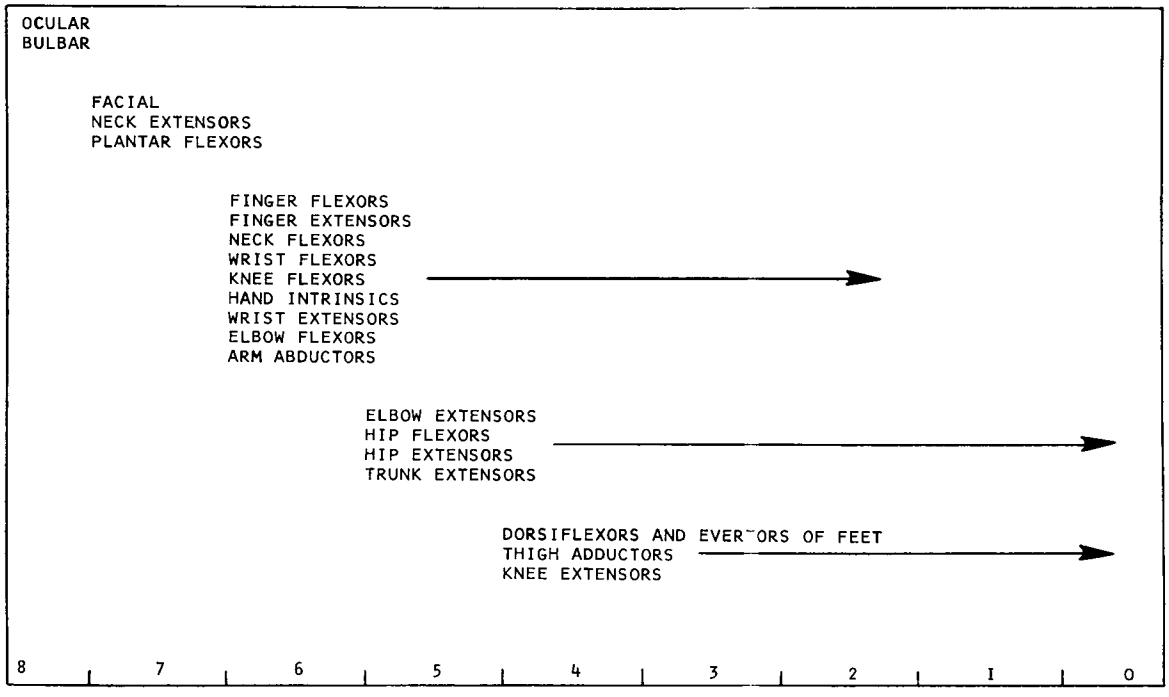

FIG. 2—Relative severity of muscle weakness in Duchenne-type dystrophy: Muscles with normal power are grouped on the left. In any one column, the muscles are arranged in order of decreasing strength from above downwards. Those muscle groups which weaken further as the disorder advances, are identified with an arrow. The figures refer to the muscle power ratings listed in Fig. 1.

the limbs were stronger than the extensor muscles, but this distinction could not be made with confidence in very advanced cases or in the presence of severe contractures. The adductors of the thighs and the quadriceps muscles were generally the most severely affected and in some advanced cases, no contractile power was detectable clinically. Another constant finding was the relatively early and severe involvement of the neck flexors and the intrinsic muscles of the hands.

Although some muscles were occasionally described as enlarged, the term "pseudohypertrophy" was avoided unless there was accompanying weakness.

In most respects, the findings are very similar to those described by Tyler and Stephens, 1951, and Walton and Nattrass, 1954. However, the neck extensors and calf muscles were thought to be normal or slightly weakened even in very advanced cases, whereas the intrinsic muscles of the hands were significantly weakened at an early stage. Although it has been shown that clinical testing gives only an approximate guide to the degree of myopathic involvement (Pearson, 1962; Bonsett, 1963), the finding of such a consistent pattern of muscle weakness has to be regarded as one of the most distinctive features of the disease. On the basis of a post-mortem anatomical study, Bonsett suggested that the selective muscle involvement was the result of the physical stresses and total workload to which certain muscle groups were subjected. Such factors could easily influence the rate of degeneration of muscle fibres whose ultimate destiny had been otherwise determined but, since an identical pattern of muscular weakness does not develop in other progressive multifocal myopathies, alternative explanations must be sought.

Tendon Reflexes

The tendon reflexes were absent in 11 patients, but the Achilles reflex was retained in the rest of the patients in whom the other tendon reflexes were either depressed or absent.

Contractures of varying severity were present at the ankles in all cases, but were an inconstant finding at other joints. Thus, in patients who were chair-bound, these were also present at the knees and in about half of the cases, flexion contractures eventually developed at the elbows. In some advanced cases, the hips, wrists and fingers were also involved. It was clear that the disproportionate involvement of opposing muscle groups acting at a joint was the major factor determining the development of a contracture (Walton and Nattrass, 1954), but the degree of immobility at such a joint was also important (Fig. 3) and probably accounted for individual differences.

In five patients, there was some degree of right bundle branch block.

Serum Enzymes

The serum creatinine phosphokinase and aldolase levels were elevated in all cases. The serum levels of S.G.O.T. were abnormal in 90 per cent and the values of lactic acid dehydrogenase were abnormal in only 60 per cent.

Electromyography

Routine muscle sampling revealed the characteristic pattern of a myopathy, with an increased incidence of short duration and polyphasic motor units which recruited more rapidly than normal during the initiation of effort, and whose overall amplitude tended to be lower than normal. In 22 patients, motor and sensory nerve conduction studies were also performed, and these invariably fell within the normal range. It was noted, however, that in the

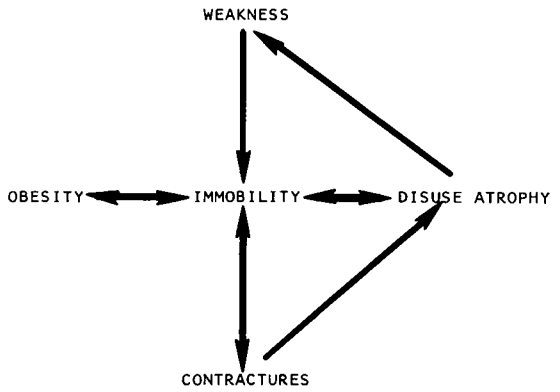

Fig. 3—A diagrammatic illustration of several factors influencing the functional status of affected muscles.

Intelligence

Wechsler Intelligence Scale tests were applied to 12 patients. Of these, the Full Scale Performance rating was less than 100 in nine and more than 100 in three. This finding supports the clinical impression that the majority of sufferers fall within the dull-normal range of intelligence.

Electrocardiographic Examination

Electrocardiograms were recorded in 20 patients; of these, 15 were regarded as normal.

majority of cases the velocity of the mixed nerve action potential recorded at the elbow, following stimulation of the nerve trunk at the wrist, exceeded that of the motor conduction velocity by a factor greater than that determined in normal subjects (Preswick and Jeremy, 1964). When this difference was sufficiently great, two distinct peaks were recorded which corresponded to the contribution of the ortho-dromic sensory volley and the slower antidromic motor volley (Fig. 4). The significance

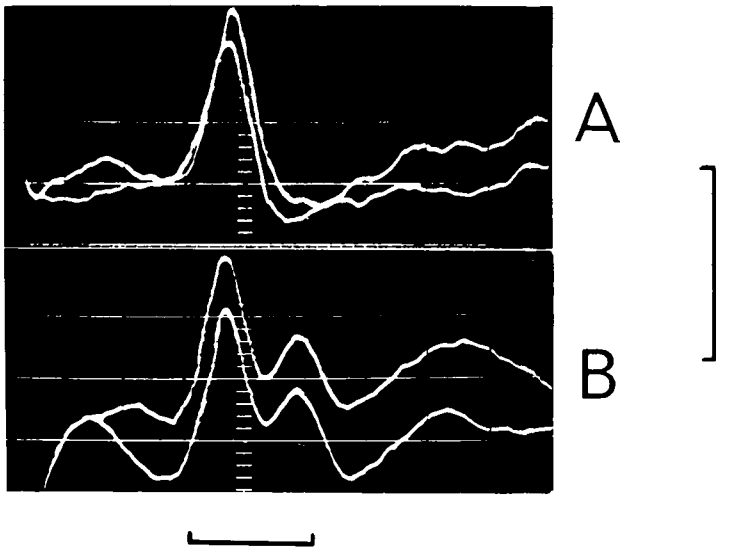

FIG. 4—Afferent volleys recorded over the median nerve trunk at the elbow following stimulation at the wrist. In (A), the nerve action potential was recorded when the stimulus was below the motor threshold and is therefore a pure sensory volley. In (B), a supra-maximal stimulus was used and two negative peaks are evident in the mixed nerve action potential. The second peak represents the anti-dromic motor volley. Calibration: 2 msec and 50 uV.

of this finding is not immediately obvious, although it could imply that the calibre of the largest motor nerve fibres decreased following the degeneration of the muscle fibres which they had innervated.

MUSCLE BIOPSY

Some histological specimens were unavailable at the time of examination, but had been examined elsewhere and considered to be diagnostic of a myopathy. In others, a muscle biopsy was obtained from either the calf or the tibialis anterior muscle, and when examined by traditional techniques, the diagnosis of a primary myopathic process was made with confidence in all cases.

THERAPY

Currently, therapeutic regimes involving a wide variety of drugs have proved to be valueless (Pearce, *et al.*, 1964). Reference to Fig. 3 will show that beneficial results can only be expected from activity, and it has been my experience that progressive, resistive exercises, aimed at promoting hypertrophy of surviving muscle fibres, yield the most rewarding results. Since such exercises are tedious, they should be applied only to the most functionally useful muscle groups and never to the stronger of two opposing muscle groups in the presence of any degree of contracture.

Patients should be kept ambulant as long as possible, with or without the aid of long-leg-irons with knee straps, however great the difficulty, since the transition towards being totally chair-bound is accompanied by a tremendous decline in morale, and consequently, performance.

Developing contractures should be anticipated and prevented by intermittent stretching and splinting, together with resistive exercises applied to the extensor muscles. In those patients who are already chair-bound, spinal and thoracic deformity may be prevented by adequate lateral padding in the sitting position. In some cases, a spinal brace may be helpful provided that this is carefully constructed with a minimum of strapping so as not to impede respiratory excursions. Breathing exercises are desirable throughout because of the progressive

decrease in vital capacity and the ever-present risk of succumbing to respiratory insufficiency, which may be precipitated by infection or retained secretions.

Finally, while it is difficult to maintain a cheerful and optimistic attitude in the face of an advancing process whose termination is so predictable, this is necessary if parental co-operation is to be maintained in efforts to retain some potential muscular activity until specific therapy becomes available.

Conclusion

Selective clinical findings in 25 patients with Duchenne-type muscular dystrophy have been discussed and the more useful, physical therapeutic measures outlined.

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Discussion

Dr. Kakulas: I would just like to ask Dr. Preswick if, in his very careful analysis of the degree of involvement of muscle groups, whether he gained the impression that the muscles which were under the greatest work load, or the greatest additions of work load, seemed to be more involved or not by the dystrophy? Was there a factor responsible for this distribution?

Dr. Preswick: Mr. Chairman, Bonsett was the first pathologist to do a full muscular survey from necropsy of a patient with Duchenne dystrophy. It was his contention that the greatest degree of wasting and weakness was proportional to the work load. If one examines his findings there is something in what he says, but I don't think that he is entirely right because the muscles that have a constant work load, for instance the neck extensors which maintain the head against gravity, are normal throughout. Finally, when these children become inactive their weakness progresses at a far greater rate than otherwise. I am certain that there is a clue for the pathologists and the anatomists in that distribution of weakness. For instance, in the calf muscles there is a far greater proportion of red fibres than in any other muscles within the body, and this type of selective muscle weakness is something that occurs in all the myopathies, but may not be clinically recognizable; this is explained by Pearson's findings written up in *Brain* in 1962, that one needs to have involvement of at least 50 per cent of muscle fibres before any weakness is clinically detectable. Clinically, we have gone as far as we can go. I think the answer will come from the laboratory.

Dr. Burke: I may have missed this in Dr. Preswick's comments about other special findings, but did he have anything to say about the E.E.G. or incidence of epilepsy in these people? Three of my own series had epilepsy and abnormal E.E.G.'s.

Dr. Preswick: Mr. Chairman, we did not do any E.E.G.'s. I cannot recollect one patient with epilepsy, nor do I know anything of E.E.G. findings in these.

Dr. Lorenz: Mr. President, Walton in his work has had published the E.E.G. findings in various myopathies, and I think the only condition where E.E.G. abnormalities and epilepsy were commoner than in the normal population was dystrophia myotonica. I think that in the other form of muscular dystrophy there was no significant rise in E.E.G. abnormality.

McARDLE'S DISEASE: THREE CASES IN AN AUSTRALIAN FAMILY

J. F. HAMMETT, PATRICIA BALE, L. S. BASSER, and F. C. NEALE

The energy required for muscular contraction is largely derived from the breakdown of glycogen and subsequent metabolism of the resulting glucose-1-phosphate fragments. Despite the many enzymes required for this process of anaerobic glycolysis, few disorders are known in which there is a defect in the pathway. The segment about which we are concerned in this paper is that which is responsible for the synthesis and degradation of the storage substance, glycogen.

According to an annotation in *The Lancet* of September 11, 1965, there are now seventeen cases on record of muscle disease due to a disturbance in glycogen metabolism starting in, or persisting into, adult life. The first three cases reported showed similar clinical features, weakness, stiffness with aching and cramps in muscles after exercise being predominant. In one instance there was associated myoglobinuria and in each of the three there was a failure of the lactic and pyruvic acid in the venous blood to rise after ischaemic exercise.

We wish to deal with a family in which three sons have such a clinical history as to suggest an inherited disorder of muscle metabolism. Noel, Barry and Andrew are three sons in a family of nine. The eldest of these, Noel, aged 19 years, presented to Dr. L. Basser in November 1964 with a seventeen-year history of recurrent, severe, exercise-induced muscle cramps, associated at the time of presentation with episodes of pigmenturia.

Since the age of two, he had been thought a lazy child, wanting to be carried more than his slightly older brother. A diagnosis of acute rheumatic fever was made at one stage and enforced bed rest for six weeks certainly abolished his muscular pain. When, at seventeen years of age, after an episode of moderate exertion, the passage of dark urine containing benzidine-positive material and protein was noticed, he was at first though to be suffering from glomerulonephritis. However, the significance of exercise-induced pain and pigmenturia was later recognized, and laboratory studies characterized the urinary pigment as myoglobin.

Apart from tender, tense muscles following exertion, there were no abnormal signs on physical examination, and at no time has objective muscle weakness been demonstrated in the absence of exercise-induced pain.

Barry, aged 17 years, had a history similar to that of Noel and his parents had long recognized his low effort tolerance. Until last year, however, he had been able to perform moderate exercise without marked discomfort and only recently had he shown myoglobinuria after sustained vigorous exertion.

The youngest afflicted child is Andrew, now aged 11 years. He has tired easily and complained of muscle cramps after exercise since the age of three. However, he had never passed myoglobin in his urine and until we saw him, enjoyed a game of soccer each week, although he played as "goalie", and never took "the kicks".

The other siblings, two boys and two girls aged 20, 13, 12 and 8, were free from any symptoms of muscle disease and all seven children showed normal physical development.

There is no consanguinity for at least three generations, nor any known maternal or paternal relatives suffering from similar disease.

The process of investigation of these subjects occurred in two stages. First, an attempt was made to identify the urinary pigment and, later, tests were carried out to determine the underlying metabolic defect.

To obtain samples after exercise, N.W. was subjected to twelve deep knee bends under supervision and serial samples of serum and urine collected. Three main features emerged:

(a) The rapid onset and short duration of pigmenturia and proteinuria.
(b) Lack of discoloration of the serum despite intense urinary pigment excretion.
(c) The pigment was positive to haem-testing.

Such heavy pigmenturia is seen most commonly in three conditions: haemoglobinuria, porphyria and myoglobinuria.

The clinical features suggested that the urinary pigment was myoglobin. To establish this by biochemical methods was then necessary.

The recognition of myoglobin by spectroscopy is difficult as the absorptive maxima at 582 and 548 closely approximate those of haemoglobin (582 and 542). Other methods depend chiefly on differences of molecular size to distinguish between the muscle oxygen storage protein (M. Wt. 17000) and haemoglobin (M. Wt. 68000).

Blondheim (1957) suggested a salting-out method, using 80 per cent saturation of a sample of pigmented urine with ammonium sulphate. The heavier haemoglobin molecule is usually precipitated out of solution while the smaller myoglobin remains in solution. However, both false positive and false negative tests have been reported. By this test, the urinary pigment in our cases remained in solution.

Ultrafiltration, using pores of appropriate size will allow only myoglobin to pass and on ultracentrifugation the urinary pigment may be found to have a sedimentation coefficient of 2 in the case of myoglobin or 4 should it be haemoglobin.

A simpler technique than these is available. On cellulose acetate electrophoresis of the serum of one of the two subjects showing myoglobinuria, a band of haem-staining pigment was seen to migrate in the medium gamma position.

Haemoglobin when present in the serum becomes attached to the haptoglobins and travels in the slow $\alpha 2$ position. Before the individual's haemoglobin-binding capacity has been exceeded some haem-staining material is seen in the β zone, attached to the haematin binding protein "haemopexin" and some is seen in association with albumin. Myoglobin is not bound by the serum proteins and there was a significant difference in the serum proteins pattern of a patient with haemoglobinuria and that of these subjects, revealed by immunoelectrophoresis.

By these means it was shown that myoglobin was present in the serum and the urine and hence it may be deduced that muscle cell lysis was occurring.

Confirmation of this was obtained by serial enzyme measurements on the same serum samples. Table 1 illustrates the results obtained for Noel's serum aldolase, creatine phosphokinase and lactic dehydrogenase levels.

You will see that the pre-exercise values for lactic dehydrogenase were within normal limits, but five hours after exertion were increased seven fold. The most impressive rise occurred in creatine phosphokinase figures. Blood urea nitrogen, potassium, haemoglobin, haemotocrit and white cell count estimations on the serum samples showed no changes.

Microscopic examination of each urine sample was done at once, and failed to show more than 0 to 3 erythocytes per high power field on any occasion.

A diagnosis of Idiopathic Familial Recurrent Exertional Rhabdomyolysis was thus reasonable. More than one hundred cases of this condition have been described.

Attempts were then made to find the cause of the muscle breakdown.

In view of the excellent work done by McArdle in 1950 on a patient at Guy's Hospital in whom the first metabolic lesion of muscle to be specifically suggested was present, we wondered whether these patients might suffer from a similar defect in phosphorylase activity.

Evidence for an abnormality in the pathway of anaerobic glycolysis was obtained by measuring serum lactic acid and pyruvic acid levels before and after ischaemic exercise.

No rise in these values occurred in the post-exercise serum from Noel, Barry or Andrew. Other members of the family exhibited a normal rise varying from 200 to 500 per cent of the pre-exercise levels except in one instance.

For more specific enzyme histochemical studies, each member of the family agreed to a muscle biopsy. Dr. Patricia Bale was able to

recognize specimens from the patients' muscles amongst those from apparently normal subjects.

Thus it was demonstrated that in Noel, Barry and Andrew there is a deficiency of muscle phosphorylase activity, and that they are three examples of a rare inborn metabolic disorder of glycogen metabolism, which was first recognized by McArdle in 1950.

In 1957, Schmid and Mahler reported a case with myoglobinuria in which a similar glycogenolytic defect in muscle was demonstrated. Pearson (1961) described a further case (this time the patient was a weight lifter) and detailed the very neat metabolic studies performed. This patient showed great increase in work tolerance following the intravenous administration of sufficient glucose to cause a rise in blood sugar levels to above 110 mg per cent. Continuous infusion of sodium lactate provided a measureable, but unpredictable improvement in work output in contrast to the lack of effect of infusion of either saline or bicarbonate. Infusion of fructose in 10 per cent solution produced a response equal to or better than that observed with glucose, and serum fructose levels of about 40 mg per cent were obtained. Conversion of fructose to glucose may have occurred, although as the blood glucose figure did not rise above a basal level of 80 mg per cent, this may not have been the case.

There was improvement also following fat emulsion infusion that could not be attributed to the vehicle used—in this instance 4 per cent dextrose.

No benefit was derived from infusions of 5 per cent glycerol in saline or 5 per cent galactose, nor was insulin of value alone or in combination with glucose.

These workers next undertook extensive *in vitro* studies of muscle biopsy specimens, and confirmed:

(1) a four-fold increase in muscle glycogen content;

(2) ready utilization of glucose -1- phosphate and hence no defect in phosphoglucomutase;

(3) absence of any phosphorylase a and b activity;

(4) an intact UDPG system for glycogen synthesis.

In 1961, at the time of writing their paper, Pearson, Rimer and Mommaerts considered only one other case had been clearly proven to be due to complete phosphorylase deficiency, and McArdle had seen no other examples of the disease bearing his name. In none of the reported cases had evidence for an inherited disorder been found.

Then Schmid and Hammaker (1961) reported further studies on the family of their previously described patient. His mother and eight siblings were available for study, and all but the 85-year-old mother underwent an ischaemic exercise test. The founders of this family were first cousins. There was good clinical and laboratory evidence that two sons suffered from McArdle's disease, and the second-hand history available on a sister suggested that she may have also been a sufferer. The authors concluded that the disorder appeared due to a single, completely recessive rare autosomal gene. They suggested that management in mild cases should be to limit physical exercise, while in the more severe and disabling disease, the production of hyperglycaemia in the presence of adequate insulin appears to afford substantial though transient improvement.

Mellick *et al.* (1962) reported the fourth case of muscle phosphorylase deficiency. An ischaemic exercise test on this patient was abnormal, and muscle biopsy with appropriate staining failed to show evidence of phosphorylase activity. In this patient, however, there was no increase in muscle glycogen, and it seems likely that there was at least one other associated metabolic defect. Apart from a possible but slight increase in effort tolerance following exhibition of fructose, no effective therapy was found and in particular, glucose administration was not helpful.

Their suggestion that 30-45G fructose be given three times daily and before exercise seems unjustified by their findings.

The three cases here reported are instances of a rare, inherited, metabolic disorder of muscle, McArdle's disease. There is growing evidence that other enzyme deficiencies are the basis for certain myopathies, and the field is fertile for a physician who is prepared to adequately investigate patients with muscle disorders to plant his eponym.

TABLE 1

SERUM ENZYME RESULTS OF TWO PATIENTS

Date and time	Lactate dehydrogenase	Aldolase	Creatine phosphokinase
B.W.			
13.5.65 11 a.m.	403	15·3	10·75
13.5.65 12 noon	420	17·1	22·4
13.5.65 1 p.m.	668	14·0	97·5
13.5.65 2 p.m.	1200	39·5	209·4
13.5.65 3 p.m.	1380	48·0	237·4
13.5.65 4 p.m.	880	56·0	237·4
14.5.65 8 a.m.	960	77·5	—
N.W.			
13.5.65 11 a.m.	317	13·8	11·8
13.5.65 12 noon	417	14·8	43·7
13.5.65 1 p.m.	980	8·9	212·8
13.5.65 2 p.m.	1810	67	429
13.5.65 3 p.m.	1520	95	416·6
13.5.65 4 p.m.	2170	108	—
14.5.65 8 a.m.	1630	183	—
Normal	233–442 (Karmen units)	1–2·5 Method of of Sibley & Lehninger	0·75–1·5 M moles creatine/ ml/hour

TABLE 2

RANDOM SERUM SAMPLES OF FAMILY

Date and time	Lactate dehydrogenase	Creatine phosphokinase	Aldolase
Mr. W.	334	0·60	4·1
Mrs. W.	325	0·39	2·2
Noel	317	11·8	13·8
Barry	403	10·75	15·3
Andrew	585	2·85	50·0
Geoffrey	290	0·61	2·7
Colleen	384	0·56	2·4
Jennifer	550	0·81	2·7
Ken	399	0·30	4·0
Normal	233–442 Units	0·75–1·5 Units	1–2·5 Milliunits/ ml

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TABLE 3

VENOUS LACTIC ACID LEVELS
(mg %)

	Pre-Exercise (2·5 mins. ischaemic exercise)	Post-Exercise With Tourniquet	Post-Exercise Without Tourniquet
Mr. W.	11·3	11·2	22·8
Noel (only 1·5 mins. exercise)	13·5	11·0	8·8
Barry	10·8	12·5	10·8
Ken	4·5	7·9	15·0
Jennifer	2·9	4·6	20·4
Colleen	1·6	5·8	—
Andrew (1)	17·9	15·8	13·4
(2)	10·7	10·0	
Mrs. W.	11·1	51·5	32·2
Geoffrey	16·5	18·4	—
Control (1) (J.F.H.) (2)	5·8	40·9	35·4

Normal = 6·16 mg %.

DISCUSSION

Dr. G. Preswick: Mr. Chairman, I should like to inform Dr. Hammett that there is yet one other patient in Australia who has been almost completely investigated by Dr. Woolridge and others. What I would like to know is why you think there is this muscle lysis that clearly must account for the tremendous increase in serum enzymes and the myoglobinuria that occurs with what appears to be trivial exercise. How can this single defect produce so much devastation, if this were the only defect, with so little effort?

Dr. Hammett: I think that perhaps some of the explanations that have been given for this massive muscle lysis are a little facile. There is undoubtedly impaired oxygenation of the cell, and it may well be that maintenance of the

integrity of the cell wall depends on continuous metabolic activity. I have been impressed by the story of thirst that these boys experience associated with their exercise, and once or twice they have been known to drink many pints of water once they start to get their muscle action cramps. I think there are very significant fluid shifts in this disease, but whether they come before or after the underlying cause of the lysis, I am not certain.

Dr. B. A. Kakulas: If I may make a comment and ask a question. With regard to the morphology of the muscle biopsy findings, I think it is true to say that the vacuoles themselves are quite non-specific, and that they are observed in a number of diseases including periodic paralysis, but also commonly as an artefact; but the vacuoles observed in muscle phosphorylase deficiency have been shown to be due to the presence of glycogen. The question I have is, in the biopsy of your patient with the myoglobinuria, was there evidence of muscle fibre necrosis in the form of macrophage activity as one might expect with the tremendous rise in enzymes parallelling the myoglobinuria?

Dr. Hammett: I have not done Dr. Bale justice, in that I have not shown all the slides that she made. The biopsy from the boy most seriously affected shows relatively little muscle necrosis and certainly no cellular infiltration. The biopsy from the second case shows significant muscle cell breakdown with fragmentation. There is no significant cellular infiltration in any of the specimens.

Dr. Game: First of all may I ask, apart from myoglobinuria as seen with other recognized conditions of muscle destruction, is there still a specific entity of paroxysmal myoglobinuria apart from McArdle's disease? Secondly, you did not stress the familial aspect of this. I think I have read of one other familially linked case. Are there others?

Dr. Hammett: There is a group of disorders that are a little like the cardiomyopathies perhaps, for which we have no other better name than paroxysmal myoglobinuria. Some are associated with exercise, some with the intake of alcohol, some with barbiturate intoxication, some following crush are more obviously secondary, but some appear to be primary, and we don't know the underlying biochemical disease. I am very suspicious that some are labelled thus and investigation ceases. The only well-established family is that of Schmidt and Hammaker. Two boys in the family, brothers of the first case described after McArdle's original case, were affected. Historical evidence suggests that a sister was also affected, but she was not available for study. Now this means that our present knowledge of the inheritance of the disease, which has been suggested to be a rare autosomal recessive, is very much one of speculation. The source is from Schmidt, who has only studied two cases in one family. He did study the whole of that family for three generations, and noted that the patients were offspring from a consanguinous marriage. The two subjects affected were in the second generation. In the third generation he studied some 31 patients, none of whom had any sign of the disease.

Dr. G. Lamoureux: I have noticed on your electrophoresis that there is one or two pre-albumins in these patients.

Dr. Hammett: Yes, with the cellulose acetate that we have been currently using, or at least that was being used at Sydney at that time, it was common to find two or three pre-albumins. With immunoelectrophoresis it is relatively common to find two pre-albumins, by the technique employed for this study.

AUTOIMMUNE ASPECTS OF MYASTHENIA GRAVIS*

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Two fields of endeavour, clinical and immunological, contemporaneously contributed to the evolution of the concept of autoimmunity in myasthenia gravis.

Clinical studies on 440 patients led Simpson (1960) to suggest that myasthenia gravis was an autoimmune disease: he observed that the course fluctuated, the incidence was twice as high in women as in men, the age distribution curve was smooth with a peak incidence between 10 and 30 years of age, and the thymus contained germinal centres or showed tumour formation. Simpson emphasized that thymectomy was beneficial, especially if performed during the first five years of the disorder, and stated that "the 'active' state of the disease is limited to this period and the subsequent course depends on the extent of the damage occurring then". Sixteen of Simpson's 440 cases had "rheumatoid" arthritis and there was a high incidence of diabetes mellitus and thyrotoxicosis in relatives. In some cases treatment with corticosteroid drugs proved of benefit.

Immunological studies relating to abnormalities in the level of serum complement led Nastuk, Plescia and Osserman (1960) to the concept that myasthenia gravis may be an autoimmune disease; a fall in the level of serum complement occurred in patients in relapse. In earlier experiments Nastuk, Strauss and Osserman (1959) had shown that serum from patients with myasthenia gravis had a cytolytic effect on frog sartorius muscle. Strauss *et al.* (1960) demonstrated serum autoantibodies which reacted by immunofluorescence with the lateral parts of the A-bands of skeletal muscle. These antibodies were characterized by Beutner *et al.* (1962) as a complement-fixing "S-antibody" reactive with skeletal muscle and a non-complement-fixing "SH-antibody" which reacted with both skeletal and cardiac muscle. The antigen present in the lateral parts of the A-bands of striated muscle is present also in the cytoplasm of "epithelial" cells of thymus (Van der Geld, Feltkamp and Oosterhuis, 1964). Striated muscle cells have recently been demonstrated in thymus of very young animals and man (Henry, 1966; Strauss, Kemp and Douglas, 1966; Feltkamp-Vroom, 1966), and the cells in the thymus which react with myasthenia gravis serum are now considered to be myoid in nature. Antibodies reacting with thymus and skeletal muscle are specific to sera from patients with myasthenia gravis with the rare exception that some cases of thymoma without myasthenia gravis also show reactivity (Strauss *et al.*, 1965). There is a 30 per cent incidence of positive muscle-thymus antibody tests with random cases of myasthenia gravis, and the incidence is 95 per cent when thymoma is associated with myasthenia gravis (Strauss *et al.*, 1966).

Serological work in the Clinical Research Unit has likewise demonstrated the presence of abnormal muscle-thymus antibodies in five of 17 patients with myasthenia gravis.

METHODS

Immunofluorescence tests for antibodies were performed on sera diluted 1/10 from 17 patients with myasthenia gravis. The sources of antigen were calf thymus, rat diaphragm, rat

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stomach wall, and human thyroid surgically removed from a patient with thyrotoxicosis. These tissues were snap frozen in a dry-ice alcohol mixture and sectioned at 4μ in a cryostat. Sections of stomach wall and thyroid were tested unfixed, and calf thymus and rat skeletal muscle were fixed in acetone for 10 minutes at 20°C. A fixed human blood film was used to test for antinuclear factor (Hasker, Mackay and Miller, 1965).

The indirect "sandwich" technique of Weller and Coons (1954) was employed in all immunofluorescence tests. The lower layer of the sandwich is the antigen in the frozen section; the middle layer is the test serum, and the upper layer is goat anti-human globulin labelled with fluorescein isothiocyanate (Microbiological Associates, Bethesda, Maryland, U.S.A.). Fluorescence under ultraviolet light, denoting binding of serum antibody to antigen, represented a positive result.

Results

Of the 17 sera tested from patients with myasthenia gravis, five (29 per cent) reacted with myoid cells of thymus and the striations of muscle, but none reacted with smooth muscle. Eight (47 per cent) gave positive tests for antinuclear factor, two reacted with the cytoplasm of gastric parietal cells and one with thyroid cytoplasm. Table I shows the incidence of positive reactions with various tissue antigens given by sera from patients with myasthenia gravis, other autoimmune diseases including systemic lupus erythematosus, lupoid hepatitis, pernicious anaemia and thyrotoxicosis, and blood donors.

Discussion

Autoimmune disease was defined by Mackay and Burnet (1963) as "a condition in which structural or functional damage is produced by the action of immunologically competent cells or antibodies against normal components of the body". Certain "markers" characterize autoimmune disease: these are a serum gamma globulin level greater than 1·5 gm/100 ml, demonstrable autoantibody, deposits in tissues of denaturated gamma globulin, accumulation of lymphocytes and plasma cells in tissues affected by the autoimmune process, therapeutic benefit from corticosteroid drugs, and association of the given disease with other autoimmune disorders in the patient or in related family members.

TABLE 1

Diagnosis	*No. of cases tested*	*No. of Cases with Positive Reactions with Specific Antigens in Different Tissues*					
		Striated muscle	*Thymic myoid cells*	*Thyroid epithelial cells*	*Gastric parietal cells*	*Smooth muscle*	*Nuclei*
Myasthenia gravis	17	5 (29%)	5	1	2	0	8 (47%)
Systemic lupus erythematosus	17	0	0	0	1	0	17 (100%)
Lupoid hepatitis	17	0	0	0	0	16 (94%)	16 (94%)
Pernicious anaemia	17	0	0	9 (53%)	14 (82%)	0	3
Thyrotoxicosis	17	0	0	16	4	0	4
Healthy subjects (blood donors)	100	0	0	4 (4%)	3 (3%)	0	3 (3%)

Immunofluorescence reactions of sera from patients with myasthenia gravis and other autoimmune diseases. It will be noted that antibodies against muscle and thymus are present only in myasthenia gravis, and that there is a high incidence (47%) of antinuclear factor in myasthenia gravis.

In myasthenia gravis the level of gamma globulin is not usually raised, being rarely above 1·5 gm/100 ml.

Circulating antibodies to thymus and muscle are present in the serum of 30 per cent of patients with myasthenia gravis, and have not been reported in any other diseases or in normal people (Strauss *et al.*, 1965); 30 per cent of sera from unselected cases of myasthenia gravis react with the lateral parts of the A-bands of striated muscle and with the cytoplasm of myoid cells of thymus. The antibodies are present in the immunoglobulin G fraction of serum, are placenta-permeable and are autoantibodies in that antigen-antibody reaction occurs between the patient's own serum and the patient's own muscle biopsy. Moreover Ricken (1966) showed that the autoantibodies of myasthenia gravis react in complement fixation and agar gel diffusion tests with myosin and to a lesser extent with actomyosin but not with actin. Myosin filaments are aggregated in the A-bands to form the dense banding seen by light microscopy, and myosin could well be the antigen in the myoid cells of the thymus.

Muscle biopsies taken from patients with myasthenia gravis have been shown by Beutner *et al.* (1966) to have gamma globulin bound to alternate striations, but only at the edges of the fibres near the sarcolemma.

The characteristic cellular reaction of myasthenia gravis occurs in the thymus. Thus 80 per cent of patients with myasthenia gravis show germinal centre formation in the thymus, a change rarely seen in other diseases. Fenichel (1966) reported lymphorrhages in 12 of 37 muscle biopsy specimens from patients with myasthenia gravis. He stated that the incidence of lymphorrhages was higher in muscle taken at autopsy, and was associated with muscle atrophy. Engel and McFarlin (1966) found lymphorrhages in only seven of 30 muscle biopsy specimens but in all 30, abnormalities could be demonstrated by histochemical means.

Von Reis, Liljestrand and Matell (1966) and Osserman and Genkins (1966) have shown that a short course of high doses of adrenocorticotrophic hormone (ACTH) can produce improvement in severe refractory generalized myasthenia gravis. Patients may suffer rapid deterioration and require special care during administration of ACTH, but after cessation of the drug, clinical improvement lasting three months and longer was observed in 50 per cent of cases. Osserman and Genkins (1966) observed only a slight fall in titre of antibodies to muscle and thymus even though clinical improvement was excellent.

Myasthenia gravis is frequently associated with other autoimmune diseases. In a recent review of 74 patients with myasthenia gravis, Downes, Greenwood and Wray (1966) reported coexisting lupus erythematosus in two cases, Sjögren's syndrome in one, proven rheumatoid arthritis in two, probable rheumatoid arthritis in three, and thyroid disorders in several cases. Simpson (1960) also observed similar associations.

Probably the most decisive evidence for autoimmunity in a given disease is the existence of a valid experimental model in animals, e.g. as exists in the case of "autoallergic" thyroiditis and "autoallergic" encephalomyelitis. Such a model has been developed recently in this Unit by Goldstein and Whittingham (1966) by immunization of guinea pigs with thymus or muscle in Freund's complete adjuvant (*vide infra*).

The reality of autoimmunization in myasthenia gravis is well accepted, but there is still controversy as to the significance of the muscle antibodies in pathogenesis, particularly since the site of the immune reaction in muscle does not correspond to the end-plate region. Simpson (1960) proposed that there may be an as yet undetected incomplete antibody reactive with an end-plate constituent, possibly acetylcholine. Van der Geld, Feltkamp and Oosterhuis (1964) claimed that the thymus was the site of an autoimmune reaction whereby an antibody was produced which cross-reacted with muscle. Strauss, who has pioneered the work on muscle antibody in myasthenia gravis, does not believe this antibody to be the cause of the myasthenic state (Strauss *et al.*, 1966). He considered that a thymus diseased by an unknown process elaborated a substance which interfered with metabolic processes in the region of the A-band; this substance not only produced the primary defect in neuromuscular transmission but acted as antigen to stimulate the production of antibody. Strauss stated "the presence of antibodies to muscle A-bands and thymus might, thus, merely be an incidental,

otherwise unimportant, concomitant of the entire process", and he thought the antibodies could even be protective rather than destructive. Strauss's hypothesis would explain three puzzling facts: (a) the relatively low (30 per cent) incidence of serum autoantibodies in patients with myasthenia gravis, (b) the presence of antibodies in patients with thymoma without myasthenia gravis, and (c) the facts of neonatal myasthenia gravis wherein babies of mothers with myasthenia gravis but no detectable antibody are myasthenic, and babies of myasthenic mothers with antibody are asymptomatic (Namba and Grob, 1966).

Recent experimental work in our Clinical Research Unit by Goldstein and Whittingham (1966) may clarify the nature of myasthenia gravis. Guinea pigs immunized with muscle or thymus in Freund's complete adjuvant developed a "myasthenic" block to neuromuscular transmission, and this block could be corrected by administration of neostigmine. There was evidence that the immunizing procedure set up an "autoimmune" thymitis and the damaged thymus presumably liberated a humoral neuromuscular blocking agent. Similarly in man germinal centre formation in the thymus is evidence of an autoimmune reaction—a thymitis—which damages the thymus and results in the release of a humoral substance which blocks neuromuscular transmission (Figure 1). On this concept the autoantibody to muscle is significant only in so far as it points to the existence of an autoimmune reaction directed primarily against a target antigen, the myoid cells, in the thymic medulla.

SUMMARY

The concept of autoimmunity in myasthenia gravis developed in 1960 from clinical studies by Simpson and immunological studies by Nastuk, Strauss and colleagues. The serum of 30 per cent of cases of myasthenia gravis contains autoantibodies to antigen(s) shared by

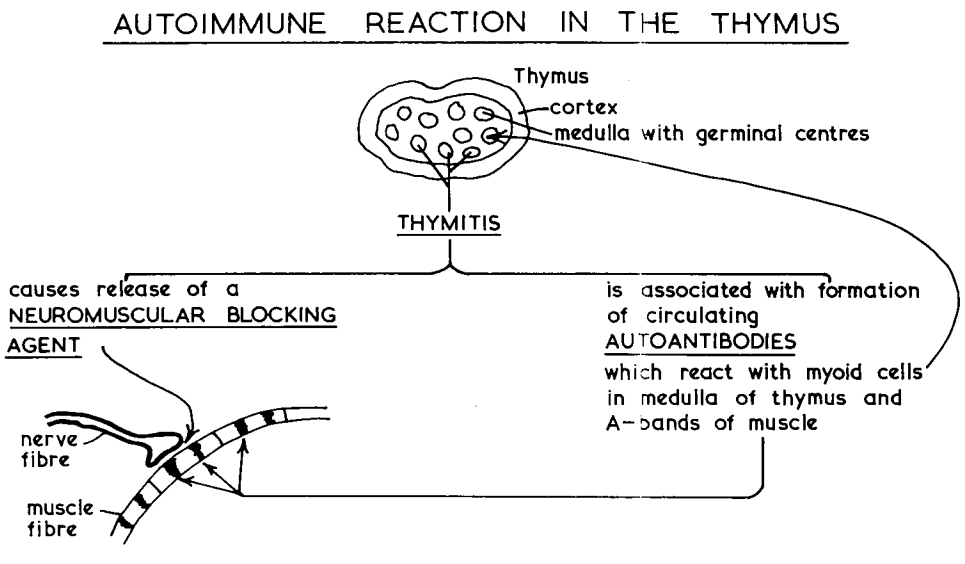

FIG. 1—A postulated explanation for the clinical and serological features of myasthenia gravis. It is suggested that a primary disorder in the thymus is perpetuated as an autoimmune reaction—a "thymitis"—and this is manifested by the formation of germinal centres in the medulla. This "thymitis" results in the release of a blocking agent and is associated with the formation of an autoantibody to thymic myoid cells which cross-reacts with skeletal muscle cells.

thymic myoid cells and the A-bands of striated muscle; there are germinal centres in the thymic medulla and lymphorrhages in skeletal muscle; there may be a response to ACTH; myasthenia gravis is occasionally associated with other autoimmune diseases; and an experimental model has been produced by immunizing guinea pigs with thymus or muscle in Freund's complete adjuvant.

The significance of the autoimmune reaction in myasthenia gravis is still controversial; the essential lesion is a thymitis, and it seems likely that the thymitis causes the myasthenic state through the liberation of a humoral substance which "blocks" transmission of nerve impulses at the neuromuscular junction. Myasthenic myopathy is probably due to poor transmission of nerve impulses and the damaging effect of autoantibody reacting at the A-band region of striated muscle.

ACKNOWLEDGEMENT

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DISCUSSION

Dr. J. F. Hammett: I have always been a little worried about some of the non-specific or cross-reactive properties of the antibodies concerned in these types of reactions. Antibodies are profitable in immunology for these two purposes, one specific and the other non-specific demonstrations, and we make use of them for various laboratory tests because of these two properties. Have you absorbed out the anti-A band antibody with muscle cell and

still find the immunofluorescent antibodies to thymus, or are these identical antibodies? Are they in fact gamma globulins or have you characterized the antibody further and is it a macroglobulin, and are you certain that this is a specific effect of this antibody rather than a non-specific cross-reactive one similar to the antibodies of lupus erythematosus?

Dr. Whittingham: The first question, can the antibody be absorbed by the muscle, and does it react against thymus; there is a shared antigen and the antibody can be completely absorbed by muscle or by thymus. The absorption is difficult with thymus because the antigen is present in the thymus in rather small quantities. It is an immunoglobulin G. It can be isolated and it is specifically in this component of serum. It is specific to myasthenia gravis serum. When testing for this antibody to the muscle and thymus, but especially to muscle, it is important to dilute. All normal serum will give this reaction to some degree. I do not think anyone has characterized the normal reaction because, quite frankly, it is a mess on the slide, but when you dilute to 1/10 of the serum you get a specific reaction, and these antibodies in myasthenia gravis will titrate up to a much higher titre and they are specific (Fig. 2a & b).

Dr. R. McD. Anderson: You showed a thymoma with germinal centres alongside. Is this the only time this has been reported? I have never seen it before.

Dr. Ian Mackay: This was a section sent to us from elsewhere, and it was not a case we studied. It was just fortuitous that this thymoma did have a bit of the so-called normal thymus attached to it. When thymectomies are done for tumors, what seems to come to the pathologist is just the thymoma and not the accompanying compressed, presumably normal thymus, so we do not often get a picture like this, but most of the pathologists experienced in thymus do make reference to the presence of germinal centres in a thymus which contains thymoma. I do not want to elaborate on Dr. Whittingham's findings—I think they speak for themselves—but we have been extremely interested in the two or three recent reports of muscle in the thymus. It is rather strange that muscle fibres should be present in thymus. They are apparently there in early life at least, and although we have never seen them, we have seen very convincing photographs, and going back over early literature, the greatest I suppose of the early thymologists, Hammar described them very clearly. He did not photograph them, but drew them—striated muscle fibres in the thymus of the chicken, frog, and man, and this gives us a reason now for this peculiar so-called cross-reactivity between the cells of the thymus and the skeletal muscle fibre.

Dr. G. Preswick: Could I ask Dr. Whittingham if this absorbed antibody could be located more precisely in the region of the A-bands? At what depth is this antibody absorbed? Could it be concentrated round the sarcotubular system, is it within the sarcoplastic reticulum or is it bound on myofibrils in this region?

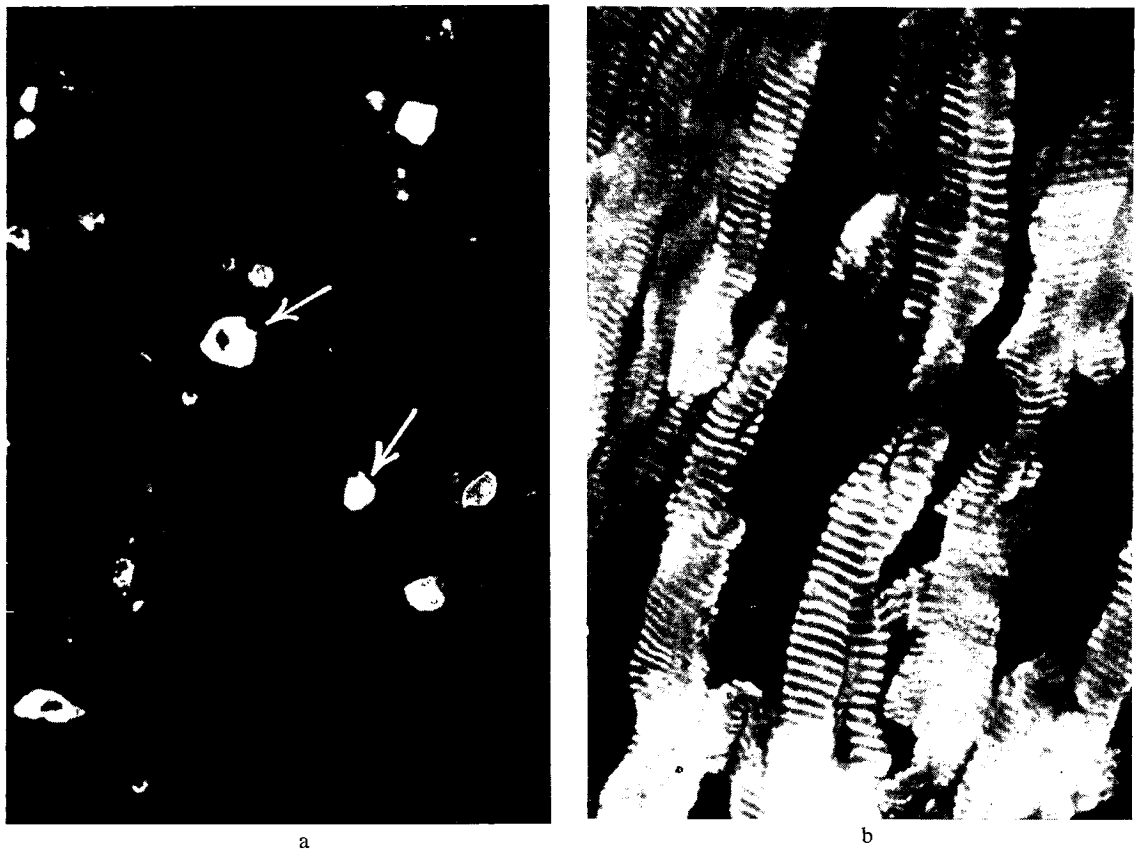
Dr. Whittingham: I have not done any work on this myself. I have only seen pictures. There is quite a definite band which seems to be on the lateral part of the A-band, but exactly where this fluorescence is located I do not know.

Dr. Preswick: There are fairly important implications in the exact location of this because Simpson sticks to the post-synaptic hypothesis to sustain everything else contrary to other views expressed by electrophysiologists that the defect is pre-synaptic and could still be along the excitation pathway rather than contractile or with a muscle disturbance, if this antibody has anything to do with it at all—with the phenomenon of myasthenia.

Dr. Lorenz: May I ask Dr. Whittingham if she considers the autoimmune hypothesis is the cause of myasthenia or perhaps an effect from damaged muscles or some other cause. Is this the primary explanation or the secondary phenomenon?

Dr. Whittingham: I think it is the primary cause of myasthenia gravis, and I think the greatest evidence for this is the germinal centre formation in the thymus.

Dr. Mackay: We are often confronted with this suggestion that the auto-antibodies which we feel are in some way implicated in the genesis of these diseases are consequential to damage, but Strauss has reported on a very



a b

FIG. 2a & b—Positive immunofluorescence reactions of a serum from a patient with myasthenia gravis with myoid cells of the thymic medulla (a), and the cross-striations of skeletal muscle (b).

large number of muscle-destroying diseases, including various types of myopathy, and in none of these has he been able to demonstrate any trace of auto-antibody to the A-bands of muscle, whereas in myasthenia gravis, where muscle damage is quite minimal, on ordinary light microscopy at any rate, the autoantibody is present in high titre, so I don't think we can say it is purely a result of damaged muscle. However, most of us interested in the autoimmune hypothesis are very worried by the fact that the antibody we are demonstrating does not seem to be relevant to the physiological site of the disease, and this is something where I think our common interests may help us to resolve the problem together.

Dr. Rail: I take it then that the A-band involvement is of a patchy nature, or does it involve all the muscle fibres in a selected section?

Dr. Whittingham: The technique I described was an indirect technique. In this particular test you can show that the antibody does react with all muscle fibres.

Dr. Game: Is there any explanation whatsoever as to why the different muscles are affected at different times? This is one of the things that always intrigues us as clinicians. Does your work throw any light on that question?

Dr. Whittingham: I think that would be best answered by a neurologist.

TYPE AND INCIDENCE OF LESIONS FOUND IN A HUMAN NECROPSY SURVEY OF SKELETAL MUSCLE

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Perth

In recent years greater interest in myopathic diseases has led to the appreciation that muscle may be affected in systemic disorders. It is well known to clinical medicine that weight loss with loss of muscle bulk is often the result of many serious diseases, including chronic suppurations, endocrine disorders and particularly malignant neoplasms. Therefore, a large survey of muscles from routine hospital necropsies was undertaken to investigate this problem further and thus define the spectrum of reaction of the muscle fibre to disease. Information of this type is also useful for the development of concepts of pathogenesis of muscle lesions.

Very few human postmortem surveys of skeletal muscle have been reported and most were undertaken to answer a specific question. The value of such studies is both epidemiological and definitive. Such information also provides a baseline for more accurate interpretation of muscle tissue obtained by biopsy.

One of the early reports of such a muscle survey is that of Clawson, Noble and Lufkin (1947). These workers concentrated on the presence of nodular, inflammatory and degenerative lesions in muscles obtained from 450 necropsies. Such interest was stimulated by the study of Steiner *et al.* (1946). Seven muscles were collected from each of the cases and evidence of inflammation was sought as well as various stages of degeneration. The muscles selected were the pectoral, sterno-cleido-mastoid, deltoid, diaphragm, intercostal, psoas and sacrospinalis. Of the 450 necropsies 118 disclosed inflammatory myositis. In 83 of the 118 cases (70 per cent) there were also degenerative changes. Atrophy, cytoplasmic changes or nuclear changes were noted in 256 (57 per cent) cases. One or more of the degenerative lesions or inflammatory nodular lesions were present in 293 (65 per cent). While either change occurred singly there was also definite overlap, which suggested that inflammation and degeneration resulted from a common cause. The inflammatory lesions were more common in the biopsy specimens of the deltoid muscle obtained in cases of rheumatoid arthritis than in necropsy specimens of the same muscle, but it was doubtful whether the lesions could be considered to be a specific reaction to an infective agent either of acute rheumatic arthritis or rheumatoid arthritis on morphological grounds. The lesions probably were part of the rheumatic and rheumatoid state. The sexes were equally involved, and they considered that no particular disease except rheumatic fever and rheumatoid arthritis seemed to increase the frequency of inflammatory and degenerative lesions. Lesions were more frequent when death occurred in the upper decades of life.

In 1952 Cruickshank reported on focal lesions in skeletal muscle and peripheral nerves in cases of rheumatoid arthritis and various other conditions following the work of Curtis and Pollard (1939-40) who were the first to describe perivascular collections of lymphocytes in muscle in rheumatoid arthritis. Cruickshank in 1952 found lymphorrhages and occasionally subacute arteritis in rheumatoid arthritis, but these changes were seen in other related diseases such as systemic lupus erythematosus and scleroderma more often than in rheumatoid arthritis, and the incidence in all other rheumatic diseases was not significantly less than in rheumatoid arthritis. They were also seen in approximately 50 non-rheumatic diseases of widely differing nature such as

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tuberculosis, poliomyelitis, malignant tumors, cirrhosis of the liver, nephrosclerosis, diabetes mellitus and even in a stillborn anencephalic foetus and in apparently normal individuals. Muscles from 93 cases of rheumatoid arthritis were examined as well as from 73 cases of other rheumatic diseases and 419 patients with non-rheumatic disease. He concluded that no lesion specific for rheumatoid arthritis was present and that the incidence of lesions could not be correlated with age, duration, activity or stage of the disease. He, therefore, considered the reaction to be non-specific and probably due to local influences rather than to a systemic factor such as a circulating toxin.

In 1959 Pearson reported on the incidence and type of alterations of muscle in a routine necropsy survey. Muscles from nine predetermined sites were sampled from a series of 110 necropsies and he recognized only three specific entities in the series. These were trichinosis, metastatic malignancy and polyarteritis nodosa. Fifty-four cases (49 per cent) showed an abnormality, including various degenerations and signs of muscle fibre regeneration, as well as interstitial or perivascular leucocytic infiltrations. There were most commonly observed in connective tissue disorders or in myasthenia gravis, but were also observed in a wide variety of unrelated diseases. Trichina were found in five cases and metastatic tumor nodules in 16 per cent of 38 cases with malignancy. He attributed the non-specific changes to the general loss of muscle strength and debility in the chronic disorders.

Marin and Denny-Brown (1962) described the changes in skeletal muscle associated with cachexia and confirmed the association of fatty-granulo-pigmentary degeneration as described by Klippel in 1889. This change was associated with severe cytoplasmic loss. Fragmentation of myofibrils developed into the cytoplasmic granules which appeared to pass through a fatty lipid stage in the process of dissolution, eventually becoming converted to free unsaturated fats.

In the present survey deltoid and quadriceps muscle from 397 consecutive necropsies performed at the Royal Perth Hospital were examined. The right deltoid and right vastus lateralis were sampled and a block 2 cm^3 was fixed in Susa and embedded in paraffin. Five micron sections were stained by haematoxylin and eosin (H. & E.), Van Gieson (V.G.) and Periodic Acid Schiff (P.A.S.) before and after treatment with diastase. Blocks embedded in gelatin were cryostat sectioned and stained with Oil red O. Microscopic examinations were undertaken before details of the necropsy findings were sought so that observations were not influenced by knowledge of the primary disease. Average muscle fibre diameter was measured and the presence of clumps, chains and centrally placed sarcolemmal nuclei, muscle fibre necrosis with macrophage reaction, degenerative changes, regenerative changes, interstitial and intra-fibre fat and glycogen and interstitial connective tissue were assessed and all the parameters were given an approximate quantitative grade from 1 to 4. After these data were entered the age, sex, nature of primary and associated diseases, together with the duration of the patient's illness, time spent in hospital, height and weight measurements were added to the chart. The findings were tabulated and a summary of these is presented in Tables 1 to 5.

Results were as follows. Of the 397 cases, 249 (63 per cent) showed abnormalities. One hundred and two (26 per cent) showed simple atrophy, i.e. average muscle fibre diameter was below 30 micra. Denervation atrophy was present in 51 (13 per cent) and inflammatory lesions with round cells, polymorphonuclear or plasma cell infiltrates, perivascular or interstitial in position were present in 39 (10 per cent) of subjects. Necrosis and degeneration was present in 40 (10 per cent) and 17 (4 per cent) showed other types of muscle change, including polyarteritis nodosa, polymyositis and fatty-granulo-degeneration.

The findings in the Circulatory Group of disorders are summarized in Table 1. Of the 136 cases in this group, 68 (50 per cent) were abnormal, 35 (26 per cent) showed simple atrophy, 13 (10 per cent) showed denervated atrophy and 7 (5 per cent) showed inflammation and 5 (4 per cent) showed necrosis and degeneration. Other abnormalities were detected in 8 (6 per cent).

In Table 2 the findings in the inflammatory group are given. Of the total of 81, 57 were abnormal (70 per cent) with 18 (22 per cent) showed simple atrophy, 16 (20 per cent) showed denervation atrophy, 9 (11 per cent)

TABLE 1

Summary of Findings in Circulatory Disorders.

Group	*Total*	*Number Abnormal*	*Types of Abnormality*				
			Simple Atrophy	*Denervation Atrophy*	*Inflammation*	*Necrosis and Degeneration*	*Other*
Myocardial ischaemic necrosis	64	27	13	3	4	4	3
Hypertensive cardiovascular disease	15	12	8	1	1	—	2
Large arterial aneurysms	8	7	4	1	1	—	1
Other types of heart disease	10	4	3	1	—	—	—
Cerebro-vascular disease	39	18	7	7	1	1	2
Totals	136	68	35	13	7	5	8

showed inflammation and 9 (11 per cent) showed necrosis and degeneration. Five (6 per cent) showed other lesions.

There were 122 patients in whom malignant neoplasia was the cause of death and of these 87 were abnormal (71 per cent), 35 (28 per cent) showed simple atrophy, 18 (15 per cent) showed denervation atrophy, 11 (9 per cent) showed inflammation, 21 (17 per cent) showed necrosis and degeneration and there were 2 (2 per cent) with other lesions (Table 3).

In Table 4 the results for other disease groups are given. These included rheumatoid arthritis, scleroderma, Hashimoto's disease and haemopoietic disorders, as well as a miscellaneous group. Of the 58 in the group 37 were abnormal (64 per cent) and 14 (24 per cent) showed simple atrophy, 4 (7 per cent) showed denervation and 12 (20 per cent) inflammation and 5 (9 per cent) showed necrosis, while 2 (3 per cent) showed other changes.

It is instructive to compare the findings within each of the groups. A higher incidence of abnormal findings was present in both the inflammatory and neoplastic groups with approximately 70 per cent in each. In the inflammatory group there was a slightly higher pro-

TABLE 2

Summary of findings in Inflammatory Group of Conditions.

Group	*Total*	*Number abnormal*	*Types of abnormality*				
			Simple Atrophy	*Denervation Atrophy*	*Inflammation*	*Necrosis and Degeneration*	*Other*
Inflammatory lung conditions	35	22	10	6	2	1	3
Septicaemia	6	6	2	2	1	—	1
Inflammatory kidney conditions	12	7	1	—	2	3	1
Conditions associated with peritonitis	21	13	3	4	3	3	—
Others	7	9	2	4	1	2	—
Totals	81	57	18	16	9	9	5

TABLE 3

Summary of findings in Malignant Neoplastic Group of Disorders.

Group	*Total*	*Number Abnormal*	*Types of abnormality*				
			Simple atrophy	*Denervation atrophy*	*Inflammation*	*Necrosis and Degeneration*	*Other*
Bronchogenic carcinoma	16	12	7	1	—	4	—
Gastric carcinoma	13	10	5	3	—	2	—
Breast carcinoma	10	7	2	3	—	2	—
Prostatic carcinoma	9	6	—	3	2	—	1
Pancreatic carcinoma	7	5	2	1	—	2	—
Colonic carcinoma	15	11	6	1	1	3	—
Uterine and ovarian carcinoma	7	2	1	1	—	—	—
Kidney and renal tract carcinoma	14	13	4	3	2	3	1
Other carcinoma	31	21	8	2	6	5	—
Totals	122	87	35	18	11	21	2

portion with denervation atrophy (20 per cent) as opposed to 15 per cent in the neoplastic group, but in the latter group there were 17 per cent where necrosis and degeneration as compared with 11 per cent in the inflammatory group. Figs. 1 to 8 illustrate many of the histological findings in the survey.

It was noteworthy that simple atrophy reflected weight loss and that denervation atrophy was manifest in many patients with diabetes mellitus as well as those with poor nutrition. The high incidence (15 per cent) of denervation atrophy in the neoplastic group may also be significant, reflecting the incidence of motor neuropathy in such patients, possibly related to the underlying cancer. The non-specific changes of necrosis and inflammation are due to the presence of rheumatoid disease, connective tissue disease and possibly endocrine disorders (Åström *et al.*, 1961; Hed *et al.*, 1962). Furthermore, the findings have underlined the very high incidence of subclinical polymyositis in

TABLE 4

Summary of findings in other disease groups.

Group	*Total*	*Number Abnormal*	*Types of abnormality*				
			Simple atrophy	*Denervation atrophy*	*Inflammation*	*Necrosis and Degeneration*	*Other*
Rheumatoid arthritis	6	2	—	1	1	—	—
Scleroderma	2	1	1	—	—	—	—
Hashimoto's disease of the thyroid	14	10	5	1	2	2	—
Haemopoietic and related diseases	19	9	3	2	3	1	—
Miscellaneous	17	15	3	—	6	2	2
Totals	58	37	14	4	12	5	2

TABLE 5

Summary of findings for all Groups.

Group	*Total*	*Number abnormal*	*Types of abnormality*				
			Simple atrophy	*Denervation atrophy*	*Inflammation*	*Necrosis and Degeneration*	*Other*
Circulatory disorders	136	68	35	13	7	5	8
Malignant neoplasms	122	87	35	18	11	21	2
Inflammatory conditions	81	57	18	16	9	9	5
Other disease groups	58	37	14	4	12	5	2
Totals	397	249	102	51	39	40	17

patients with visceral carcinoma if this be defined as muscle fibre necrosis, regeneration and leucocytic infiltrates. It is possible that such patients did have clinically significant proximal muscular weakness overshadowed by the fatal disease. All the "wasting diseases" revealed simple atrophy with myofibrillar loss, endomysial condensation, nuclear darkening and aggregation with some loss of subsarcolemmal nuclei. Such findings were similar to those

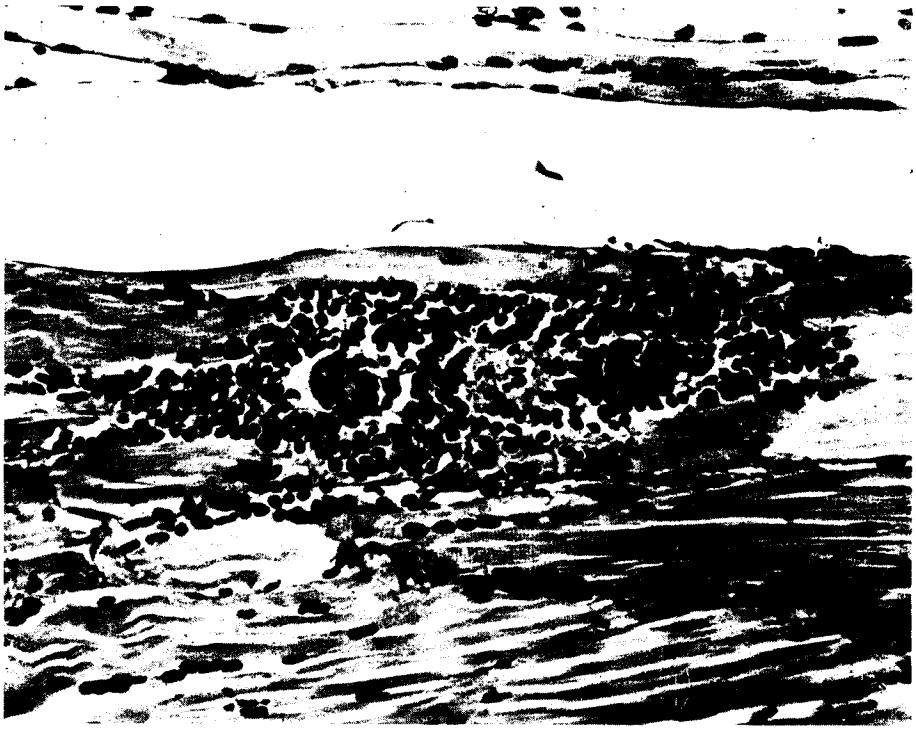

FIG. 1—Muscle fibre necrosis with round cell infiltration from a patient with Hashimoto's disease, rheumatoid arthritis and polyarteritis nodosa (A62/55). Longitudinal section, deltoid muscle, Van Gieson stain. × 360.

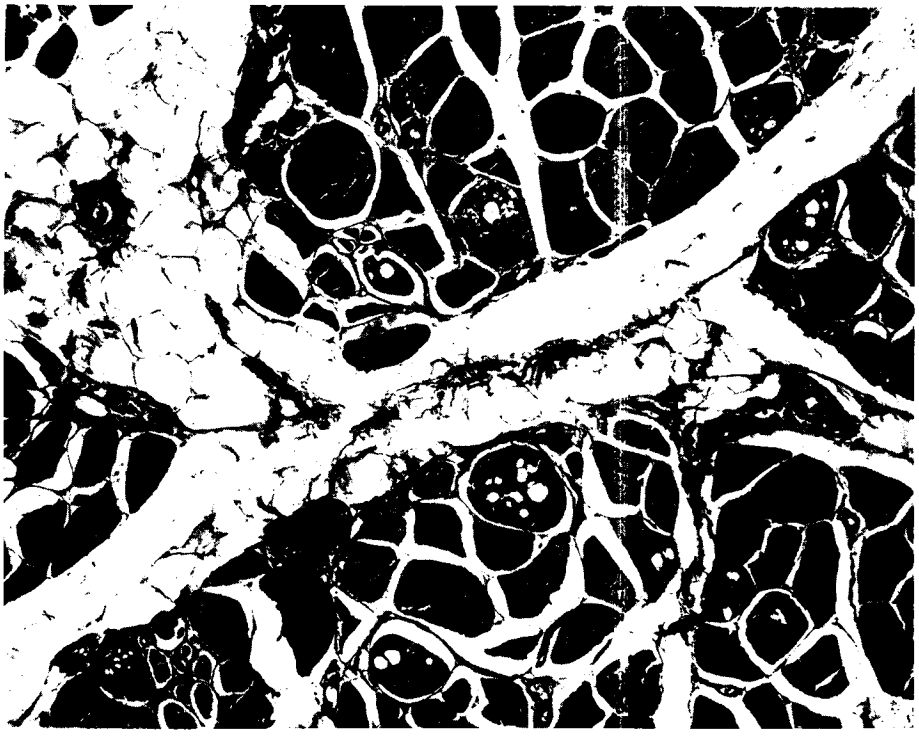

FIG. 2—Swollen necrotic and vacuolated muscle fibres. Deltoid muscle of patient with recent and old myocardial infarcts (A62/5). Cross section haematoxylin and eosin stain. × 130.

described by Montgomery (1962) in infantile protein malnutrition. A possible further factor to be considered in the pathogenesis of the lesions found in the present study is the effect of local ischaemia due to pressure, particularly in unconscious patients. Biochemical derangement in the agonal process was probably responsible for many of the acute changes. The findings support the known limited mode of reaction on the part of the muscle fibre and in general the observed histological changes present were of a similar type as those observed in myopathic diseases.

SUMMARY

Muscle lesions were sought in 397 human necropsies in the right deltoid and right vastus lateralis muscles. Of these 249 (63 per cent) were found to be abnormal and 102 (26 per cent) were categorized as simple atrophy, 51 (13 per cent) were shown to have signs of grouped or denervation atrophy, 39 (10 per cent) showed inflammation, 40 (10 per cent) showed necrosis and degeneration, and there were 17 (4 per cent) with other lesions. The high incidence of abnormalities indicates the rapid response of the muscle fibre to disease reflecting its high metabolic rate and its great susceptibility to toxic and nutritional disorders. In the group of patients with malignant neoplasms the high incidence of denervation atrophy as well as muscle fibre necrosis, signs of regeneration and leucocytic infiltrates suggested many of these patients may have had clinically undetected carcinomatous neuropathy and polymyositis. Furthermore, the study highlights the stereotyped mode of reaction of the muscle fibre to disease which suggests that a characteristic morphology occurring in a particular myopathic entity, e.g. progressive muscular dystrophy, is the result of the pattern

formed by the various components of the lesions which in themselves are quite non-specific.

Acknowledgements

We are indebted to Professor R. E. J. ten Seldam who first suggested the value of such a postmortem survey. Part of the work was prepared during tenure of a James and Sith Annie Chesters Scholarship by Dr. F. L. Mastaglia. The photomicrographs were prepared by Mr. H. Upenieks, A.R.P.S. The technical assistance of Mr. C. Owen and Mr. C. Raven is gratefully acknowledged.

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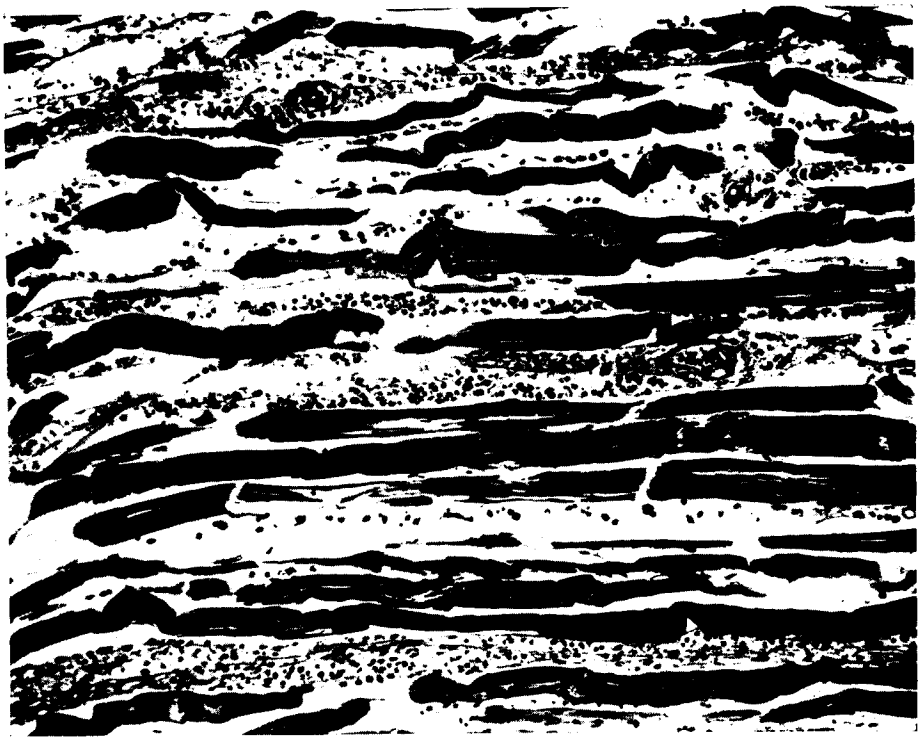

Fig. 3—Muscle fibre necrosis with loss of sarcoplasm and associated leucocytic infiltration. Patient with gastrointestinal haemorrhage (A62/106). Longitudinal section, vastus lateralis muscle, haematoxylin and eosin. × 136.

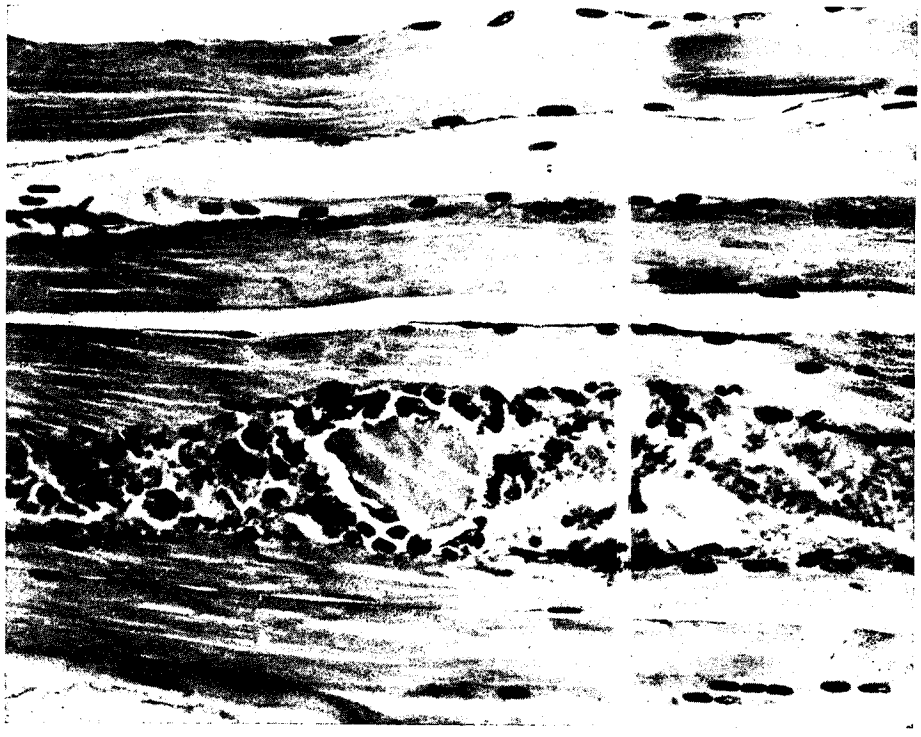

FIG. 4—Recent muscle fibre necrosis and macrophage reaction. Patient with viral meningoencephalitis (A62/104). Longitudinal vastus lateralis muscle, Van Gieson. × 310.

DISCUSSION

Dr. G. Preswick: This was a most fantastic survey. Could I seek answers to two questions, please? One, how do you know that the denervation changes in the carcinomatous group are likely to have been due to a motor neuropathy any more than they are to a myelopathy? Secondly, in the cases where you found focal changes suggestive of secondary myopathy, can you be certain that these sites had not previously been the site for an intramuscular injection which could give rise to this sort of change?

Dr. Kakulas: Whether or not this grouped atrophy is due to denervation, which must surely be involved in the motor supply to the nerve, or whether it be due to a subclinical myelopathy or a myelopathy which was not detected during life deserves comment. I would like to ask Dr. Preswick what he means by carcinomatous myelopathy, because I believe that this is quite a rare disease, that it has not been well categorized, and in the few reports which exist affects the white columns of the spinal cord. I would qualify these statements and say that if he has in mind the condition which picks out anterior horn cells selectively, then of course this picture may certainly be due to such a lesion; but grouped atrophy of muscle fibres does not result from lesions of the upper motor neuron. I would like to hear his comments on this question, and I think that this is quite an important and useful point. Whether or not the necrotic lesions are due to trauma is, of course, an open question. You will note that other people who have done surveys, such as Pearson, selected nine muscles, and he found a very high incidence of changes in such patients, and so have many others. I think it would be unlikely for an intramuscular injection to cause such a widespread effect, and I would look for support to the findings of myopathy in association with cancer which is

at least 20 per cent, probably a lot higher. These are clinical series. In autopsy series you would find that there may even be a higher incidence. The value of this survey is to indicate the non-specificity of the lesions, that the muscle fibre reacts very rapidly, and that there are certainly difficulties in interpretation and inference. I think that it is quite useful to bring to the attention of practising neurologists the fact there are all sorts of changes present in muscles which have not been given attention during life.

Dr. Preswick: Patients are presented to us frequently in the late stages of carcinoma at a stage when they are wasted from cachexia and non-specific effects of carcinoma, and those that are wasted from other causes. These patients are frequently demented, or nearly so, and their lack of co-operation limits the usefulness of the clinical examination. However, in many of these people that I have seen, I have come to the conclusion that whether you are dealing with a myopathic process, myelopathic in terms of anterior horn cells and associated pyramidal disease, or whether indeed you are dealing with a mixed or motor polyneuropathy, can only be resolved by determination of motor conduction velocity. In other words, if one finds electromyographically that you are dealing with denervation, and this is a simple process, the lesion may be at any level—the peripheral nerves, anterior nerve roots, or anterior horn cells. If, however, the motor conduction velocity is normal, then the lesion must be either in the roots or in the anterior horn cells. Now, when you have a combination of weakness together with evidence of denervation with the preservation of normal conduction velocity and increased or normal reflexes, then I think this is very good evidence to suggest on combined

FIG. 5—Swelling roundness and vacuolation of degenerate fibre on left with macrophage ingestion of necrotic sacroplasm on right. Patient with cerebral thrombosis (A62/184). Cross section deltoid muscle, haematoxylin and eosin. × 520.

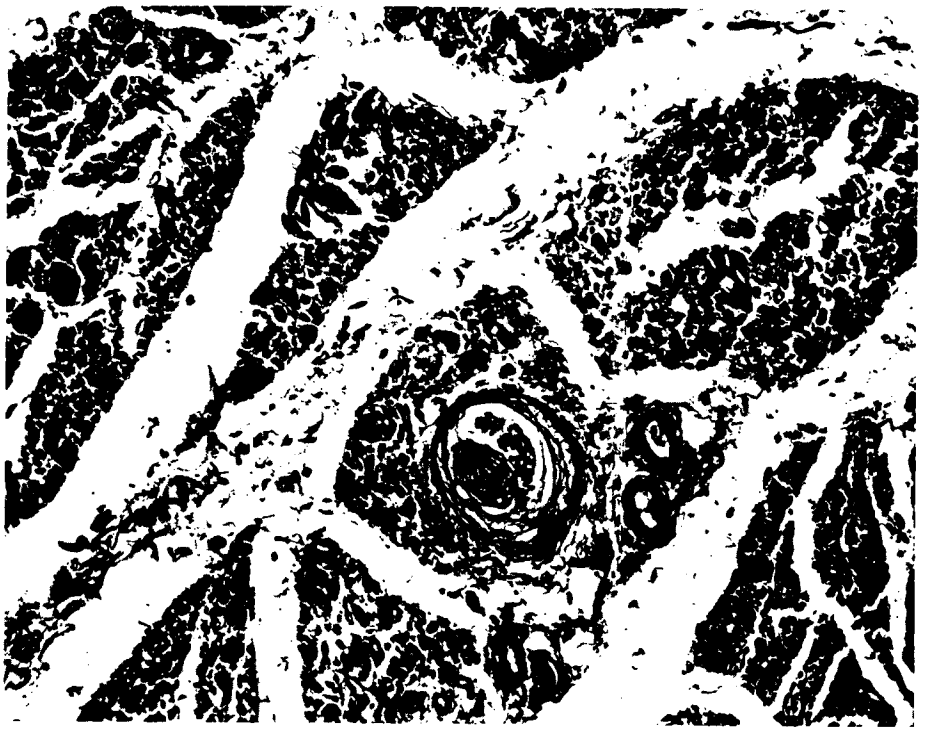

FIG. 6—Extreme generalized atrophy; compare size with muscle spindle near centre. Patient with rheumatoid arthritis and suppurative myelitis (A62/78). Cross section deltoid muscle, haematoxylin and eosin. × 150.

clinical and electromyographic grounds that you are dealing with a myelopathy, and in my experience of the carcinomatous patient, this is a reasonably common occurrence.

Dr. Kakulas: I would urge Dr. Preswick to obtain autopsies on his patients and to document his impressions on the very high incidence of carcinomatous myelopathy affecting anterior horn cells. I believe that the changes that have been described in the literature have been of a necrotizing process in the spinal cord rather than one selecting anterior horn cells particularly, and I will be very much looking forward to the results of a prospective investigation of this sort. I would suggest that it is indeed possible for a pathologist to distinguish between a primary muscle disease and a secondary denervation atrophy in disease; that when the muscle fibre architecture is disturbed, then there is a cellular reaction and one may infer that there is something wrong with that muscle fibre *per se*. In denervation atrophy the muscle fibre becomes reduced in size, but architectural markings are preserved at least until the very late stages. I do not know if you did intend to imply that one could not distinguish these two conditions.

Dr. Preswick: No, I did not say this. My idea was that in clarifying denervation you cannot detect by histological means alone without looking at the nerves and the anterior horn cells, the level which was responsible for the denervation, and this was the point I was making. You suggested that the motor neuropathy was present subclinically or clinically. I would venture to say that you cannot make this distinction on histological grounds without examining the spinal cord at the appropriate level, or the peripheral nerves.

Dr. Kakulas: I would say that the changes present are very similar to those in known documented cases of motor neuropathy. That is as far as I am prepared to go in that case, and I thank you for your comments.

Prof. Lance: Could Dr. Kakulas summarize these changes that he has found so frequently by giving us the percentage change of denervation atrophy or inflammatory polymyositic change in carcinomatous and non-carcinomatous groups, and the statistical significance of the difference between these two groups?

I do not think you grouped together the whole of the non-carcinomatous group. I was quite surprised by the number of changes you found in the non-carcinomatous group, and I was just wondering whether there was any possibility of the carcinomatous changes in any way being non-specific.

Dr. Kakulas: In the presentation I had to summarize and highlight certain of these findings, Mr. Chairman, and the details of the percentages will appear in the proceedings. They were not subjected to statistical analysis because the findings are very close between all groups. I think that Prof. Lance's point is well made, and it is indeed the point which I would like to bring forward that the changes are non-specific, and if one does define polymyositis as muscle fibre necrosis and regeneration with cellular reaction, then we are seeing this in all sorts of other conditions, so that we have to be careful in our criteria morphologically, and that this diagnosis can not be made on a small sample of muscle alone.

Prof. Lance: Could I say that is it possible that we clinicians are missing subclinical polymyositis and neuropathy in all manner of chronic disease or wasting disease, and it is even conceivable that these changes could not be specific to carcinoma, but may be held in common by many different disease processes?

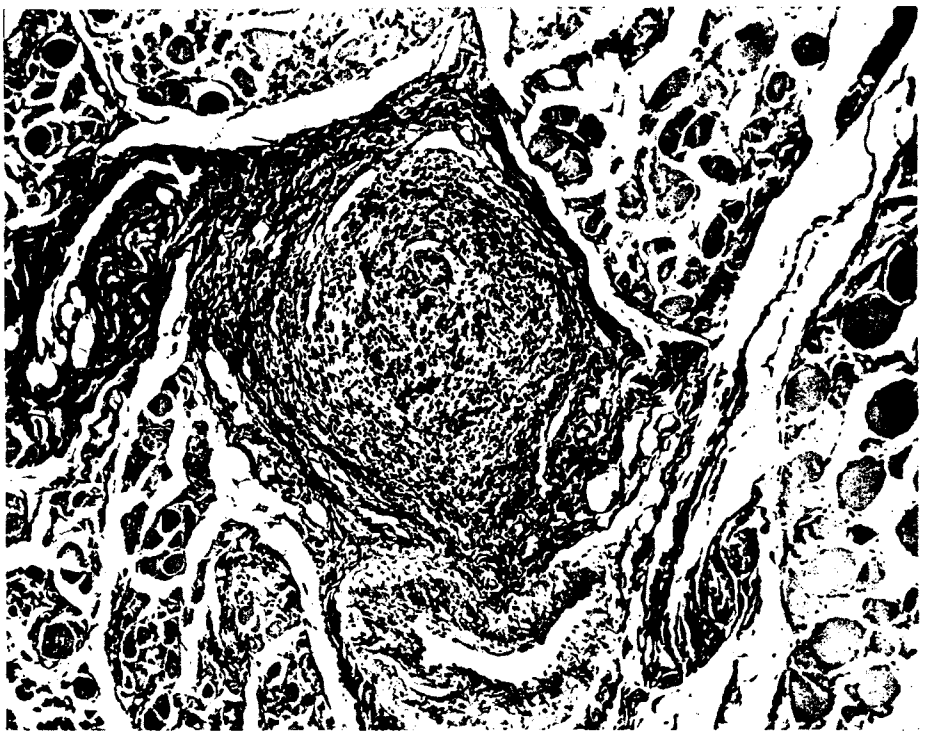

FIG. 7—Arterial lesion of perarteritis nodosa and muscle atrophy (A62/116). Cross section vastus lateralis muscle, haematoxylin and eosin. × 104.

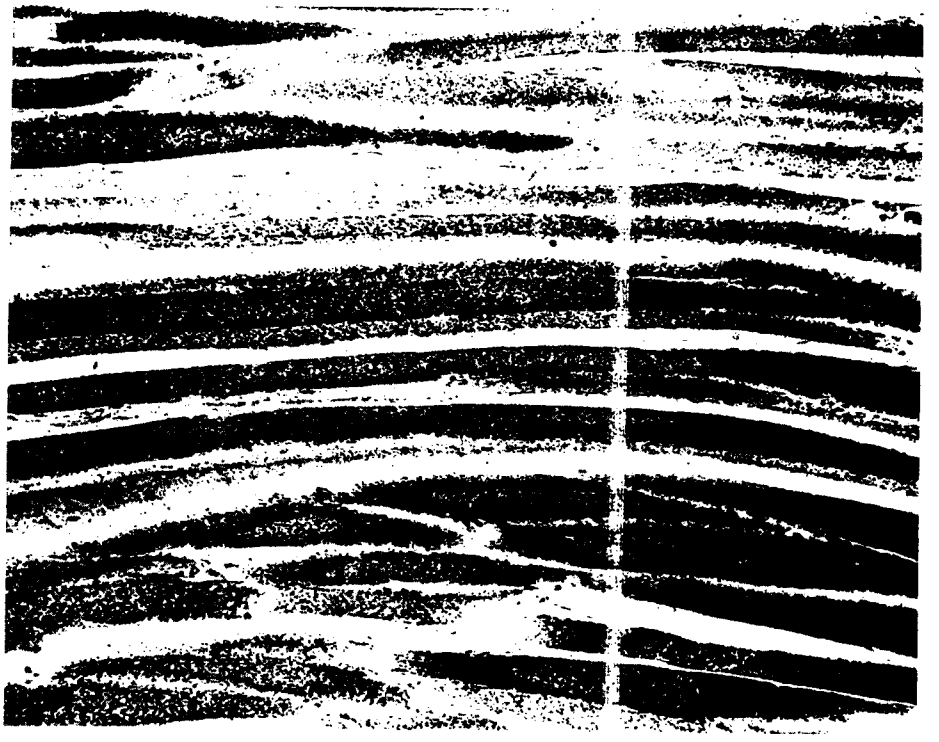

FIG. 8—Abundant granules of neutral fat within muscle fibres. Patient with cerebral thrombosis (A62/184). Longitudinal section deltoid muscle, Oil red O stain. × 150.

Dr. Kakulas: The only value of the survey is to suggest that perhaps closer examination for the incidence of neuropathy, myelopathy and myopathy be given. In all sorts of conditions the muscle fibre does react very rapidly. While in the past attention has been restricted very largely to muscular dystrophy, following the work of Eaton and later Walton and Adams, attention has been given to polymyositis. More recently the endocrine myopathies have emerged, and now I think we should look at the broad spectrum of muscle fibre changes in disease.

Dr. Anderson: I wonder if you could just tell me how you know this is a regenerating fibre, and what it looks like?

Dr. Kakulas: It was postulated late in the last century and early this century that certain changes in muscle were due to regeneration, but it was first clearly shown by Le Gros Clark in 1946 that muscle fibre had in fact a great potential for regeneration, and he subjected various experimental animals to a variety of traumatic injuries, and followed the regenerative sequence at great length. This work has been followed by other workers in regenerative activity, and more recently electron-microscopists have entered the field, and we are lucky now to have Professor David Allbrook in Perth, Western Australia, who was one of the first people, in fact I think the first, to examine the regenerating muscle fibre electron-microscopically with very neat preparations from a bat's wing, and he has not only documented the full sequence of regeneration, but has shown that there probably exists a satellite cell which we are unable to identify as such in light microscopic preparations. These are subsarcolemmal nuclei as far as we can determine, but these cells are probably the source of regenerating fibres. Walton in combination with Adams in 1956 proved, on repeated biopsy of

a patient with progressive muscular dystrophy, undoubtedly that regeneration occurs, and the morphologic criteria which Dr. Anderson asks for are firstly that the architectural markings are distinct. The muscle fibre has a blue or basophilic tint, due to the very high concentration of basophilic tint, due to the very high tration of ribonucleic acid, and there is a prominence of longitudinal markings, and these markings are due to the presence of protomyofibrils. The sarcolemmal nuclei proliferate, enlarge, become vesicular. Nucleoli also have a very high content of R.N.A. There has been personal experience in this field with the regeneration of muscle fibre in the Rottnest Quokka, which is subject to a spontaneously occurring nutritional myopathy which is reversed by vitamin E, and these histologic criteria apply equally as much in this observation in the experimental animal as they do in humans. There is a very nice report in one of the late issues of the *Journal of Pathology and Bacteriology* by Gilbert and Hazard, where a regenerative sequence in human tissue was followed and presented.

THE REFLEX EFFECTS OF MUSCLE VIBRATION

JAMES W. LANCE*

Sydney

INTRODUCTION

Our Association has chosen a Waratah as its insigne because of its geographical and historical significance. If we had had to select a symbol of the art of the clinical neurologist, we may well have chosen the percussion hammer. By a flick of the percussion hammer the clinician initiates a synchronous volley of impulses in afferent nerve fibres to test the reflex excitability of motor cells at many different levels of the nervous system. The sudden impact of the hammer on a tendon produces a direct stretch of muscle fibres of sufficent velocity to stimulate the large (group Ia) afferent nerves arising in the intrafusal fibres of the muscle spindle. If the blow be applied to a firm bodily structure, a vibration wave is set up which propagates through the limb or trunk and stimulates every muscle spindle of sufficient sensitivity, thus producing reflex contraction of muscles which are distant from the point of impact (Lance and de Gail, 1965). Tendon jerks may be described as phasic muscle reflexes because of the brevity of the evoked contraction. Electrical stimulation of group Ia afferent fibres produces a similar phasic reflex contraction, known as the H reflex after Paul Hoffman who described it in 1910. The H reflex tests the monosynaptic reflex arc without the intervention of the muscle spindle, required to translate a mechanical stimulus into a nervous impulse. The study of the tendon jerk and the H reflex has thrown much light on phasic spinal mechanisms, but how relevant is a synchronous neuronal discharge to the normal functioning of anterior horn cells in man, since it may not be possible to elicit tendon jerks in a champion athlete? Tonic stretch reflexes are thought to be of much greater importance in the maintenance of posture, but they cannot be obtained in a normal subject who is completely relaxed. It takes a certain degree of nervous tension or physical alertness to provide a "starter function" for tonic stretch reflexes in intact man. Tonic stretch reflexes involve sustained asynchronous discharge of anterior horn cells in contrast to the single synchronous burst of motor activity which characterizes phasic reflexes.

Whatever part the stretch reflex arc plays in the normal control of movement, its importance in contributing to the physical signs of motor disorders is undeniable, but changes in phasic muscle reflexes do not always run parallel with changes in tonic stretch reflexes. For example, muscle tone is increased in Parkinson's disease without much alteration of tendon jerks, and not all spastic patients show the expected increase in tendon jerks. On the other hand, patients with cerebellar hypotonia may exhibit brisk phasic muscle reflexes. How does this divergence in the intensity between tonic and phasic stretch reflexes come about when, as far as is known, both share the same monosynaptic reflex pathway? Are there separate populations of tonic and phasic anterior horn cells which react differently to afferent stimulation as demonstrated in the cat by Granit, Henatsch and Steg (1956)? Or is there only one type of alpha cell which discharges once in response to a synchronous afferent volley but fires repetitively when subjected to a sustained asynchronous barrage?

At the Canberra meeting of this Association in 1964, it was reported that the application of continued vibration to muscle suppressed phasic reflexes, while a reflex tonic contraction developed in the muscle concerned (Lance, 1965). At about the same time Hagbarth and Eklund (1965) independently found that vibra-

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tion of muscle tendon would induce a slow reflex contraction of the appropriate muscle. Recently Matthews (1966) has produced the same phenomenon in the decerebrate cat. To explain the differential effect of vibration upon tonic and phasic spinal mechanisms, de Gail, Lance and Neilson (1966) found it necessary to postulate the presence of both tonic and phasic anterior horn cells in man. This concept has now been studied further, and the present paper summarizes vibration-induced effects upon spinal reflexes. It now appears possible to explain these effects by a unitary hypothesis based on a single anterior horn cell population.

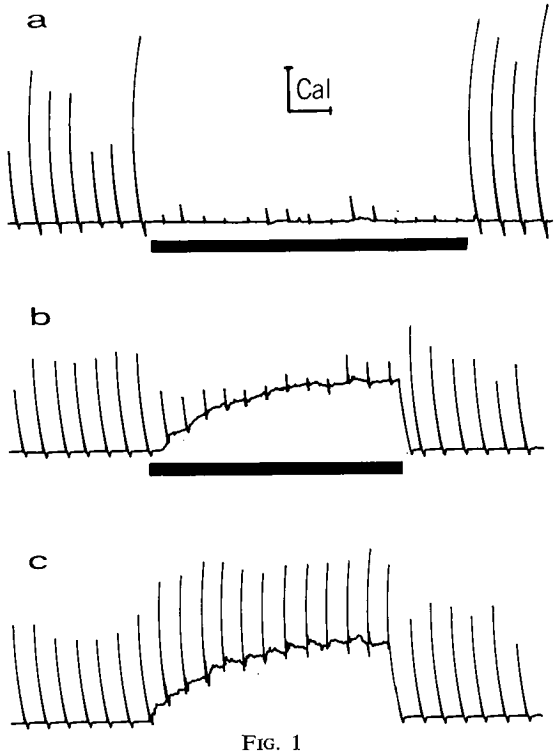

FIG. 1

(a) Strain gauge recording from ankle while knee jerk is elicited once every 5 seconds. Knee jerks are suppressed for duration of quadriceps vibration (black bar), although a tonic contraction is not obtained in this instance.

(b) As above, showing the tonic contraction of muscle which commonly develops during vibration.

(c) Voluntary contraction of quadriceps, showing that knee jerks are not suppressed.

Calibration:

vertical = 0·4 kg for (a), 0·6 kg for (b) and (c).

horizontal = 10 sec.

VIBRATION-INDUCED REFLEX TONIC CONTRACTION

The application of an orbital physiotherapy vibrator to muscle belly or tendon evoked a slowly augmenting tonic contraction of muscle in 19 out of 20 normal subjects (Fig. 1b). The contraction reached its maximum in about 30 seconds and declined rapidly after the removal of the vibrator, taking about one second to return to the baseline. The force of contraction was sufficient to elevate the limb against gravity and, in the case of the quadriceps, reached 2·2 Kg. in isometric contraction as recorded by a strain-gauge at the ankle. The contraction could be relaxed voluntarily, but recurred when the subject's attention was distracted. The force of contraction increased as the frequency of vibration was increased up to 80 c.p.s.

Infiltration of the muscle with procaine diminished the force of tonic contraction in proportion to the depression of tendon jerks, without reducing muscle power. The contraction could not be obtained in patients on the side of an absent tendon jerk resulting from nerve root compression by lumbar disc degeneration. Since there was no loss of muscle power or sensation in these patients, it was concluded that the afferent pathway of tonic contraction was the group Ia fibre which also serves the tendon jerk.

While the quadriceps was being vibrated and tonic contraction was in progress, a second vibrator was applied to the tendons of its antagonist, the hamstrings muscles. When the second vibrator was switched on, the quadriceps contraction ceased after a short latent period, even though quadriceps vibration continued (Fig. 2). When the second vibrator was switched off, the quadriceps contraction recurred. On some occasions a limited quadriceps contraction recurred even while vibration was being applied to the hamstrings, but it reverted to its full force only when hamstrings vibration stopped. The converse was also demonstrated in that a vibration-induced contraction of hamstrings was abolished by vibrating the quadriceps or its tendon, thus illustrating that the principle of reciprocal innervation applies to vibration-induced tonic contraction.

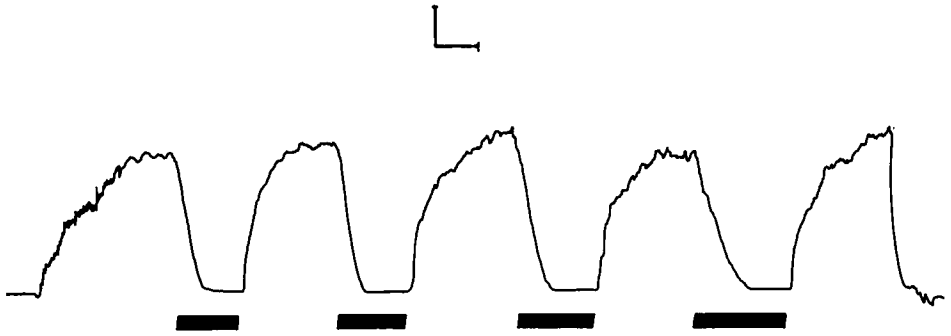

FIG. 2—Tonic contraction of quadriceps produced by vibration of the muscle belly, which is continued throughout the record. Black bar indicates the duration of vibration applied to the hamstrings. Inhibition of quadriceps contraction by vibration of hamstrings illustrates principle of reciprocal innervation.

Calibration: vertical = 0·6 kg
horizontal = 10 sec.

Repetitive stimulation of the posterior tibial nerve at 500/sec. for 10 seconds at an intensity sufficient to evoke the H reflex was interpolated in the middle of a tonic contraction of gastrocnemius-soleus while vibration of the tendo Achillis was continued. After a brief suppression of tonic contraction, it recurred more forcefully than before (Fig. 3). This post-tetanic

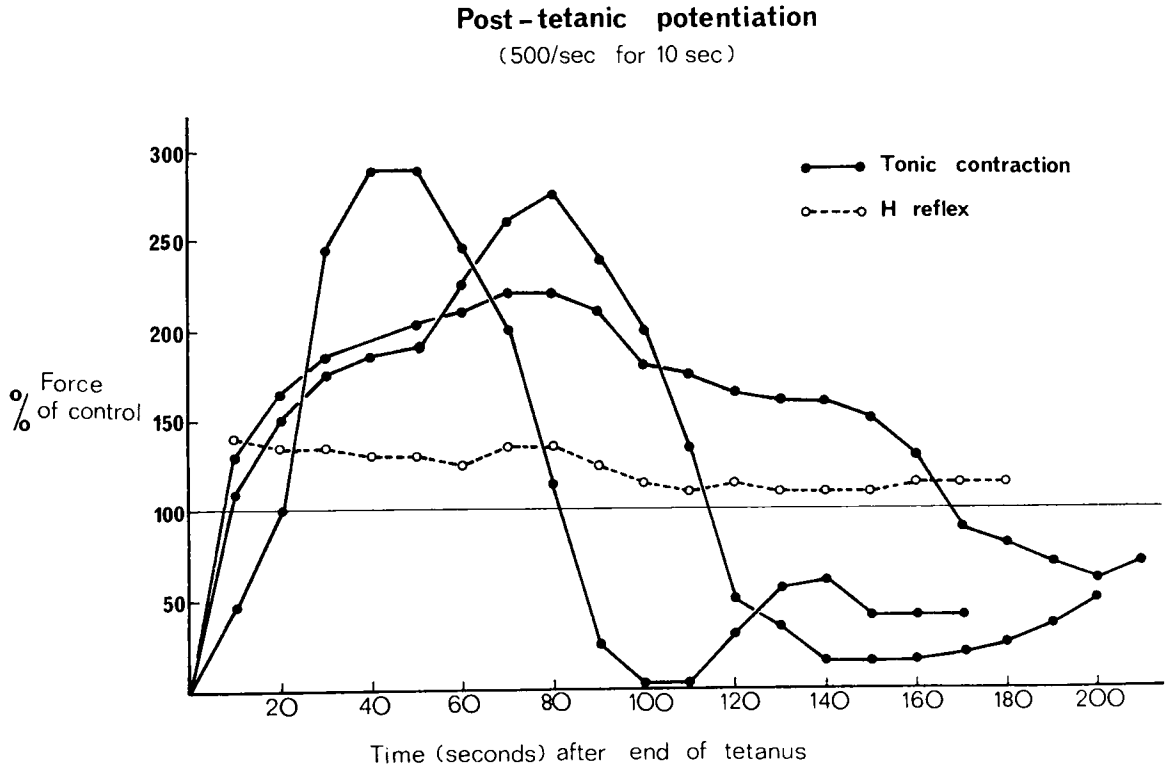

FIG. 3—Comparison of post-tetanic potentiation of vibration-induced tonic contraction of triceps surae, and of the H reflex, in the same subject.

potentiation of tonic contraction was more consistent than post-tetanic potentiation of H reflexes in three out of the four normal subjects in whom it was studied. Such a striking degree of post-tetanic potentiation suggests that vibration-induced tonic contraction is mediated, at least in part, by the monosynaptic reflex arc.

Tonic contraction was suppressed by various drugs such as Thiopentone, Ciba 28,882 Ba (Bein and Fehr, 1962), Diazepam (Valium) and benztropine methanesulphonate (Cogentin) without any alteration of tendon jerks.

In 11 out of 12 patients with chronic transection of the lower cervical spinal cord, tonic contraction could be obtained from muscles innervated from the cord above the level of the lesion, but not from flexor or extensor muscles innervated from the cord distal to the site of damage. In one out of the 12 patients, a variable and feeble tonic contraction could be elicited from the quadriceps. The majority of patients with partial upper motor neurone lesions also showed a diminished tonic contraction on the affected side, in contrast to patients with Parkinson's disease in whom the reaction was normal. Tonic contraction was diminished or absent on the side affected by a unilateral cerebellar disturbance. It was concluded from these clinical observations that tonic contraction depended in some way upon supraspinal structures for its full development.

The Suppression of Phasic Reflexes by Muscle Vibration

Tendon jerks and H reflexes maintained a fairly constant amplitude when elicited once every five seconds. When the appropriate muscle was vibrated, the amplitude of tendon jerks or H reflex was markedly diminished, whether or not a tonic contraction was evoked (Fig. 1a, b). The suppression of phasic reflexes was relatively less in patients with hyperreflexia from upper motor neurone lesions, and, in normal subjects, suppression could be overcome by reinforcement with the Jendrassik manoeuvre. Phasic reflexes were not suppressed by a voluntary contraction exerting the same force as reflex contraction (Fig. 1c).

When thiopentone, Ciba 28,882 Ba and diazepam were administered intravenously to normal subjects, abdominal reflexes were abolished at the same time as tonic contraction, suggesting that polysynaptic reflex pathways were blocked. The suppression of tendon jerks by vibration persisted at the time of maximal drug action, implying that this phenomenon did not rely upon polysynaptic pathways.

After cessation of vibration at 20 or 50 c.p.s., the excitability of the H reflex returned to normal after about five seconds. This prolonged depression after vibration is of similar duration to the recovery of the H reflex after a single conditioning H reflex or a conditioning tetanus at 50 c.p.s. Suppression of phasic reflexes by vibration takes place in the same way below the level of spinal cord transection, unlike tonic contraction, and therefore depends upon spinal or peripheral mechanisms.

Single Unit Recording in Tonic and Phasic Reflexes

The apparent paradox of phasic reflexes being suppressed while a tonic reflex was elicited brought up the possibility of two different types of motor neuron which were affected differentially by the reflex effects of vibration. It therefore became of importance to determine whether a given motor unit played a part in both tonic and phasic reactions. In three normal subjects a Disa bipolar electrode was inserted deeply into the medial and proximal part of the calf so as to pass through gastrocnemius into soleus near its origin. An H reflex was produced by stimulation of the posterior tibial nerve, and the electrode position altered until a single motor unit was recorded and photographed. A tonic contraction was then initiated by vibration of gastrocnemius tendon and the resulting unit discharge photographed. This process was repeated until a single unit was found which discharged consistently with both tonic contraction and H reflex. The procedure was time consuming since the muscle shortening produced by phasic or tonic contraction shifted the electrode position slightly so that a single unit discharge was frequently lost or altered in appearance in the transition from one form of stimulation to another. Fig. 4 illustrates a single unit which discharged consistently as part of the H reflex as well as with vibration induced tonic contraction and sustained voluntary contraction.

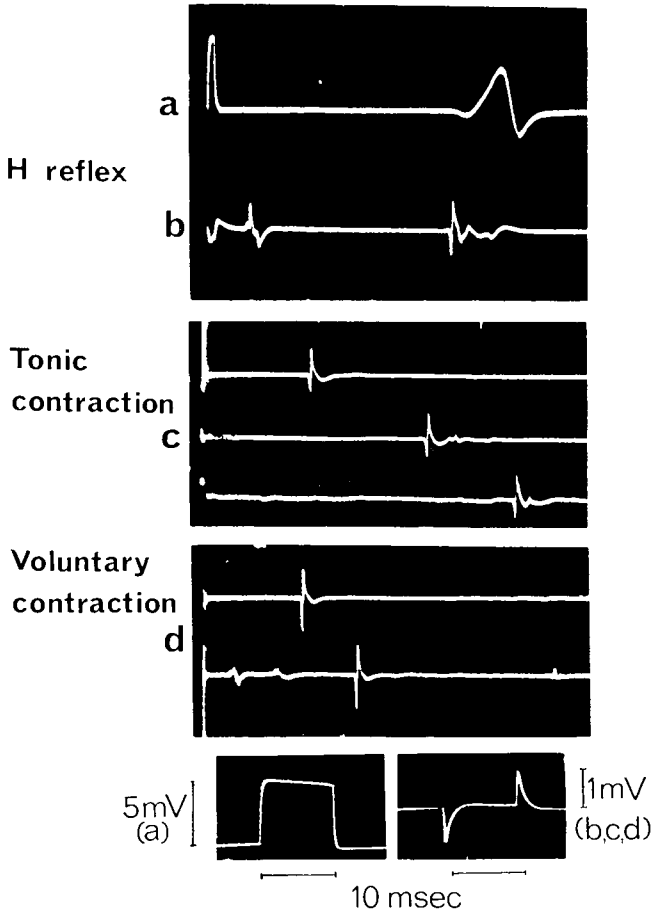

FIG. 4—Recordings of the same soleus motor unit participating in the H reflex (b), vibration-induced tonic contraction (c), and voluntary contraction (d). The surface-lead EMG of the H reflex is shown in (a) recorded simultaneously with the single unit response of (b).

DISCUSSION

It has been shown that a given motor unit may participate in both tonic and phasic reflexes. Is there any way in which phasic reflexes may be occluded by the effects of vibration and still permit a tonic contraction to develop?

Vibration is known to stimulate muscle spindle receptors and induce a discharge in group Ia afferent fibres (Echlin and Fessard, 1938; Granit and Henatsch, 1956; Bianconi and Van der Meulen, 1963). The suppression of phasic reflexes could not be explained solely by the vibratory stimulus engaging the muscle spindle receptors and making them unresponsive to tendon tap, because H reflexes, which do not depend directly upon the spindle, are also suppressed. However, Hunt (1952) demonstrated that steadily increasing the amount of muscle stretch reduced the size of the monosynaptic response progressively in spinal cats. He attributed this to central effects of the asynchronous afferent discharge evoked by steady stretch. Recently Henneman (personal communication) has shown that the electrical excitability of group I afferent fibres is diminished by stretch of their muscle of origin,

even when these fibres are sectioned central to the recording site, i.e. that sufficient afferent activity is generated by stimulation of spindle endorgans to produce a "busy line". This would account for diminution of the monosynaptic reflex in the intact animal, without the necessity for postulating a central inhibitory effect. It is probable that the same mechanism is operating in our experiments, since sustained vibration of muscle would be expected to produce continuous afferent activity in the same manner as steady stretch, thus suppressing tendon jerks and H reflexes by reducing the number of afferent fibres available for stimulation. If this is the case, two problems remain:

(a) The five second period required for recovery of H reflexes after vibration ceases. This prolonged depression of excitability is much the same after a single conditioning H reflex or after repetitive afferent stimulation, and probably depends upon a central mechanism.

(b) The reversibility of tendon jerk suppression by reinforcement and the relative ineffectiveness of vibration in reducing the hyperactive reflexes of a spastic patient. It is now considered that reinforcement increases excitability of alpha cells as well as gamma, since the H reflex, which by-passes the spindle mechanism, may be reinforced (Landau and Clare, 1964a). The same authors (1964b) have also shown that the H reflex is augmented on the affected side of hemiplegic patients, indicating a state of facilitation of alpha cells. If this be the case the synchonous burst of afferent activity produced by tendon tap or posterior tibial nerve stimulation may produce an increment of activity above the asynchronous afferent discharges of the "busy line" produced by vibration, which is sufficient to produce a significant reflex discharge of the facilitated alpha cells.

The studies of experimental tonic contraction presented here indicate that it depends upon vibration stimulating muscle spindle receptors to produce impulses in group Ia afferent fibres. Because muscle spindles are scattered throughout muscle bellies, each having an afferent path to the spinal cord of different length, and because the vibration wave propagates irregularly through muscle, it is probable that vibration-induced afferent activity is asynchronous by the time it arrives at the alpha anterior horn cell. In spinal man, although the alpha cell is known to be in a state of facilitation for the passage of phasic reflexes, the asynchronous vibration-induced afferent activity does not usually induce a tonic contraction. This is surprising, since the presence of post-tetanic potentiation indicates that vibration-induced tonic contraction has a monosynaptic component, although it is dependent upon supraspinal centres for its full development. The supraspinal structures which appear to be essential for the augmenting nature of vibration-induced tonic contraction are the cerebellum and the upper motor neurone. It is postulated that the extensive group Ia afferent projection to the cerebellum may reflexly reinforce spinal mechanisms through the upper motor neurone, since stimulation of deep cerebellar nuclei is known to influence gamma and alpha motor neurones (Henatsch, Manni, Wilson and Dow, 1964; Henatsch, Manni and Dow, 1964).

Various drugs used in this study abolished tonic contraction without affecting phasic responses. This could be explained by polysynaptic blocking action with the exception of benztropine methanesulphonate (Cogentin) which did not reduce the abdominal reflexes. All these drugs may impair the ability of the anterior horn cell to respond to asynchronous afferent stimuli, i.e. decrease its susceptibility to temporal summation in a non-specific manner like anaesthesia. Alvord and Fuortes (1953) remarked "anaesthetized preparations, though giving reflex reactions to single shocks . . ., do not respond to stimuli other than sudden and can only react with unsustained activity. Apparently they lack the properties which normally allow reactions to gradual stimuli and secure sustained reflexes". Alvord and Fuortes went on to show that in decerebrate cats there was no relation between the frequency of afferent activity recorded from the dorsal nerve roots in response to stretch and the regular frequency of reflex unitary motor discharge at about 10/sec. Sustained reflex responses to repetitive stimulation were more consistent in decerebrate cats than the monosynaptic reflex after a single shock, but long latencies were found between start of stimulation and initiation of the reflex discharge. Alvord and Fuortes postulated that asynchronous afferent discharges

from muscle built up a central excitatory state which was necessary for the generation of sustained reflexes.

The vibration-induced tonic contraction in man has characteristics different from those of spasticity or extrapyramidal rigidity. It is more variable in latency and intensity than the abnormal tonic stretch reflexes found in these conditions, and is abolished rather than augmented by the application of sudden stretch to the muscle. Vibration-induced tonic contraction is a physiological reaction found in its most complete form in normal subjects, and may be considered as a means of testing the pathway for tonic stretch reflexes in intact man. The fact that this reaction is diminished in patients with upper motor neurone lesions who show the increased tonic stretch reflexes of spasticity, implies that the latter are not simply exaggerated normal responses, but employ only part of the normal pathway, being deprived of the supraspinal connections required for the normal graded augmenting reflex contraction.

In conclusion, there does not appear to be any need to postulate separate tonic and phasic anterior horn cells in man. The anterior horn cell pool may react phasically if it is presented with a synchronous afferent volley when in an adequate state of excitation. Under normal circumstances when the afferent barrage is asynchronous, from muscle stretch or an artificial stimulus such as vibration, the anterior horn cell pool has to be raised progressively to a higher state of excitation in order to respond with sustained tonic discharge. The manner in which this process depends upon supraspinal structures in intact man remains uncertain.

Summary

Sustained vibration of muscle in normal man supresses phasic reflexes (tendon jerks, H reflexes) and provokes a reflex tonic contraction in the muscle vibrated. This tonic contraction is abolished by vibration of the muscle antagonist and potentiated by a conditioning tetanus applied to the muscle nerve. Evidence is presented that vibration stimulates the muscle spindle, thus producing activity in group Ia afferent fibres. The presence of post-tetanic potentiation implies that tonic contraction is mediated at least in part through the monosynaptic reflex arc. The fact that the reaction is diminished in lesions of the cerebellum or upper motor neurone indicates that it is dependent upon connections with supraspinal structures in contrast to the abnormal tonic reflexes found in spasticity.

The diminution of phasic reflexes by vibration is best explained by most of the group Ia afferent fibres being occupied with the asynchronous activity produced by vibration, so that only a small increment of activity can be produced by a synchronous volley. This explanation for occlusion of phasic responses in the peripheral nerve obviates the necessity for postulating two separate groups of tonic and phasic motor neurones affected differentially by vibration. This is supported by the demonstration of the same single motor unit participating in voluntary contraction, vibration-induced tonic contraction and the H reflex.

Acknowledgements

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DISCUSSION

Dr. Eric Davis: On the clinical side, do you believe that Valium or Cogentin have any lasting effect in reducing spasticity?

A./Prof. Lance: Unquestionably, during a short term when given intravenously, I think that there are beneficial results to be obtained from these drugs, but, like all the drugs used for spasticity, I think the benefit is marginal. One may prevent flexor spasms and certain other unpleasant symptoms, but one can not ameliorate the main process of spasticity in my view by any drug unless it is given repetitively by intravenous drip or something of this sort.

FINE CONTROL OF HUMAN MUSCULAR MOVEMENT

D. J. DEWHURST*

Melbourne

The complexity and speed of execution of most co-ordinated muscular movements are such that visual observation by the subject is clearly insufficient to account for their control in other than a strategic sense; the tactics are evidently managed by sequences learned as a result of previous training. In an endeavour to investigate in a normal human subject the relationship between these learned sequences, the spinal reflexes and the visual or other feedback pathways from the environment, equipment has been set up in the Biophysics Unit to establish an initial constant load on the flexor muscles of the elbow, and then to abruptly change this load to a new constant value without warning to the subject. This technique (Dewhurst, 1961; Dewhurst and Axford, 1965) results in the appearance of the components of the reflex response in sequence; the spinal components first, followed by the learned central response, and finally by corrections made by visual observation.

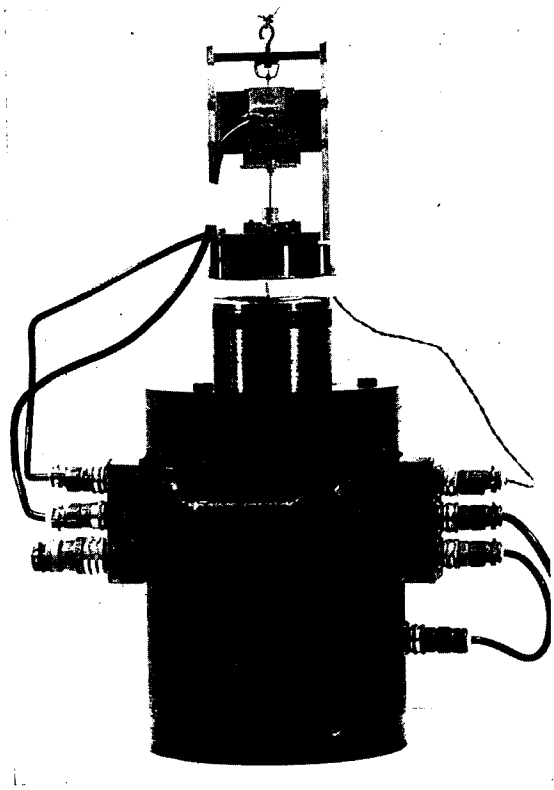

FIG. 1—Electromagnet for loading human muscle.

The apparatus consists of a large moving coil electromagnet, whose armature is wound with insulated aluminium wire (Fig. 1). This armature travels freely in a vertical direction. Its position at any time is detected by a linear potentionemeter, the displacement transducer shown in Fig. 2. The output of this is added to that of a time mark generator, and the result displayed on one trace of a four-trace cathode ray oscilloscope. The other three traces are used to record the electromyograms from three intramuscular bipolar needle electrodes, which may be inserted as desired into the muscle groups being studied. The force being exerted at any instant is measured by a force transducer consisting of an unbonded strain gauge attached to a steel bar, which is deflected by the load being applied to the subject. The output of this transducer is used to control the driving current being passed through the moving coil, so that irrespective of acceleration or external changes, the force applied to the subject remains constant. Attachment to the subject is made at the wrist, which is immobilized by a plaster cast to which the moving coil system is clamped. This yields a slight but reproducible mechanical latency between the application of a change in current to the moving coil, and a change in position of the elbow joint. Independent meas-

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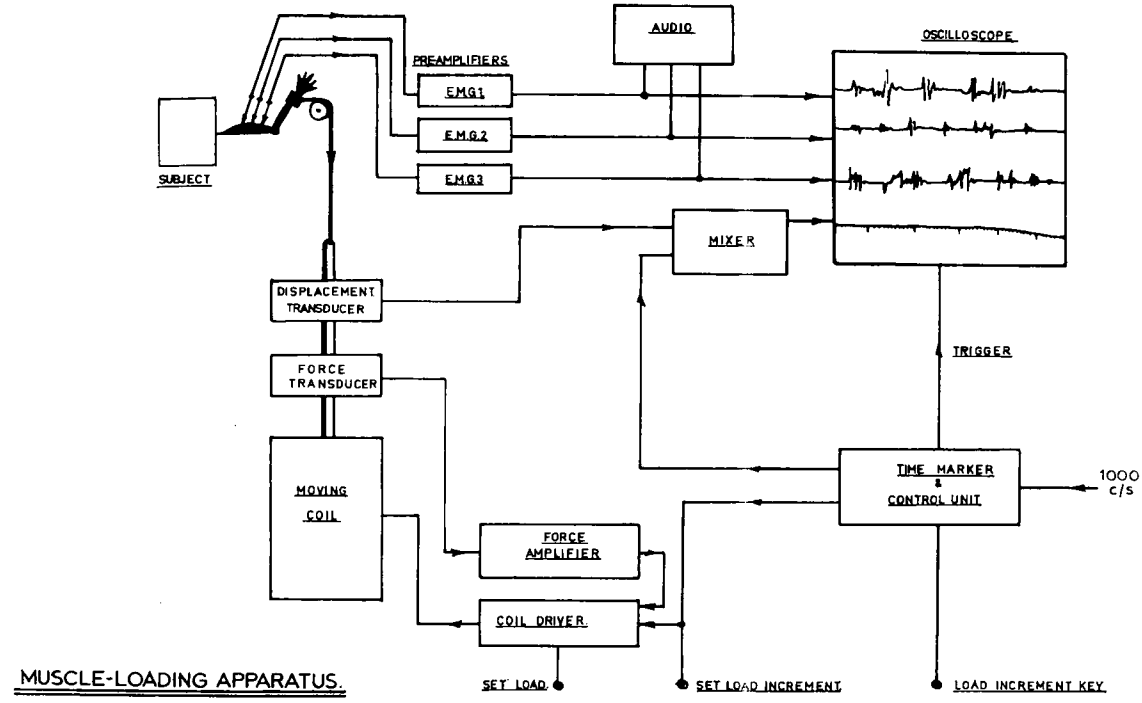

FIG. 2—Configuration of muscle-loading apparatus.

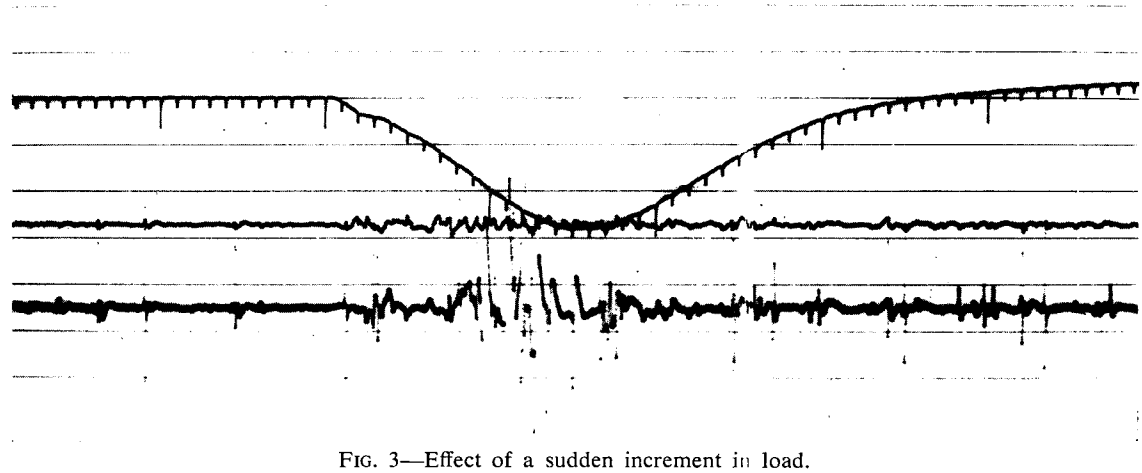

FIG. 3—Effect of a sudden increment in load.

Upper Trace: Time marker (10 and 100 msec. marks) and position of wrist. (Horizontal lines represent cm. of movement.) The load is applied at the last 100 msec. marker before the displacement commences.

Middle Trace: Right biceps EMG.
Lower Trace: Right brachioradialis EMG.
Load: Initially 1 kgmwt, increased to 2 kgmwt.

urements of this latency have been made both by a photoelectric method and by the observation of interstitial pressure in one of the flexor muscles (Buller *et al.*, 1959), and these measurements show a value of 15 milliseconds for the present apparatus (Wilcock, 1966).

A typical response to a sudden increment in load is shown in Fig. 3. The subject concerned uses the brachioradialis as the major flexor of the elbow when placed in the posture used for the test; this was true of about half the group of subjects tested. Prior to the onset of the increment in load two motor unit action potentials can be observed in the brachioradialis EMG, and this was true for a much longer period prior to the period shown in the figure. At approximately 30 msec. after the onset of load the firing rate of the units already active is observed to increase, and study of a large number of results has shown that this is invariably the first result of the stimulus. The delay is consistent with that for a spinal reflex response. From about 80 to 100 msec. a large burst of new motor unit action potentials is elicited, and this burst also appears to be spinal in origin; no modification of the subject's volition ever modifies the appearance or form of this group. The latency may be varied by a change in the increment of load, and hence in the rate of movement of the elbow joint, so the receptors initiating this burst may be position-sensitive or velocity-sensitive; further work is being done on this aspect. The subject is normally instructed to maintain his arm in a constant position, despite changes in load; however, if this instruction is not given, or if he is deliberately told to maintain the initial load, but not to attempt to correct for any changes, the elbow joint is extended when the increment occurs and the reflex muscular activity so far described is quite inadequate to prevent this extension.

Following this, a second large burst of action potentials occurs; in Fig. 3 this lies between 110 and 180 msec. Unlike the activity described in the previous paragraph, this is completely abolished if the subject is not instructed to maintain a constant position of the arm. Both this fact and its latency suggest that it is central in origin, and represents a "pre-programmed" sequence of impulses of size appropriate to the subject's estimate of the exigencies of the situa-

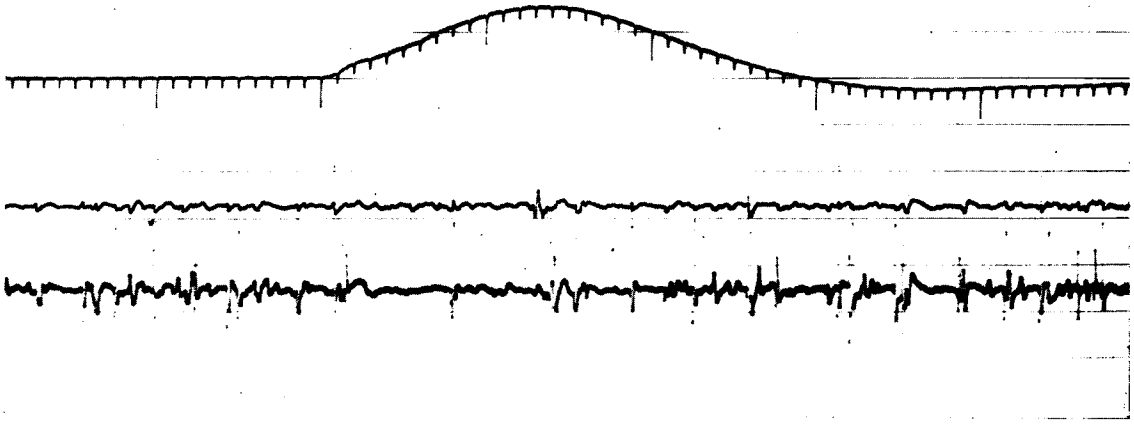

FIG. 4—Effect of a sudden decrement in load.

Upper Trace: Time marker (10 and 100 msec. marks) and position of wrist. (Horizontal lines represent cm. of movement.) The load is reduced at the last 100 msec. marker before the displacement commences.

Middle Trace: Right biceps EMG.
Lower Trace: Right brachioradialis EMG.
Load: Initial 2 kgmwt, reduced to 1 kgmwt.

tion, as made prior to the stimulus. The fine structure of this burst is modified with training on a particular load increment, and concurrently the mechanical movement of the arm shows a more nearly perfect return to its initial position. If now the situation is changed without warning to the subject, a burst of impulses appropriate to the previous situation will occur. If the subject is instructed not to attempt a correction at all, and carries out this instruction, this burst is abolished completely.

Finally, if the burst of impulses "selected" by the subject from his repertoire is not quite appropriate, a further burst or bursts may occur at about 300 msec., when the required degree of correction of the limb position can be estimated. This is primarily the result of visual feedback, although proprioception evidently plays some part.

The effect of a sudden decrement in load is shown in Fig. 4. The immediate result is a complete inhibition of firing of the flexor motor units, with the latency to be expected of a spinal reflex.

An interesting feature of the records obtained by the use of bipolar intramuscular needle electrodes is the differentiation of the motor unit action potentials into two distinct categories of amplitude. The smaller group typically has an amplitude in the range 50-100 μV; the larger group lies in the range 500-1000 μV. During recruitment of a load, the smaller units always appear first, and the larger ones typically at about 25 per cent of maximum load, in the case of the larger muscle groups. In chronic cases of poliomyelitis, it is often possible to observe the presence of one group or the other exclusively. In Figures 3 and 4 the differentiation between the two types shows clearly, and it is possible that they represent two distinct classes of innervation ratio.

This work was supported by a grant from the National Health and Medical Research Council of Australia, and was carried out in collaboration with Mrs. Lynette Wilcock (nee Axford).

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DISCUSSION

Dr. G. Preswick: Mr. Chairman, with regard to Dr. Dewhurst's question about the small motor units as against the large ones in normal subjects during recruitment or in poliomyelitis, I think the answer would be more readily obvious if one were to call the small ones motor sub-units rather than motor units. The motor units that are terribly large in poliomyelitis are in fact much larger and longer in duration than those seen in normal subjects; the reason is that the motor unit territory for any one motor nerve is increased due to ramification and increased innervation of adjacent muscle fibres that have been previously denervated during the loss of anterior horn cells. I think this is the explanation for Dr. Dewhurst's query.

Dr. Dewhurst: There is certainly a wide variety of motor units observed in chronic poliomyelitis patients. I was not referring primarily to the very markedly polyphasic units, which we feel normally do accompany reinnervation, but to many cases where you can get perhaps only three or four large units, which are quite consistent with the large units which one sees in a normal muscle at maximum load. As far as sub-units are concerned, one can frequently get a recording in which the small type of units is still apparent, but with an increasing number of the larger type of units supervening. We have looked very carefully, and we never see any suggestion of one type turning into another.

A./Prof. J. W. Lance: We find that in the tonic contraction produced by vibration the small units come in first and then later on the large units come in, and Hennerman has shown this in the experimental animal too, that it is the small units that are fired first. I think this is consistent with your observation, and I would like to ask you what is the maximal rate of firing that you found at any point during the sustained phase below—what is the maximal firing rate of a motor unit? We could not

fire any of ours faster than about ten a second. The second question I would like to ask is the latency of the premature firing of the unit which you showed, and of the phasic response which came afterwards. Was it the same latency as one anticipates from the triceps or the brachioradialis jerk?

Dr. Dewhurst: In answer to the first question, on the maximum rate of firing, we have observed a great many motor units accelerating their firing rate under a rising load, and recruitment usually occurs at 5 or 6 a second. The maximum rate of firing in the large muscles is 20-25 a second. In smaller muscles it could go as high as 50, but 20-25 would be quite typical. The second point is that the latency is completely consistent with the tendon jerk reflex time.

Theme: Neurology and General Medicine

THE ASSOCIATION BETWEEN DIFFUSE SCLEROSIS AND ADDISON'S DISEASE

M. J. EADIE

Brisbane

When diffuse sclerosis and Addison's disease occur simultaneously in the one individual, it might be expected that the two conditions have become associated purely by chance. Yet Hoefnagel, Van den Noort and Ingbar (1962) found record of eight previous instances of the autopsy-proven combination of the two diseases when they reported the occurrence of adrenal cortical atrophy, virtual absence of pituitary basophil cells, and an interstitial cell tumor of the testis in a six-year-old male with sudanophil diffuse sclerosis. These authors also discovered several doubtful examples of the association in the literature. Subsequently Blaw, Osterberg, Kozak and Nelson (1964) described a nine-year-old boy with familial sudanophil diffuse sclerosis and biochemical and autopsy evidence of Addison's disease.

In this paper, I wish to record a further instance of the simultaneous occurrence of Schilder's and Addison's diseases, and to consider the nature of the association between the two conditions.

CASE REPORT

In April 1963 a 12-year-old schoolboy was referred. Increased melanin pigmentation of his skin had appeared two years previously, but extensive investigation at that time did not establish a diagnosis of Addison's disease. The boy's subsequent presentation was due to a decline in his scholastic performance. Between 1959 and 1961 his examination results had ranged between 72 per cent and 93 per cent, with a mean of 83 per cent. During 1962 he obtained 62 per cent and 76 per cent in his examinations, and early in 1963 only 54 per cent. At the time of referral he was unable to apply himself to his studies, could not copy from a blackboard, omitted words when writing, and had begun to cheat from his schoolfellows and to lie to his parents. He neglected personal hygiene, was rowdy and aggressive, and sometimes ate his dog's food as well as large amounts of salt. He complained of headache and asked to be taken to a doctor.

The boy had suffered only minor illnesses in the past. There was no family history of relevant organic disease. His mother was an over-anxious, demanding woman with obvious obsessional traits.

The boy was thin, of average height for age, with deep melanin pigmentation of his skin, and with deep brown macules on his lips. His blood pressure was 105/65. He was euphoric and facile and often appeared as though preoccupied with his own thoughts, or with hallucinations, though he denied the presence of the latter when questioned. There was no definite abnormality on clinical neurological examination, though both plantar responses were possibly extensor.

A psychiastrist observed the child in hospital for several days. The boy's full scale I.Q. was 83 and a Rorschach projective test suggested schizophrenia. The most probable diagnosis was thought to be a revolt against parental authority and overprotectiveness, but juvenile schizophrenia or organic brain disease were also possible.

Skull and chest X-rays were normal. An EEG showed well developed bilaterally symmetrical 11 c.p.s. alpha rhythms posteriorly with blocking on eye opening. There was almost continuous, irregular, often bilaterally synchronous, fairly high voltage frontal 1-2 c.p.s. activity with, in transfrontal recording, a possible focus of origin near the right frontal pole. The possibility that the frontal slow activity was artefact could not be excluded, but the appearance did suggest the presence of an actively progressing localized pathology in frontal regions (Fig. 1).

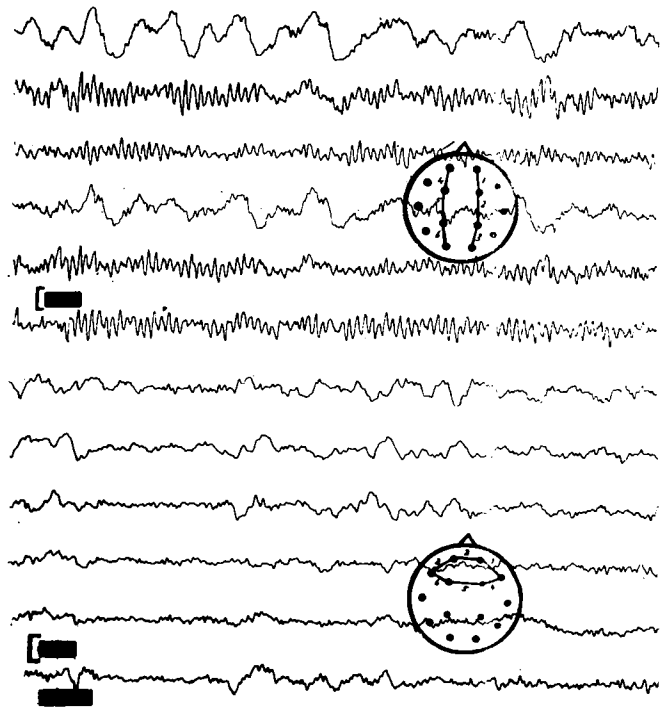

FIG. 1—Antero-posterior and transfrontal E.E.G. recordings showing bilateral frontal slow activity.

Despite the EEG appearance, it was decided to observe the child for a time rather than to investigate further. He was treated at home with chlorpromazine for several weeks. His behaviour deteriorated further, he became anorexic and lost weight. A second EEG was similar to the first, although a few generalized paroxysms of high voltage 4 c.p.s. rhythm had appeared. He was then admitted to hospital for investigation.

His haemoglobin and blood counts were normal. There was no abnormality in serum electrolytes or protein fractionation, in tests of renal or hepatic function, and there was no evidence of intestinal malabsorption. His Wassermann reaction was negative. An endocrinologist felt there was not sufficient evidence to diagnose Addison's disease, but the boy was always too uncooperative for measurement of his urinary steroid output. An EEG showed a reduction in voltage of the frontal slow activity, and occasional abortive generalized spike and slow wave paroxysms.

At air encephalography the cerebrospinal fluid was under normal pressure and was of normal composition. The ventricular system showed some generalized dilatation without displacement, and there was no widening of the cortical subarachnoid spaces.

The diagnosis remained uncertain, and the boy was sent home. Over the following three months his behaviour and physical condition progressively deteriorated, and he was brought back to hospital. By this stage he was emaciated, rarely spoke, and actively resisted any attempt at physical examination. The frontal slow activity in his EEG was of lower amplitude than previously, and there was slight slowing of the alpha frequency. A further air encephalogram showed increased dilatation of the ventricular system. During the general anaesthetic for this procedure the boy collapsed and required resuscitation. Unfortunately the clinician responsible for the boy's care was not told of this happening. On the day after the air study a right frontal lobe diagnostic cortical biopsy was performed by Mr. J. D. Yelland with the boy under general anaesthesia. The child's condition deteriorated shortly after he was returned from the theatre to a psychiatry ward. He died within a few hours.

The posthumous biopsy report stated that the cerebral cortex was normal. There was demyelination of the subadjacent white matter with some sudanophil products. The subcortical U fibres were spared.

Autopsy Findings

The autopsy was performed by Dr. George Middleton. Dr. Margaret Mead reported on the brain material.

No abnormalities were seen in the cardiovascular, respiratory, alimentary, haemopoietic and genito-urinary systems.

The pituitary, thyroid and testes appeared normally macroscopically and microscopically. At the autopsy no definite adrenal tissue could be found on either side. Sections of tissue from the suprarenal areas showed atrophic adrenal glands which consisted mainly of medulla with a thin rim of cortex containing many degenerate cells, some with giant nuclei.

The brain was oedematous and congested. The basal leptomeninges were thickened, and there were adhesions between the frontal poles and between the tentorium cerebelli and the occipital lobes. No cerebral atrophy was obvious, and there was no evidence of bleeding at the biopsy site. On coronal section the frontal horns of the lateral ventricles were dilated. Homogeneous grey-yellow tissue replaced the white matter of both frontal lobes anterior to the caudate nucleus and putamen, and also replaced the anterior limb of the internal capsule and the genu of the corpus callosum. The subcortical arcuate fibres were not involved in this appearance, and the cerebral cortex was macroscopically normal. The remainder of the cerebral hemispheres, the cerebellum, brain stem and spinal cord appeared normal.

Histologically the abnormal area of the cerebral hemispheres showed oedema and congestion, widespread demyelination with sparing of subcortical U fibres, much gliosis, and accumulations of sudanophil and granular lipid.

Thus the child had both sudanophil diffuse sclerosis and adrenal cortical atrophy, and had probably died from acute adrenal insufficiency.

Discussion

Table 1 sets out some features of the reported cases of associated Addison's and Schilder's diseases. The following points seem worth notice:

(i) All reported instances of the association have been males.

(ii) Neither Schilder's nor Addison's disease is restricted to youth yet the combination appears to be.

(iii) Nearly all patients with the disease combination have shown cutaneous pigmentation. However this pigmentation may not necessarily be an integral part of the combination as pathologists may not always study the adrenals intensively in non-pigmented cases of Schilder's disease.

(iv) The pigmentation has nearly always preceded the onset of clinical manifestations of the diffuse sclerosis.

(v) In some instances there have been family histories of Addison's or of Schilder's diseases, though the family history may be positive in either condition when it occurs alone.

When there are relatively few reports of the association of diffuse sclerosis and adrenal cortical atrophy, it could be argued that the combination represents no more than the chance occurrence of two unrelated diseases. I do not think adequate prevalence data are available to establish statistically whether the association

TABLE 1

Showing certain features of reported cases of diffuse sclerosis associated with Addison's disease.

Author	*Sex*	*Age at Onset of:—*			*Autopsy Findings:—*			*Family History*
		Neurological symptoms	*Pigmentation*	*Other Addisonian symptoms*	*Diffuse Sclerosis*	*Adrenal Atrophy*	*Other*	
SIEMERLING (1923)	M	7	3–4	—	+	+	—	—
PFISTER (1936)	M	7	3	—	+	+	—	cousin with diffuse sclerosis
	M	8	$<8\frac{1}{2}$	—	+	not examined	not examined	—
HAMPEL (1937)	M	none	15	15	+	+	—	—
ADAMS (1952)	M	9–10	not mentioned	7	+	apparently	—	—
GAGNON (1959)	M	9	7	—	+	+	pituitary basophils abnormal	Mother and grandmother pigmented. Maternal uncle and brother both died at 13 of progressive neurological disease
LICHTENSTEIN (1959)	M	5	present	—	+	+	—	—
BRUN (1960)	M	9	9	>9	+	+	—	—
HOEFNAGEL (1962)	M	$5\frac{1}{2}$	none	terminal	+	+	loss of pituitary basophils	One and possibly two sibs had Addison's disease
BLAW (1964)	M	9	none	$9\frac{1}{4}$	+	+	—	Brother had diffuse sclerosis
PRESENT CASE	M	11	10	Terminal	+	+	—	—

is more than an effect of chance. However the occurrence of all eleven reported instances of the disease combination in young males is unlikely to be merely due to coincidence. There seems to be no acknowledged differential sex incidence of either juvenile Addison's or Schilder's diseases and statistically the probabality of the combination occurring eleven times in males and never in females, and still being due to chance, is less than one in 4,000.

If, as seems likely, the combination of the two diseases is not due to chance, the association may have arisen in several ways. Blaw, Osterberg, Kozak, and Nelson (1964) suggested the following possible mechanisms:

(i) Primary hypopituitarism (ACTH failure) may lead to adrenal insufficiency and the cerebral changes. There is really no evidence whatsoever that pituitary disease causes diffuse sclerosis.

(ii) Primary adrenal failure may lead to demyelination. There is again no evidence for this. Both Brun and Voigt (1960) and Blaw, Osterberg, Kozak and Nelson (1964) treated their patients with combined Addison's and Schilder's diseases with adrenal steroids without improvement in the neurological condition.

(iii) There may be a familial metabolic error affecting both adrenal and myelin metabolism. There is no direct evidence for this, but it would be a convenient explanation for the age, sex, and familial incidence of the disease combination. At least we do know that manifestations of adrenal insufficiency may occur in childhood as a result of abnormal steroid metabolism.

Two further possibilities should be considered

(iv) Before neurological manifestations have become apparent clinically, diffuse sclerosis may have affected the hypothalamus to cause pituitary and thus adrenal insufficency. This mechanism, though unproven, seems feasible, but does not explain the sex incidence. Also, in at

least the case of Hoefnagel, Van den Noort and Ingbar (1962) the hypothalamus was said to be normal histologically.

(v) Both diffuse sclerosis and Addison's disease may arise from allergic or autoimmune mechanisms. Autoimmunity has been suggested as the cause of idiopathic Addison's disease, and under the guise of allergy, as producing primary demyelinating disease (e.g. Adams, 1959). It is believed that autoimmunity sometimes involves more than one tissue in the one individual. There is no proof that such autoimmune mechanisms do apply in the combination of Addison's disease and diffuse sclerosis and the sex incidence would be difficult to explain if these were the cause, but the possibility of autoimmunity might be worth further consideration.

Despite this speculation we must be clear that we do not know the meaning of this combination of adrenal cortical atrophy and diffuse cerebral sclerosis, though the association is probably a genuine phenomenon and not merely an effect of chance. The aetiology of primary demyelinating disease is unknown, and it may be that this rare event, the joint occurrence of Addison's and Schilder's diseases, could provide an answer to the problem. Because of this, if for no other reason, it is important that in future cases of the combination of Schilder's and Addison's diseases should be recognized during life, so that possible aetiological, and particularly metabolic factors, may be fully evaluated while the opportunity exists. If we could explain the association of diffuse sclerosis and Addison's disease we might be able to explain much more.

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DISCUSSION

The President: Thank you Dr. Eadie for your paper, the first today on our theme for the meeting—the relationship of neurology and general medicine.

Your paper prompts two thoughts.

The first is the importance of a full autopsy, rather than just "taking" the brain and spinal cord.

The other thought is that one often hears us referred to as "one of the splinter groups", in a critical sense.

I believe that speaking of "splinter groups" is behind the times, and that we are really taking part in a synthesis of medicine. We find ourselves all the time in contact with other disciplines—here a common interest with the endocrinologist.

Your paper is an example of the neurologist contributing knowledge to general medicine.

Dr. Rischbieth: All our diffuse sclerosis patients were males and I believe that the disease occurs chiefly in males.

Dr. Eadie: There is little diffuse sclerosis in Brisbane, hence I have insufficient data to comment on this point.

Dr. Anderson: Was there more sudanophil material than the slides show?

Dr. Eadie: That was biopsy material. The autopsy material showed more.

Dr. Rail: Was there more pigmentation of mucosa?

Dr. Eadie: No, it was present only on the lips.

Dr. Rischbieth: Were there any records of blood pressure?

Dr. Eadie: The child was too unco-operative to obtain readings for much of his illness, and the record of his anaesthetic, with the blood pressure fall, cannot be obtained.

HYPOGLYCAEMIA RESULTING FROM INSULIN SECRETING TUMOURS OF THE PANCREAS

J. M. SUTHERLAND, J. H. TYRER and M. J. EADIE*
Brisbane

In 1869 Paul Langerhans described the islets of pancreatic tissue which now bear his name, and in 1924, some two years after the discovery of insulin, Harris (1924) directed attention to a syndrome of hunger, weakness and neurotic symptoms which simulated the effects of insulin overdosage. In 1927, Wilder made a preoperative diagnosis of hyperinsulinism in a patient who was subsequently operated on by W. J. Mayo (Wilder *et al.*, 1927). The tumour was malignant and the patient died some four weeks after operation, but in 1929 a successful operation was carried out by Graham of Toronto (Howland *et al.*, 1929), and in 1930 Harvey Cushing removed the first definitely benign islet cell adenoma (Cushing, 1930).

Since this time a large number of patients with insulin secreting pancreatic tumours have been successfully treated but, as emphasized by Morley (1952), these tumours are not very common and it is probable that a good many patients suffering from them die undiagnosed, perhaps because the symptoms of hyperinsulinism can mimic so many other conditions. Early diagnosis and operative treatment is, however, of great importance since operation may produce a complete cure, since in some instances the condition is malignant and early operation may prevent dissemination and, lastly, since repeated attacks of hypoglycaemia may result in irreversible neurological damage. Because the symptomatology of hypoglycaemia is so closely related to the nervous system many patients with islet cell adenomas are seen at an early stage by neurologists and psychiatrists who must therefore be familiar with the clinical syndrome, appreciate the implications and be prepared to diagnose and advocate appropriate treatment.

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We propose to review this subject briefly on the basis of three patients suffering from islet cell adenoma of the pancreas seen during the past few years. These patients were diagnosed preoperatively, and successfully treated by surgical colleagues.

CASE MATERIAL

CASE 1. G.H., a married female, was seen first in 1961 when she was aged 37 years. At 14 years of age she had experienced a fainting attack diagnosed as "sunstroke" and from which she made an uneventful recovery after a few hours. When 24 years of age she was involved in a car accident in which she sustained a neck injury but was not rendered unconscious. Since this time, however, she had been subject to headaches which were episodic in character, occurring sometimes during the day, at other times awakening her from sleep at night. The pain was described as throbbing in character and was sometimes associated with vomiting. Paraesthesiae were experienced from time to time in both hands. Three months before being examined she commenced to have episodes in which she would waken from sleep feeling nauseated and sweating excessively. These symptoms would generally subside spontaneously after an hour or so, but on two occasions she lost consciousness for 30-45 minutes and her husband observed clonic movements of her limbs during these attacks. Her previous history was otherwise normal and there was no family history of either migraine or epilepsy.

Neurological examination at this time revealed no abnormality. Radiograms of the skull revealed no unusual features, but electroencephalography indicated a focal abnormality with low voltage theta and high voltage sharp activity arising from the right midtemporal region. On the basis of episodic autonomic symptoms occurring at night, a history consistent with two nocturnal major epileptic attacks, and a focal E.E.G. abnormality, the patient was diagnosed as having temporal lobe epilepsy. The headaches were regarded as being migrainous in nature and treatment with phenytoin sodium and "Bellergal" was advised. When seen again two months later marked improvement was reported; she had experienced no further attacks of impaired consciousness and had suffered from very few headaches. She still had occasional disturbed nights in which she would waken from sleep sweating excessively. Clinical examination was again

negative, and it was decided not to investigate her further at this time.

Four months later (July 1962) the patient was admitted to a local hospital with this history: for the past 6-8 weeks her headaches had increased in severity and frequency. She frequently felt drowsy, particularly on wakening, but improved after breakfast. On the morning of her admission to hospital she had felt more drowsy than usual and this feeling had steadily increased in degree. There was no evidence to suggest that she had taken an excessive dose of any drug. She was transferred to the Brisbane Hospital.

On examination, the patient was stuporose and although she would obey some commands sluggishly, she would not answer questions. The pupils were equal and reacted to light and the optic fundi were normal. The deep reflexes were all sluggish, the plantar responses being flexor.

The blood sugar was 40 mg per 100 ml. Twenty ml of 50% glucose were given intravenously with immediate lessening of stupor, but despite adequate intake of glucose and fluids and correction of a mild electrolyte imbalance the patient remained confused with a tendency to confabulate for some 5 days. Bilateral carotid angiography carried out during this period revealed no abnormal features.

CASE 2. D.R., a 52-year-old housewife, was seen in July 1963. Her health had been good until September 1962, when she began to experience episodes of double vision. In November 1962 whilst travelling by car she lost consciousness and remained in coma for 36 hours. She was admitted to a local hospital and apparently made a spontaneous recovery following a lumbar puncture. She was discharged one week later with a diagnosis of "encephalitis", but since this time she had felt unduly sleepy, experienced a tendency to stagger when walking and had episodes of diplopia lasting for several hours at a time. On some 10 occasions she had become stuporose and on at least one occasion "twitching and jerking" of her limbs had been observed by her husband. The patient had no recollection of these events, which might last from one to several hours. In October 1962, two attacks of diplopia had been associated with loss of postural tone in which her legs suddenly "gave way". Blurring of vision associated with a throbbing occipital headache had occurred if she became excited.

The patient's previous history was otherwise not relevant. Both her parents had died from "strokes".

On examination the patient was somewhat obese and her blood pressure was 200: 100 mm of mercury. The deep reflexes were symmetrically brisk, the plantar responses being flexor. Physical examination was otherwise negative and the patient was admitted to the Brisbane Hospital with the differential diagnosis, ? vertebrobasilar insufficiency; ? spontaneous hypoglycaemia; ? space occupying lesion upper brain stem. Skull and chest X-ray films were normal and her E.E.G. showed only non-specific abnormalities.

CASE 3. W.M., a P.M.G. technician, aged 43 years, was examined in October 1964. The following history was obtained: for the past 3 years he had experienced episodes which tended to occur whilst he was in bed in the early morning. In these attacks, his wife reported that he was not fully conscious; he would throw his arms about and carry out purposeless movements, on some occasions falling out of bed. His speech would be mumbling and he would sweat excessively. Very rarely, similar attacks would occur during the day and then usually before a meal. This fact had prompted him to take a meal immediately before retiring for the night, with some lessening in the number and severity of the attacks. During the day "taking even a jelly bean" would ward off an attack. On direct questioning, the patient admitted to feeling light-headed and experiencing paraesthesiae in the mouth and lips in relation to these incidents. He was never incontinent of urine.

His previous health had been good. At 32 years of age he had suffered from mild concussion, being unconscious for only 2 to 3 minutes. The family history was not relevant.

Physical examination revealed no unusual features and neurological examination was entirely negative. Skull and chest X-ray films and an E.E.G. were normal. He was admitted to the Brisbane Hospital for investigation of ? hypoglycaemic attacks.

DISCUSSION

1. *Clinical diagnosis*

Case 1 was initially misdiagnosed as suffering from temporal lobe epilepsy, the correct diagnosis being achieved only when the patient was admitted to hospital with drowsiness progressing to stupor. In retrospect, more significance should have been attached to the history of episodes of wakening from sleep feeling nauseated and sweating excessively.

In *Case 2*, the symptoms of diplopia, staggering gait and drop attacks, and the family history of "strokes" suggest a diagnosis of vertebrobasilar insufficiency, and the attacks of impaired consciousness with convulsions could be ascribed to epilepsy resulting from cerebral or brain stem ischaemia. On the other hand, Cairns (1952) reported that tumours in the upper brain stem might result in intermittent or continuous disorders of consciousness and fits of various kinds; consideration was therefore given to the possibility of a space-occupying lesion involving the upper brain stem or region of the third ventricle. The history of drowsiness, of amnesia extending over several hours, and of coma lasting for 36 hours was sufficiently consistent for hypoglycaemia to be included in the differential diagnosis.

The history, in *Case 3*, of nocturnal attacks of impaired consciousness, confusion and clonic movements associated with excessive sweating strongly suggested hypoglycaemic attacks and this was rendered the more likely by the protection afforded by food or the ingestion of "jelly beans". Functional hypoglycaemia, rather than an islet cell adenoma, is possible in such instances, but consciousness is rarely lost in functional hypoglycaemia and the symptoms tend to vary with emotional stress. In this case the weight of clinical probability favoured a diagnosis of islet cell adenoma.

The signs and symptoms in 193 patients with insulinomas have been summarized by Crain and Thorn (1949)—see Table 1. Kepler

TABLE 1

SIGNS AND SYMPTOMS EXHIBITED BY 193 PATIENTS WITH INSULINOMAS

(From CRAIN, E. L., Jr., and THORN, G. W., 1949, *Medicine,* **28**, 427)

Symptom/Sign	%
Loss of consciousness	58
Confusion	54
Weakness and fatigue	41
Coma	40
Sweating	36
Drowsiness and stupor	35
Visual disturbances	30
Amnesia	28
Clonic convulsions	24
Headache	20
Noisy behaviour	20
Tremor	14
Irritability	11
Transient hemplegia	10

and Moersch (1937) have indicated that the whole range of psychiatric symptomatology can be observed—mild neurotic manifestations, automatic behaviour, mania, hallucinations and compulsions, and Moorhouse (1956) has studied the many neurological manifestations —loss of conscoiusness, convulsions, psychomotor attacks, pyramidal, extra-pyramidal and cerebellar signs, diplopia and hemianopia. It is therefore little wonder that the syndrome of hypoglycaemia is frequently mistaken for other conditions. Thus Cohen (1950) refers to hypoglycaemic attacks attracting a diagnosis of cerebral tumour, neurosis, psychosis or alcoholism. In like vein, Crain and Thorn (1949) found that some 25 per cent of patients had symptoms for more than five years before the condition was recognized, and Priestly (1962) lists the most common erroneous diagnoses as being epilepsy, psychosis, cerebrovascular disease, hysteria, brain tumour and drunkenness.

A diagnosis on clinical grounds is often possible provided one thinks of hypoglycaemia as a possible cause of the patient's symptoms. It is our impression that it is necessary to probe deeply by direct questioning before an adequate clinical history is unfolded. Freeark and de Peyster (1963) summarize the position well—see Table 2.

TABLE 2

CONSIDER A DIAGNOSIS OF HYPOGLYCAEMIA

in patients of either sex, and particularly between the ages of 30 and 60 years who present with

1. Disturbances of consciousness
2. Voracious appetites
3. Paroxysmal disorders of behaviour
4. Transient neurological defects
5. Distal motor neuropathy
6. Symptoms of alcoholism but who deny taking alcohol.

(After FREEARK and DE PEYSTER, 1963, *Surgical Clin. N. America,* **43**, 79)

2. *Laboratory diagnosis*

Cohen (1950) has emphasized that the diagnostic problems of hypoglycaemia resolve themselves into two groups: (a) the recognition of hypoglycaemia and (b) the unmasking of its cause. The possible causes of hypoglycaemia of particular value to the clinician are indicated in Table 3, and in Table 4 the laboratory investigations most likely to be of assistance are listed.

TABLE 3

CAUSES OF HYPOGLYCAEMIA

(After CONN, 1947, *J. Amer. Med. Assoc.,* **134**, 130)

I ORGANIC
1. Hyperinsulinism
2. Severe hepatic disease
3. Anterior pituitary hypofunction
4. Adrenal hypofunction
5. Lesions of hypothalamus and brain stem
6. Fibrosarcomata, retroperitoneal or thoracic

II FUNCTIONAL
1. Imbalance of autonomic nervous system
2. Alimentary—following gastric resection
3. Renal

III MISCELLANEOUS
1. Overdose of insulin
2. Inanition
3. After prolonged intake of alcohol
4. Idiopathic hypoglycaemia of infancy

TABLE 4

LABORATORY INVESTIGATIONS IN SUSPECTED HYPOGLYCAEMIA

(After FREEARK and DE PEYSTER, 1963, *Surg. Clinics N. America,* **43**, 79)

I FASTING TEST (Whipple's Triad)—to establish hypoglycaemia
II X-RAY SKULL—to exclude pituitary adenoma
III X-RAY CHEST AND ABDOMEN—to exclude fibrosarcoma (+ or — Barium Meal)
IV TOLBUTAMIDE TOLERANCE TEST—to establish insulinoma

And, if indicated on clinical grounds:

V LIVER FUNCTION TESTS (? liver disease)
VI URINARY KETOSTEROIDS (? adrenal insufficiency or hypopituitarism)
VII RADIOACTIVE I^{131} UPTAKE (? thyrotoxicosis)

In our own practice we have found the *prolonged fasting test* of value. It must be emphasized that a blood sugar estimation after 12 hours fast is not sufficient unless the patient is displaying symptoms suggestive of hypoglycaemia. The fast must be continued for 18 hours, 24 hours or even longer. Blood sugar estimations may be performed at intervals and whenever symptoms suggestive of hypoglycaemia occur. Should this eventuate, intravenous glucose is administered after removal of the blood sample, and the effect noted. In this way the requirements of Whipple's triad may be fulfilled. Whipple (1935) emphasized: (1) periodic attacks of nervous disturbance in the fasting state, (2) blood sugar value of 50 mg per cent or less, and (3) prompt relief by administration of glucose. It is Priestley's experience (Priestley, 1962) that when an islet cell tumour produces symptoms the blood sugar is invariably less than 45 mg/100 ml during an attack. Following an overnight fast, blood sugars in excess of 50 or 65 mg/100 ml are commonly encountered in these patients.

The results of the *fasting test* in *Case 1* are indicated in Figure 1 and, for comparison, a six-hour glucose tolerance test, which we have found of little value in this condition, is also shown.

The intravenous tolbutamide tolerance test (Fajans and Conn, 1959) is of outstanding value in the diagnosis of insulin-secreting tumors since the degree and the persistence of tolbutamide-produced hypoglycaemia is more marked in patients with islet cell tumours than in patients with hypoglycaemia from other

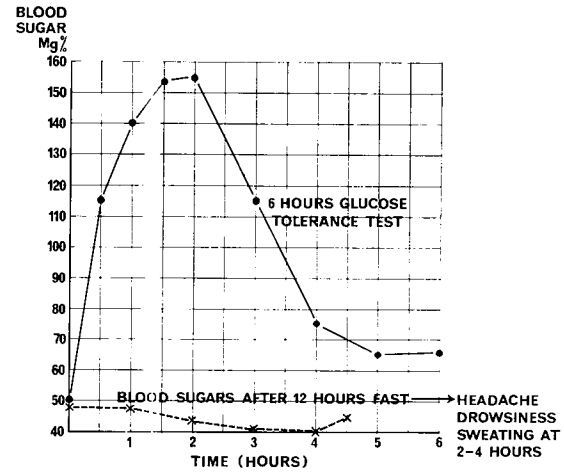

FIG. 1—Case 1, 6 hours Glucose Tolerance Test and Fasting Test.

causes. L-leucine, taken orally, apparently produces similar results, but we have no personal experience with this test. Freeark and de Peyster (1963) have found that occasionally the tolbutamide test may fail to demonstrate a characteristic response in patients with islet cell adenomas, but they have never known a false positive reaction to occur.

It would seem that at least in part the action of tolbutamide and leucine is to increase the elaboration of insulin by the tumour. The results of tolbutamide tolerance tests in *Cases 2 and 3* are shown in Figure 2, and the results of the

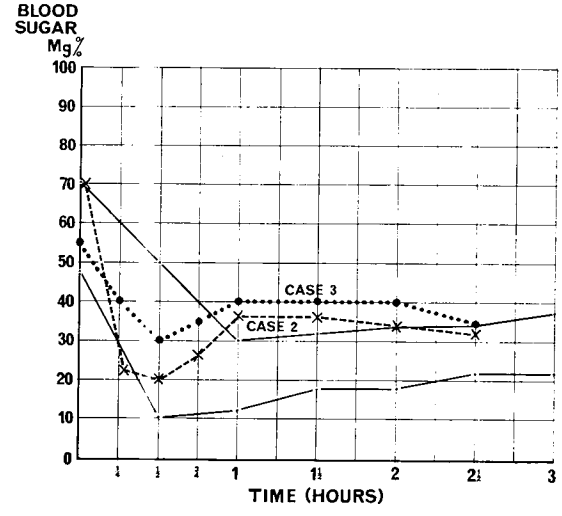

FIG. 2—Tolbutamide Tolerance Test in Cases 2 and 3.

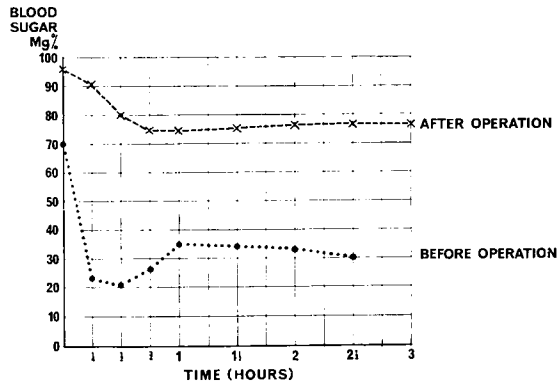

FIG. 3—Tolbutamide Tolerance Test before and after operation in Case 2.

test before and after operation in *Case 2* are indicated in Figure 3.

3. *The tumour*

Table 5 indicates the situation, size and nature of the tumours found at operation. Patients 1 and 2 were operated on by Mr. D. B. Leaming, M.S., F.R.C.S., F.R.A.C.S., and Case 3 by Mr. B. H. Courtice, M.B., B.S., F.R.C.S.

TABLE 5

ISLET CELL ADENOMA

Case	*Situation*	*Size (approx.)*	*Nature*
1	Lower border body of pancreas	1" diameter	Poorly encapsulated. Locally invasive.
2	Posterior surface superior border neck of pancreas	1" diameter	Poorly encapsulated. Locally invasive.
3	Posterior surface body of pancreas	1" diameter	Well encapsulated.

In these three patients the tumours were single; in some 10-12 per cent of cases more than one adenoma is present but rarely more than two (Morley, 1952; Freeark and de Peyster, 1963).

The gross appearance of the adenoma from *Case 3* is shown in Figure 4 and the histology in Figure 5 from *Case 2*.

FIG. 4—Gross appearances of islet cell tumour removed from Case 3. Both halves of bisected tumour are shown (the vertical lines are part of a centimetre scale).

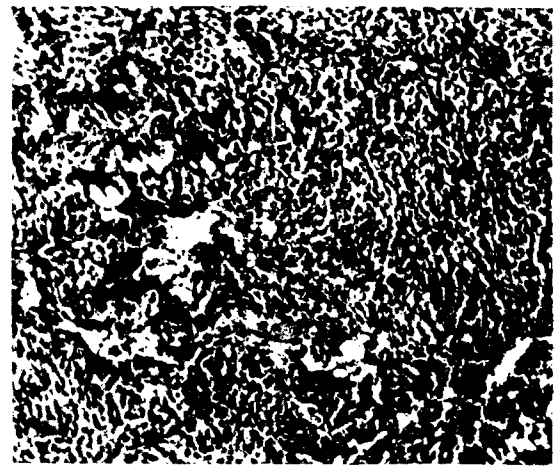

FIG. 5—Islet-cell tumour showing masses of tightly packed cells with scanty vascular stroma. Peripherally this tumour was poorly encapsulated and was locally invasive. × 120.

All three patients had a normal convalescence and have since remained well with the exception of *Case 1* who continues to have headaches. There is no evidence of continuing hypoglycaemia or of hyperglycaemia in this case and physical examination is otherwise negative. It is not known whether the continuance of this symptom is an expression of the patient's underlying personality or whether it reflects irreversible changes in the nervous system due to hypoglycaemic episodes in the past.

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DISCUSSION

The President asked the pathologists to comment upon the permanent structural damage described by Dorothy Russell in hypoglycaemia.

Dr. R. Anderson: I have seen such damage in patients with insulin over-dosage and insulomas. The main changes are in the territory of the middle cerebral artery in the cortex and putamen. The neurone is affected primarily and demyelination follows. There may also be cerebellar cortical degeneration. The histology suggests anoxia in a particular vascular supply. Garland comments that the recorded blood sugars may never be less than 40 mg/100 ml in these patients, yet children and adults in insulin coma with no blood sugar may recover.

Dr. B. Turner stated that he had diagnosed hypoglycaemia once on low sugar in the cerebrospinal fluid. He pointed out that coma was a biochemical death, but we also saw effects of heart failure, etc. He recommended that the blood sugar should be done if the cerebrospinal fluid sugar is low. He considered the cerebrospinal fluid sugar to be reliable, and he was uncertain how soon the sugar should be estimated after taking cerebrospinal fluid.

Dr. John Gordon: In cases of insulinoma I have seen the diagnosis was either temporal lobe epilepsy or hysteria. Attacks at night are suggestive, but a random fasting blood sugar may not clinch the diagnosis, therefore the patient should be fully investigated. He illustrated this point by showing fasting and tolbutamide tests in a child with hypoglycaemia who was sensitive to leucine. During removal of the tumour the blood sugar rose to a high level.

Dr. J. Ailsop: Only 36 per cent of 193 cases reviewed by Conn had sweating. Sweating, tachycardia and tremor may be absent in many cases.

Dr. Prestwick suggested that the first patient had temporal lobe epilepsy and the second basilar insufficiency and hypoglycaemic symptoms.

Dr. Eadie: The first patient probably had temporal lobe epilepsy, but the second did not have vertebro-basilar insufficiency.

Dr. Wolfenden asked what are the risks of the tolbutamide test, and secondly, whether it is possible to differentiate anoxic and hypoglycaemic cerebral damage on pathological grounds?

Dr. Eadie: A risk does exist, and the pathologist must be prepared to give glucose.

Dr. Turner stated that it was impossible pathologically to distinguish between anoxic and hypoglycaemic damage.

In answering a question by Dr. Davis, *Dr. Gordon* said that 12 per cent of the adenomas were malignant, and 12 per cent of adenomas were multiple.

Dr. Selby described another case with an electroencephalographic focus before operation, and after the tumour was removed, the focus disappeared.

Dr. Rischbieth suggested that vascular effects were responsible for localized effects in hypoglycaemia.

THE NEUROLOGICAL MANIFESTATIONS OF SUBACUTE BACTERIAL ENDOCARDITIS

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Sydney

INTRODUCTION

Certain neurological complications of subacute bacterial endocarditis are well known. These include cerebral and spinal embolism and subarachnoid haemorrhage due to mycotic aneurysm. One might add hypertensive encephalopathy and uremia due to the diffuse glomerulonephritis which may complicate subacute bacterial endocarditis.

In the last twelve months I had the opportunity of observing three cases exhibiting a generalized subacute encephalopathy. Following this the cases of subacute bacterial endocarditis admitted to the Royal Prince Alfred Hospital between January 1958 and July 1964 were reviewed. Some of the literature was examined.

CASE REPORTS

1. R.S., male, 54, R.P.A.H. No. 150104. This patient was examined first on 1/4/64 and died on 8/6/64. In February 1963 he sustained abrasions of the face with brief unconsciousness in a motor accident. Two months later he noticed headache and insomnia. It was observed that his intellectual functions were progressively and gradually deteriorating. He complained of joint pains and his walking became slow. He noticed difficulty in swallowing and became anxious and tremulous. He began to sweat excessively and progressively lost weight. Memory, thought and speech progressively deteriorated. On examination marked dementia was apparent. He was disorientated in time. The serial 7 test and interpretation of proverbs could not be commenced. Voluntary movement was slow and clumsy though there was no frank cerebellar ataxia. He did not swing his arms freely and his face was immobile. There was a rapid tremor of the outstretched hands and cogwheel rigidity of the lower limbs and of the right upper limb. The deep reflexes were exaggerated but the plantar responses flexor. His blood pressure was 120/80 mm of mercury and there was clubbing of the fingers. No cardiac murmur could be heard. It was suspected that he might have carcinoma of the lung, and he was admitted to hospital where for the first time a mitral systolic mumur became apparent. He was noticed to be febrile. His cerebrospinal fluid contained 13 lymphocytes per cubic mm, the protein was 30 mg/100 ml, and the globulin test negative, chlorides were 655 mg/100 ml, and glucose 75 mg/100 ml. The Lange curve was 0000000000 and the Wassermann reaction was negative. E.E.G. showed bilateral slow waves which became more apparent in a later tracing. Repeated blood cultures showed a growth of streptococcus faecalis. The rheumatoid latex test was positive as were the blood Wassermann, Kline and V.D.R.L. tests. Despite massive and appropriate penicillin and streptomycin therapy his condition progressively deteriorated. Right-sided facial weakness appeared with a right extensor plantar response. Shortly after this he developed a left hemiplegia and gross confusion of thought. Right-sided Jacksonian seizures heralded his death. Autopsy revealed healing subacute bacterial endocarditis of the aortic and mitral valves, infarcts of the spleen and kidneys and extensive softening in the right hemisphere with less marked softening in the left hemisphere. There was atheroma of the cerebral vessels. Detailed neuropathology was not performed.

2. D.P.B., male, 56, P.R.U. No. 960. Admitted 10/9/64, died 3/10/64. This man had been a heavy drinker for many years up till two months before his admission to the Rozelle Psychiatric Centre. He was admitted to this Centre on 22/7/64 from a general hospital because of mental confusion. He had been well until October 1963 when he suffered a coronary occlusion. After this he became increasingly forgetful and rambling in speech. One week before his admission he became more confused and complained of severe frontal headache. His appetite was poor. On examination his temperature was normal but rose subsequently as high as 101·5°F. He was dysphasic, disorientated in time and place, and perseverated. His pupils were small and reacted poorly to light. The ankle jerks were absent. There was a grade 3-4 mitral systolic murmur. The liver and spleen were enlarged. His fingers were clubbed. He was pale and a purpuric rash was visible on his legs. Streptococcus faecalis was grown on repeated blood cultures. He was treated by means of penicillin and streptomycin after return to the general hospital. He was confused and noisy and after one month was returned to the Psychiatric Centre. An initial E.E.G. showed a left-sided slow

wave abnormality. A subsequent tracing showed generalized slow wave changes with some left-sided predominance. The cerebrospinal fluid contained 3 cells per c.mm. The protein content was 74/100 ml and the globulin test positive. The Lange curve was 4322110000. Despite treatment by means of penicillin and streptomycin he continued to deteriorate. A series of grand mal seizures occurred, followed by cardiac arrest, coma and subsequent death.

Autopsy Findings: The significant general findings were the presence of small vegetations on the free surfaces of the mitral and aortic valves and some distortion of the mitral valve. There was septic infarction of the spleen and old infarcts in the kidneys. The lungs showed bronchopneumonia.

The brain was fixed and sectioned according to routine. It weighed 1283 gm. The circle of Willis was complete and the vessels at the base of the brain were free of atheroma. The carotid arteries were patent.

There was a large amount of subarachnoid blood which arose from a recent massive haemorrhage into the left frontal lobe. The head of the left caudate nucleus had been destroyed and the haemorrhage had ruptured into the left lateral ventricle. Adjacent to the haemorrhagic area there was a region of old cystic softening in the white matter. Throughout both hemsipheres there were scattered small areas of cystic infarction and more recent areas of cortical laminar necrosis.

Histological examination confirmed the above findings and revealed other interesting features. There was a quite marked fibrous meningeal reaction of some duration which had been obscured by the subarachnoid blood. There was widespread loss of cortical nerve cell, gliosis and occasional thrombosis of cerebral arteries, one of which had been recanalized. The intracerebral vessels were generally very prominent with collections of haemosiderin pigment in their perivascular spaces. Little evidence of acute inflammatory reaction could be found with the exception of one vessel, probably a vein, at the base of the pons whose wall was infiltrated with chronic inflammatory cells.

Comment: The overall findings confirmed the clinical diagnosis of subacute bacterial endocarditis.

The brain does not show the classical lesion of inflammatory nodules characteristic of metastatic encephalitis as these have probably resolved under therapy and any resultant focal damage has largely been obscured in the cortex by the period of cardiac arrest.

The meningeal thickening is consistent with an episode of meningitis at some period whilst the scattered areas of softening suggest, in the presence of a well preserved local vasculature, previous embolic episodes.

3. R.B., male, 41, P.R.U. No. 950, R.P.A.H. No. 154194. Admitted 6/8/64, died 21/5/65. This patient was admitted on 6/8/64 to the Parramatta Psychiatric centre. Twelve months before admission he developed paranoid ideas about his wife and son. He had previously drunk to excess and was known to have had a disturbed personality for many years. Several weeks before admission to the Parramatta Psychiatric Centre his appetite became poor and he became short of breath and developed a cough. On examination there he was confused and exhibited an expressive dysphasia with perseveration. There was slight weakness of the right upper limb. A loud apical systolic murmur was detected. There was some sensory disturbance of unspecified type in the right upper and lower limbs. His speech improved and the impairment of sensation disappeared. When admitted to the Psychiatric Research Unit it was noted that his teeth had been extracted in March 1964, following which he lost two stone in weight and became fatigued and suffered a decline in mental functions with increasingly paranoid features. On examination at the Psychiatric Research Unit there was weakness of the right upper limb, with exaggeration of the deep reflexes on the right side and a right extensor plantar response. The spleen was enlarged. There was a grade 3-4 mitral systolic murmur and aortic systolic and diastolic murmurs. Streptococcus viridans was discovered on repeated blood cultures. Despite intensive penicillin and streptomycin therapy he developed progressive congestive cardiac failure due to his valvular lesions and died. His E.E.G. showed a focal left-sided disturbance of slow waves at 2-3 c.p.s. The cerebrospinal fluid contained 4 cells per c.mm. The protein was 62 mm/100 ml with a positive globulin test. The colloidal mastic test was 4321000000. At autopsy there were vegetations on the mitral and aortic valves, congestion of the liver, infarcts of the spleen and kidney, and an extensive infarction of the right hemisphere of the brain. Detailed neuropathology was not performed.

A fourth case is included. This does not illustrate the generalized encephalopathy which first excited my interest. It is not contained within the survey of cases at Royal Prince Alfred Hospital.

4. W.R., male, 56, R.P.A.H. No. 152721. Admitted 25/9/64, discharged 23/11/64. This coalminer was admitted with the provisional diagnosis of cerebral abscess. Twelve months previously he had had a cholecystectomy. On 10/9/64 he reported to his

general practitioner complaining of loss of weight and energy, night sweats, scattered joint pains, cough and shortness of breath of recent onset. He had previously taken alcohol to excess. He had lost his right eye in an accident. When seen by his doctor his temperature was 101°F. The following day he developed sudden right hemisparesis and aphasia, and became semiconscious. His cerebrospinal fluid contained 1500 leucocytes per c.mm. and was turbid. The protein content was 20 mg/100 ml and the glucose 35 mg/100 ml. Some involuntary movements of the left upper limb were noted. He was transferred to the Royal Prince Alfred Hospital where it was observed that the left pupil was slightly dilated and the left eye could not be turned to the right. There was no papillodema. Neck stiffness was present and Kernig's sign positive. There was an expressive aphasia and a right hemiparesis with a right extensor plantar response. No cardiac murmurs could be detected despite careful auscultation. The cerebrospinal fluid contained 2200 white cells per c.mm., 80% being neutrophils. There were 88 red blood cells per c.mm. Left carotid arteriography demonstrated a block of the main trunk of the left middle cerebral artery. On 8/10/64 the cerebro-spinal fluid contained only 15 lymphocytes per c.mm. Aortic systolic and diastolic murmurs became heard during his hospital stay. Streptococcus faecalis was grown on blood culture. Appropriate therapy with penicillin and streptomycin was commenced and he was discharged with no signs of active infection but with residual dysphasia and right hemisparesis. The serological tests for syphilis in blood and cerebrospinal fluid were negative.

Commentary on Cases

The first three cases, of which Case 1 is the most striking, illustrate a subacute diffuse encephalopathy with some focal features. In my opinion the positive blood Wassermann reaction in Case 1 is a biological false positive, particularly as the cerebrospinal fluid Wassermann was negative. Biological false positive reactions are fairly common in subacute bacterial endocarditis (Kerr 1955). In the last two cases the picture is complicated by alcoholism. In Case 2 the loss of ankle jerks and pupillary changes are quite likely to be due to alcoholism. It is clear, however, that the later mental changes are at least partly due to the cerebral manifestations of subacute bacterial endocarditis.

Review of Cases at Royal Prince Alfred Hospital

From January 1958 to July 1964 (inclusive) 88 acceptable cases of subacute bacterial endocarditis were admitted to this hospital. In all of these a firm clinical diagnosis had been made. In some, positive blood cultures were not obtained, but no case has been included in the neurological list without a positive blood culture, autopsy evidence, or in one case only a clinical diagnosis with evidence of an embolus in a retinal artery.

There were 49 females and 39 males. A positive blood culture was obtained in 40. The organisms found were streptococcus viridans in 22 cases; non-haemolytic streptococcus in 7 cases; haemolytic streptococcus (not group A) in 2 cases; staphylococcus pyogenes in 5 cases; staphylococcus albus in 1 case; and haemophilus influenzae in 3 cases. Autopsy was performed in 17 cases.

In the rheumatic cases the aortic valve alone was involved in 20; the mitral alone in 40; and the mitral and aortic in 8. There were 15 cases of congenital heart disease, and 5 other sundry cases which were difficult to classify from the records available.

Neurological manifestations were noted in 12 cases. Amongst these cases there were two instances of subarachnoid haemorrhage, 3 of cerebral embolism, 1 of subacute encephalopathy (case 1 above), 5 of acute encephalopathy, and 2 of epileptic seizures. There are thus 13 manifestations occurring in 12 patients (Table 1).

Subarachnoid haemorrhage and cerebral embolism require no discussion. Epileptic seizures probably do not require elaboration except to say that these may occasionally be a complication of the very high doses of penicillin employed in treatment. In cases labelled acute encephalopathy it is clear that several mechanisms may be involved in different cases. Perhaps some of these should be more properly deemed to be suffering from a toxic confusional state associated with high fever as may occur in other infections. In others clearly multiple cerebral embolism may be the cause. On clinical grounds it is difficult to see how this distinction may be made, so the cases are grouped together as acute encephalopathy, although in the histories at times the diagnosis of toxic confusional state had been suggested by the clinician.

It was the cases of subacute encephalopathy which first excited my interest. It would seem likely that multiple embolism together with the effects of haemorrhage and infection within the central nervous system might explain the syn-

TABLE 1

Summarizing Neurological Manifestations in 12 of 88 cases of Subacute Bacterial Endocarditis.

Sex	*Age*	*Clinical Neurological Findings*	*CSF Findings*	*EEG*	*Blood Culture*	*Autopsy Findings*	*Remarks*
F	63	Basilar embolism	normal	not performed	not cultured	basilar embolism, mitral stenosis, bacterial endocarditis	
F	65	Acute encephalopathy (stupor and neck rigidity) ("meningoencephalitis")	352 white cells per cmm. 97% polymorphs, later 500 white cells per cmm. 98% polymorphs protein 30 mgm% globulin not increased, Cl 685 glucose 111	not performed	Staphylococcus pyogenes from valve at post mortem	Atheroma of mitral valve, bacterial endocarditis	
F	64	Acute encephalopathy (neck stiffness, mental confusion)	not performed	not performed	not cultured	bacterial endocarditis, mitral incompetence, embolism R internal carotid artery, softening of R hemisphere, haemorrhage into R lobe cerebellum	
M	79	Subarachnoid haemorrhage, coma	blood stained xanthochromic	not performed	Streptococcus viridans	not performed	Had been on anticoagulants
F	59	Subarachnoid haemorrhage	blood stained CSF	not performed	Streptococcus faecalis	not performed	
M	59	Cerebral embolism R hemiplegia	normal	not performed	Streptococcus viridans	not performed	
M	29	Acute encephalopathy embolism of L retinal artery	not performed	not performed	negative	not performed	
M	70	Cerebral embolism, hemiparesis	not performed	not performed	Staphylococcus pyogenes	not performed	
M	71	Terminal fit	not performed	not performed	Streptococcus viridans	not examined	
M	64	Subacute encephalopathy	13 lymphocytes per cmm. see Case 1	bilateral slow waves	Streptococcus faecalis	brain softening, subacute bacterial endocarditis	Case 1
M	50	Acute encephalopathy 1 epileptic seizure	40 white cells per cmm. 98% lymphocytes	normal	Haemophilus influenzae	not performed	30 million units of penicillin per day, fit may have been due to medication.
F	19	Acute encephalopathy (subacute meningo-encephalitis)	134 red blood cells, 19 white blood cells, 84% lymphocytes, protein 35	slow basic rhythm of 8–9 cps, later increasing to 9–10 cps	Streptococcus viridans	nil	Disturbed frontal lobe behaviour for 9 days before admission. Passed urine in clothes. Temperature 99·4, left facial palsy. Aortic incompetence.

drome. The detailed neuropathology in case 2 lends support to this thesis. In other cases the effects of toxaemia and hypoxia due to cardiac failure must be considered if these are present.

REVIEW OF THE LITERATURE

The neurological manifestations of subacute bacterial endocarditis are not stressed in recent standard texts. Turning to Kinnier Wilson, however, we read "Encephalitic lesions are not rare in malignant endocarditis whether accompanied by nervous symptoms or not. When present the latter include somnolence, apathy, pyramidal malfunction on one or both sides, cranial nerve palsies, optic neuritis, tremor, ataxia, fits, etc., in a varety of combinations or singly. Tissue

reactions depend in some degree on the pathogenic organism; ischemic and hyperplastic lesions may be due to streptococcus viridans (endocarditis lenta) or in more acute cases to pneumococci; minute embolic zones of necrosis or "bleaching" may be a feature, or miliary abscesses. Glial and mesodermal structures sometimes unite to form nodules resembling those of typhus. Toxic endarteritis is usual, perivascular infiltrates and glial overgrowth also; ganglion cells undergo chromatolysis and neuronophagia occurs."

Toone (1941) discussed the cerebral manifestations of bacterial endocarditis and reviewed 35 cases at the Medical College of Virginia from 1/6/32 to 31/12/38. Seventeen had neurological manifestations. Nine cases were admitted primarily as neurological or psychiatric problems. He considered the fundamental change in the nervous system to be a diffuse embolic meningo-encephalitis. He drew attention to the occurrence of a meningeal syndrome with neck rigidity and spinal fluid pleocytosis. The cellular response could be polymorphonuclear or lymphocytic. In subacute bacterial endocarditis the causative organism is not usually recovered from the spinal fluid, though in acute bacterial endocarditis the organism may be recovered. He pointed out that in the aseptic meningitis syndrome one should consider the diagnosis of subacute bacterial endocarditis.

Glecker (1958) discussed the diagnostic aspects of subacute bacterial endocarditis in the elderly. He pointed out that the clinical picture was often atypical in older patients and described 10 cases with ages ranging from 57 to 81. Three cases presented as major psychoses. In one the brain was not examined, in another there were multiple cerebral emboli, and in the third the patient was cured. A further case presented as a cerebral haemorrhage. This author suggested that the symptoms could be ascribed to a focal encephalitic reaction to multiple small emboli, or in some cases to gross infarctions.

A comprehensive review of subacute bacterial endocarditis was made by Kerr (1955). He found that clinical evidence of central nervous involvement in subacute bacterial endocarditis is frequent and that the more dramatic manifestations are usually terminal, though may be the presenting problem. This tendency for the manifestations of subacute bacterial endocarditis to be late may explain why these were more common in the literature in the pre-antibiotic era. Kerr noted apart from the better known manifastations, other less well known disturbances such as chorea, meningitis, organic psychosis and papillodema. He stressed the relatively frequent occurrence of organic psychosis. He re-emphasized the sterile reaction in the cerebrospinal fluid as pointed out by Toone (1941). He noted that not all the cerebral embolic lesions are bland and that abscess is occasionally reported. He pointed out that the retinal change often considered to be characteristic of subacute bacterial endocarditis, namely a white-centred retinal haemorrhage, is found in other conditions. He noted that swelling of the optic disc due to optic neuritis may be seen. Finally, he noted that protein abnormalities are often found in the serum in subacute bacterial endocarditis consisting primarily in an elevation or alteration in gamma globulin. This may give rise to abnormalities in liver function tests and often positive serological tests for syphilis.

SUMMARY

Four cases are presented, three illustrating a subacute generalized encephalopathy, and one a cerebral embolism with a marked meningeal reaction. Experience in subacute bacterial endocarditis at the Royal Prince Alfred Hospital between January 1958 and July 1964 is reviewed with particular reference to cases exhibiting neurological symptoms or signs. Some of the literature is discussed.

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DISCUSSION

Dr. Anderson: Did you think all the lesions were embolic?

Dr. Allsop: I think Dr. Turner considered the arterial lesions were embolic. There were neuronal changes due to cardiac arrest.

Dr. Anderson: Do you think these lesions could be related to rheumatic arteritis?

Dr. Allsop: No, my feeling is that some of the arterial lesions are associated with a septic arteritis, not a rheumatic arteritis. When further material is available we shall explore the nature of the arteritis further.

Dr. Burke: These patients were sick patients suffering malnutrition; are these perhaps the primary changes and the bacterial endocarditis terminal?

Dr. Allsop: Certainly the lowered resistance associated with malnutrition is important, but well marked embolic changes with infarction were demonstrated which would explain the mental abnormality. There were no changes in the brain indicative of nutritional disturbance. The whole picture was well known to the older clinicians who saw untreated subacute bacterial endocarditis. Most of the clinical literature is before 1941.

Free Papers

STUDIES IN MIGRAINE

JAMES W. LANCE

Sydney

A Headache Clinic was in operation at the Prince Henry Hospital, Sydney, from June 1962 until November 1964. Over a thousand patients were examined, most of whom suffered from migraine or tension headache. Patients who were subject to both forms of headache were classified under the major source of disability (Table 1). Criteria for the diagnosis of migraine were based on those of Selby and Lance (1960). Electroencephalography and radiographic studies were carried out only when headaches were of recent onset or the diagnosis appeared doubtful.

TABLE 1

PATIENTS ATTENDING HEADACHE CLINIC

June 1962—December 1964

Diagnosis	*Number of Patients*
Migraine:	
1 per month or more	500
less than 1 per month	112
Cluster headache	13
Tension headache:	
daily	354
10-30 per month	64
less than 10 per month	48
Miscellaneous	61
	1,152

The present paper will not deal with much of the clinical data accumulated about migraine and tension headache, but will summarize our studies into the mechanism and treatment of migraine.

Various chemical substances (acetyl choline, histamine, "neurokinin") have been considered in the past as possible mediators of the migraine syndrome. The most recent addition to this list is serotonin (5-hydroxytryptamine, 5HT), a natural amine which tends to constrict large vessels and dilate small ones, as well as acting on the gut, kidneys and nervous system. Serotonin is manufactured mainly by the gut, transported in blood platelets and metabolized in the liver, chiefly to 5-hydroxyindoleacetic acid (5HIAA), which is excreted in the urine. The possible relation of serotonin to various neurological disorders was discussed by Lance (1964).

Sicuteri, Testi and Anselmi (1961) drew attention to the increased excretion of 5HIAA at the time of the migraine attack. Recent work in our laboratory, using a technique developed for the purpose (Hinterberger, Curran and Bartholomew, 1964) has confirmed this in general, although the amount of the increase is not always consistent from attack to attack or patient to patient. Increased 5HIAA excretion takes place in the first twelve hours of the migraine attack and usually precedes the onset of vomiting or diarrhoea when these occur. Total plasma serotonin commonly, but not invariably, drops at the onset of the migraine attack (Curran, Hinterberger and Lance, 1965a). If this phenomenon is causally related to migraine it would account for the precipitation of migraine headache by the injection of reserpine 2·5 mg., which denudes blood platelets of serotonin, and for the relief of migraine by the injection of 5 mg. serotonin intravenously (Kimball, Friedman and Vallejo, 1960). It would also explain the spontaneous relief experienced by many patients after vomiting, since Adams (1960) observed that serum serotonin increased when intestinal motility was stimulated. The beneficial effect of methysergide in migraine could therefore depend upon it simulating the action of serotonin. This is con-

sistent with some experimental findings which suggest that the serotonin antagonism of methysergide may be competitive.

Since the blood-brain barrier prevents free diffusion of serotonin, it is unlikely that any rapid change in blood serotonin levels would produce the vascular changes of migraine by central action. Further work is in progress to determine whether changes in serotonin level affect the scalp vessels, either directly, or via the carotid body which is known to be sensitive to serotonin (Skinner and Whelan, 1962). Mr. A. Gonski has removed the carotid body in ten of our patients on the side affected by severe, frequent migraine attacks unresponsive to medical treatment. Nine of these patients have now been followed up for 3-9 months and substantial improvement has been obtained in five patients. This result must be interpreted with caution, as the recent experience of O'Rourke and O'Rourke (1964) with carotid glomectomy in asthma has shown.

It is quite possible that the ingestion of certain foods, emotional stress or hormonal changes act as trigger factors for migraine by altering gut motility or liver metabolism, thereby altering serotonin levels in the blood. Since the urinary excretion of 5HIAA is usually high at a time when total plasma serotonin is low, it is probable that metabolism of serotonin in the liver is increased at this time. Possibly the onset of vomiting may diminish hepatic breakdown of serotonin and permit the building up of normal blood levels. This hypothesis is highly speculative but provides a stimulus for further research into the mechanism of migraine.

Methysergide (Deseril), a derivative of lysergic acid which has some properties similar to those of serotonin and which blocks some actions of serotonin, has been used extensively by the clinic in the treatment of severe attacks of migraine recurring more than once each month. A preliminary report (Lance, Fine and Curran, 1963) demonstrated the effectiveness of methysergide in a controlled clinical trial. Almost 400 patients have now been treated with methysergide for up to 30 months, with sustained improvement in 58 per cent. A comparable group of patients have been treated with cyproheptadine ("Periactin"), which has both anti-histamine and anti-serotonin properties, for up to 12 months. The results were significantly better than placebo ($p < 0{\cdot}05$) but not as good as those with methysergide, in spite of the fact that the shorter follow-up period for cyproheptadine allows less possibility of relapse. Table 2 compares the results of methysergide and cyproheptadine when both were used as the initial treatment in patients presenting to the clinic with two or more severe migraine attacks each month. The table also includes results with "Bellergal", although this group is not comparable with the others as children, pregnant women and patients with milder headaches were placed on this medication first because of the infrequency of side-effects. Table 3 illustrates that "Bellergal" and cyproheptadine perform at much the same level

TABLE 2

Migraine: 1 per month or more
First Treatment

Treatment	*Headache free*	*Half improved*	*No change*	*Abandoned*	*Number in group*	*Number improved*	*% improved*
methysergide	72	115	99	39	325	187	58
cyproheptadine	6	18	26	9	59	24	41
"Bellergal"	14	23	37	2	76	37	49*
placebo	1	6	27	0	34	7	21

* selected group

TABLE 3

Migraine : 1 per month or more
First and subsequent treatments

Treatment	*Headache free*	*Half improved*	*No change*	*Aban-doned*	*Number in group*	*Number improved*	*% improved*
methysergide	80	138	112	46	376	218	58
cyproheptadine	22	47	88	22	179	69	39
"Bellergal"	20	56	120	2	198	76	38
placebo	1	9	40	0	50	10	20

when used on a wider group of patients, including those who had not responded to a previous treatment.

It may be concluded that methysergide is the most effective medication known in the prevention of severe migraine. It is most useful when the frequency of attacks exceeds 3/month (Curran and Lance, 1964). However, it is not the drug of choice for initial treatment because of the frequency and potential severity of side-effects. Some 40 per cent of patients experience symptoms such as nausea and pain in the abdomen or limbs on starting treatment and 10 per cent have to cease treatment because of side-effects. More serious reactions include vasoconstriction of limb vessels, resulting in obliteration of radial pulses or intermittent claudication. These may be overcome by reducing the dose and combination with vasodilators, but usually necessitate stopping treatment. Recently there have been reports of hypertension from spasm of the renal arteries, hydronephrosis and hydroureter, and heart murmurs developing in some patients under treatment with methysergide, all of which resolved when the drug was stopped. The numbers involved are small but emphasize the importance of keeping patients under close supervision when on long-term treatment with methysergide. Methysergide should be used with caution in patients with angina pectoris, peripheral vascular disease, hypertension or a tendency to thrombophlebitis, although atherosclerotic vessels are less liable than normal vessels to constrict as a drug idiosyncrasy. The symptoms of peptic ulcer may be aggravated by methysergide. Our own policy has been to avoid the use of methysergide during pregnancy, although there is no evidence that it can be detrimental to the foetus, and three of our own patients have given birth to healthy infants after its use in the early months of pregnancy. Methysergide is sufficiently effective in preventing migraine to warrant trial in any patient who is disabled by frequent attacks, but patients should remain under close medical supervision. Is is usually advisable to give half of one tablet (1 mg.) as a test dose, then to start treatment with one tablet three times daily, increasing to two tablets three times daily if necessary. Once a patient's attacks are controlled, the dose should be reduced to the minimum necessary to maintain control. The biochemistry, pharmacology, clinical trials, side-effects and mechanism of action of methysergide are reviewed in detail by Curran, Hinterberger and Lance (1966).

"Bellergal" and cyproheptadine are useful as an alternative treatment to methysergide, particularly in milder cases. It should be noted that some 10 per cent of patients are unable to tolerate cyproheptadine in the usual dose of 4-8 mg. three times daily, mainly because of drowsiness.

SUMMARY

Over a thousand patients with the presenting symptom of headache were examined at a Headache Clinic during the thirty months of its existence. The majority of patients suffered from migraine or chronic tension headache.

Research into the mechanism of migraine

established that total plasma serotonin falls at the onset of the attack, while the urinary excretion of its chief breakdown product, 5-hydroxyindoleacetic acid increased. The value of methysergide ("Deseril"), a serotonin antagonist, in the prevention of frequent attacks of migraine was established, and methysergide was found to be more effective than cyproheptadine ("Periactin") or "Bellergal". The importance of patients remaining under medical supervision while being treated with methysergide is emphasized.

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DISCUSSION

Dr. E. Davis: Have you met with the problem of increasing severity of migraine in patients who have been placed on oral contraceptive tablets?

Dr. J. W. Lance: I agree that many patients appear to be worse while taking contraceptive tablets, while only a few are improved. It is known that oestrogens tend to make migraine worse, and progestogens tend to improve it. This has led to the use of injections of chorionic gonadotrophin in the interval treatment of migraine. We have recently tried Gestanin, a progestogenic agent, in a small group of about 20 patients without any significant improvement being manifested.

Dr. J. Allsop: What is the relationship of retroperitoneal fibrosis and hydronephrosis to methysergide?

Dr. J. W. Lance: Graham in Boston has knowledge of 19 patients presenting with gastrointestinal and urinary tract symptoms while taking methysergide, and eight of these were found to have retroperitoneal fibrosis at operation. Intravenous pyelography demonstrated unilateral or bilateral hydroureter and hydronephrosis, which regressed three to six weeks after withdrawal of methysergide. Eleven of the nineteen patients had been treated with more than 6 mg of methysergide daily. Eight patients have also been reported as developing cardiac murmurs while on methysergide, and these too have lessened or disappeared after discontinuing treatment.

THE EARLY DIAGNOSIS OF ACOUSTIC NEURILEMMOMA

WILLIAM H. WOLFENDEN

Sydney

I will present briefly two cases of acoustic neurilemmoma which illustrate important features of the early diagnosis of these tumours.

Wylie McKissock had operated on 270 cases in the twenty years up to 1960, but only eight of these had a tumour of less than 3 cm diameter. In these eight cases neurological signs were few or absent apart from deafness, six having some 5th nerve sensory disturbance or partial facial weakness. Enlargement of the porus was shown by X-ray in only two of these patients, the cerebrospinal fluid protein was elevated in only two of the five examined, and air studies were done in each of the eight cases but showed the tumour in only one. The diagnosis was made in these patients by special otological tests performed by C. S. Hallpike and Terence Cawthorne. These tests rather than neurological examination lead to early diagnosis; all eight cases survived operation with rapid recovery, the auditory nerve and hearing were preserved in five of them, and the facial nerve was preserved in all.

As otological tests offer the best chance of early suspicion or diagnosis, it is logical that the otologist as well as the neurosurgeon should become interested in this field. This has recently been illustrated by the spectacular results of Dr. W. F. House, of Los Angeles, who has perfected the translabyrinthine approach via a postauricular incision and the mastoid, using an operating microscope.

House records 54 cases. In three the routine suboccipital approach was used and one died. A middle fossa approach was used in ten and two died. In the remaining 41 the translabyrinthine operation was used and there were no deaths at all.

CASE REPORTS

CASE I. Of two recent cases at Sydney Hospital, the first was a man aged 44 who had noticed gradually increasing deafness in the right ear for the past twelve months which was now almost complete. Recently there had been a buzzing noise in the right ear and occasionally there would be a slight unsteady feeling on standing up quickly. There had been no true vertigo.

Examination revealed only a right-sided sensori-neural deafness and the indefinite suggestion that the right corneal response may have been less brisk.

Otological tests (Fig. 1) showed an absence of recruitment in the deaf ear, there was marked tone decay and speech discrimination in the ear was nil. The caloric test in the right ear showed no reaction at 0°, 30° and 44°C.

The cerebrospinal fluid protein on two separate occasions was 244 mg/100 ml and 390 mg/100 ml.

X-rays suggested an asymmetry in size of the internal auditory meati, but this was not confirmed on tomography. A posterior fossa positive contrast study was attempted but the result was technically unsatisfactory.

A translabyrinthine operation was done and a neurilemmoma said to be the size of the distal segment of a thumb was removed piecemeal. It extended from the porus into the cerebellopontine angle and its capsule was confluent with the pia arachnoid. Some of the capsule in this region was not removed so as to avoid damage to the brain-stem.

The patient was perfectly well next day. He was completely deaf in the right ear, as the eighth nerve had been destroyed, but the facial nerve had been well seen at operation and there was no evidence of facial weakness following the procedure. The operation took 11 hours 15 minutes and was performed by two otologists, each taking occasional spells for obvious reasons.

CASE II. The second patient was a lady aged 63 who, three years previously, had first noted periods when she would feel off-balance with buzzing in the right ear for some weeks at a time. These symptoms cleared, only to recur about one year before diagnosis and, for the preceding four months, she had noticed deafness in the right ear. Again there had been no definite spinning vertigo.

On examination there was a sensori-neural deafness on the right side and a tendency to sway on toeing-the-line, but no other neurological signs. Fifth and seventh nerve functions were normal.

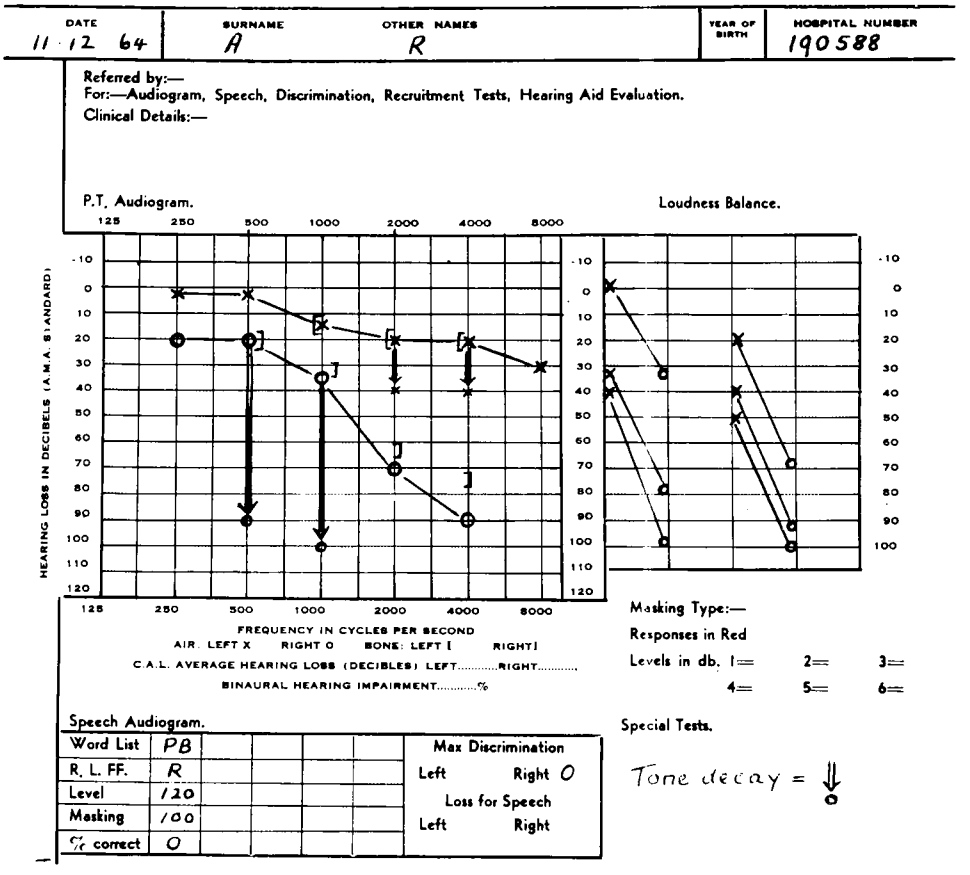


FIG. 1—Audiogram of Case I.

Otological tests (Fig. 2) again showed absence of loudness recruitment in the deaf ear, marked tone decay and no speech discrimination. Caloric tests produced no response at 0° and 30°C on the right. Lacrimation was tested but was found to be more marked on the right side.

The cerebrospinal fluid protein was 38 mg/100 ml and skull X-rays were normal. Posterior fossa positive contrast studies showed a filling defect on the right side measuring 1·8 cm × 2·3 cm (Fig. 3).

A translabyrinthine piecemeal removal of a neurilemmoma was performed by two surgeons over a period of seven hours. A small piece of capsule was attached to the pons and not removed. The anterior inferior cerebellar artery was seen deep to the tumour and not disturbed. Swelling of the cerebellum was noted at the end of the operation.

The following day the patient was conscious and co-operative, with some right facial weakness. Thirty-five hours after operation the patient suddenly became shocked and died after four hours. At autopsy (Fig. 4) a haemorrhage or haemorrhagic infarct was found in the anterior part of the right cerebellar hemisphere disrupting the middle peduncle, and involving the lateral part of the pons. There was blood in the fourth ventricle. The anterior inferior cerebellar artery entered the clot but appeared intact.

ORIGIN AND SPREAD OF TUMOUR

In the embryo a group of cells appear medial to the auditory vesicle. They constitute the acoustico-facial ganglion, the acoustic part of which divides into the cochlear and vestibular ganglia-groups of bipolar cells whose axones grow outwards to the endorgans and inwards towards the brainstem.

These cells also give rise to the neurilemmal sheath cells. The axones however extend more rapidly than the sheath cells, and the vestibular axones reach the brain-stem first, whereupon neuroglial cells from the brain-stem grow out along the vestibular nerve to sheath it and meet the neurilemmal cells in the internal auditory

meatus. It is at this point of junction of sheath cells on the vestibular division of the nerve within the porus that something goes wrong and neurilemmomas arise.

The tumour extends inwards along the auditory meatus and then enters the cerebellopontine angle, pushing the anterior inferior cerebellar artery medially and downwards so that it eventually lies between the capsule and the brain-stem.

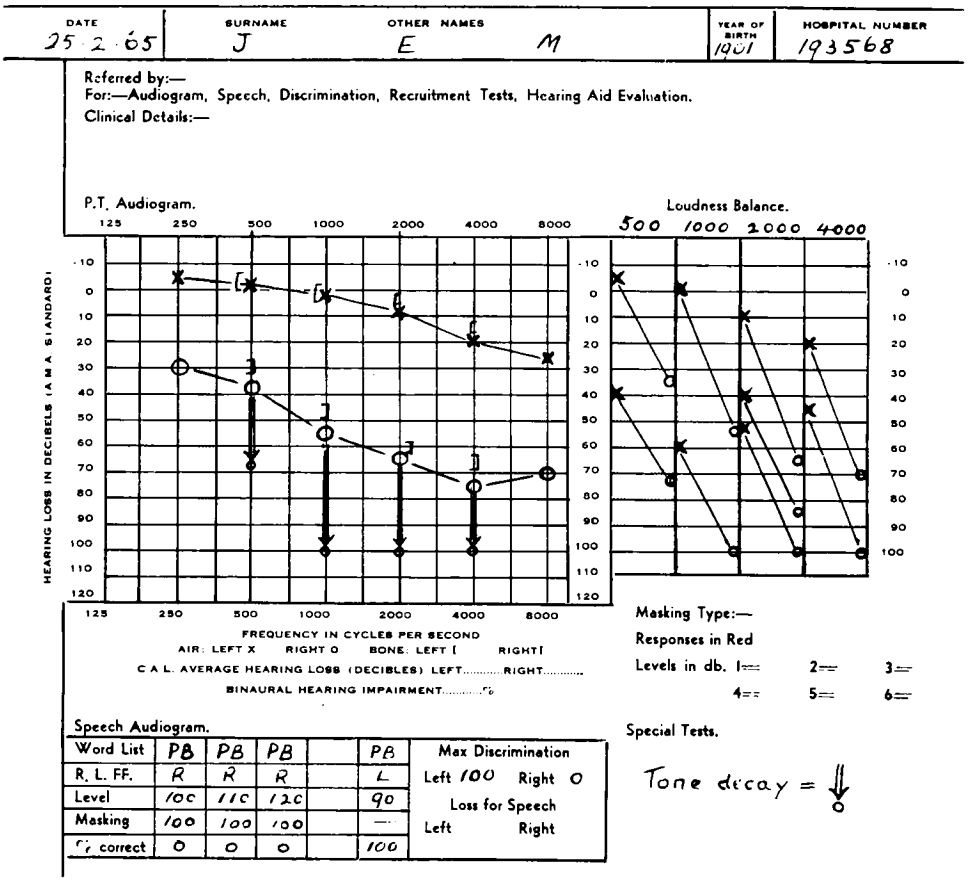
In the suboccipital approach the artery lies over the tumour and is sometimes ligated. In the translabyrinthine approach the artery lies medially and can be saved. Endeavours to remove the capsule from the brain-stem may damage the artery and, although great care was taken in these cases, some damage to the artery in the second case seems likely as the haemorrhage in the cerebellum and pons was in the distribution of this vessel. It is now thought to be an important artery of supply to cardio-vascular autonomic centres in the tegmental region of the pons.

Otological Tests

These cases illustrate the otological tests so important in early diagnosis, and they emphasize the fact that no patient should be left with a diagnosis of nerve deafness or Ménière's disease without thorough investigation.

The otological tests are designed to distinguish between the two types of sensori-neural deafness—the sensory, endorgan or cochlear deafness such as Ménière's on the one hand and the neural, nerve or retro-cochlear deafness as in acoustic tumour on the other.

One of the earliest of these test was *loudness balance* (Fig. 5). During audiometry the tone



Word List	PB	PB	PB		PB	Max Discrimination
R. L. FF.	R	R	R		L	Left 100 Right 0
Level	100	110	120		90	Loss for Speech
Masking	100	100	100		—	Left Right
% correct	0	0	0		100	

FIG. 2—Audiogram of Case II.

FIG. 3—Posterior fossa positive contrast (myodil) radiograms of Case II showing a filling defect in the internal auditory meatus on the right side (A). The shadow marked B is a metallic marker in the external canal.

is switched from one ear to the other and this is repeated with increasingly louder tones, the patient being asked to compare the relative loudness of each tone in the two ears. It is a characteristic of cochlear endorgan deafness such as Ménière's disease that the louder the tone the more nearly equal does it seem to be heard in the two ears. This is called *recruitment* and, if at a certain volume the patient hears the sound equally well in the normal and the deaf ear, it is called *complete recruitment.*

The expected finding in nerve, or retrocochlear deafness such as acoustic neuroma is absence of recruitment, the tone being heard

FIG. 4—Haemorrhage into the cerebellum and lateral part of the pons in Case II.

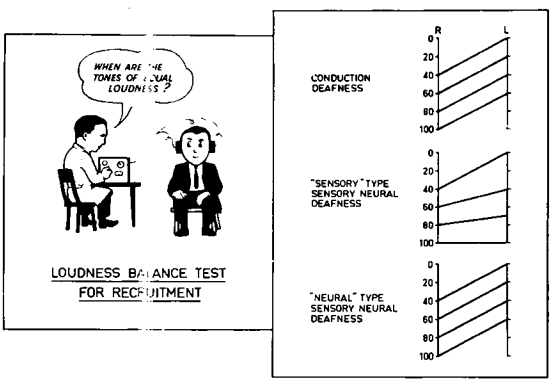
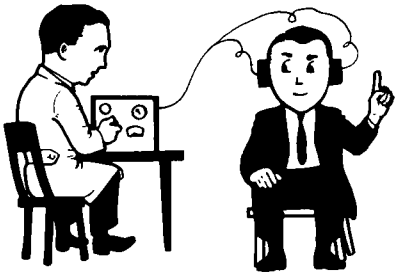


FIG. 5—Loudness balance test for recruitment.

much better in the normal ear whatever the amplitude. This was the situation in both the present cases. In tumour cases, however, partial recruitment may occur, and two proven tumours in House's series showed complete recruitment. It is assumed that these cases have endorgan or cochlear deafness due to ischaemia as the tumour compresses the internal auditory artery.

Late last century Gradenigo noted that patients with acoustic nerve tumours could hear a tuning fork only for a few seconds. This is called *tone decay* (Fig. 6) and occurs in nerve or retrocochlear lesions. A tone above threshold is sounded, the patient raising his finger while he hears it and lowering the finger when he fails to hear it. With an acoustic neuroma he rapidly fatigues and fails to hear the tone. The amplitude of the tone is then increased until he hears it and raises his finger again, and this is repeated as often as necessary for 60 seconds. The number of decibels the amplitude of the tone must be raised over this period is the amount of tone decay, and anything over 20 decibels raises suspicion of acoustic tumour.

Normal tone decay in 60 seconds is 10 decibels or less



MEASUREMENT OF AUDITORY FATIGUE

TONE DECAY IN 60 SECONDS

CONDUCTION DEAFNESS	0–5 decibels
"SENSORY" TYPE SENSORY NEURAL DEAFNESS	0–15 decibels
"NEURAL" TYPE SENSORY NEURAL DEAFNESS	20 or more decibels

FIG. 6—Auditory fatigue or tone decay.

Complete tone decay may occur in about 50 per cent of tumour cases and partial decay in a further 10-20 per cent. Marked tone decay is well illustrated in these two cases.

Speech discrimination is another valuable test (Fig. 7). The normal ear is masked with sound and the patient is asked to repeat a number of phonetically balanced words. In acoustic nerve tumours or retrocochlear deafness, the patient may be able to distinguish and repeat very few words or even none at all even though he has a fair or quite good response to a pure tone in the affected ear. This important test is again well illustrated in the present cases.

Seventy per cent of tumour cases have less than 30 per cent discrimination, and in about half the cases discrimination was less than 2 per cent. Nevertheless many proven tumours have had quite good discrimination scores.

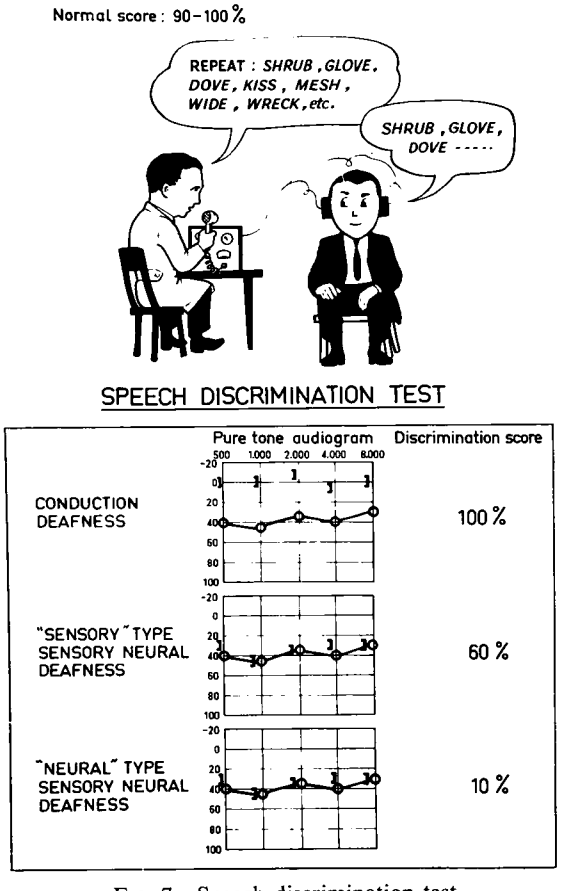


FIG. 7—Speech discrimination test.

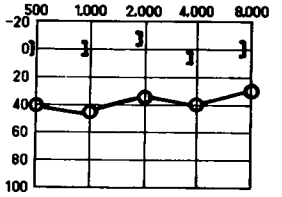
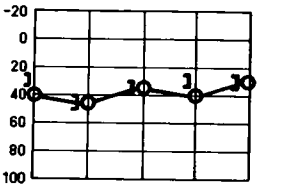
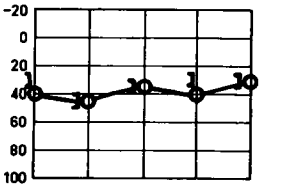
	PURE TONE AUDIOGRAM	RECRUITMENT	DISCRIMINATION	TONE DECAY
CONDUCTION DEAFNESS		NOT PRESENT	EXCELLENT	ABSENT
"SENSORY" TYPE SENSORY NEURAL DEAFNESS (OR COCHLEAR DEAFNESS)		PRESENT	POOR	ABSENT OR SLIGHT
"NEURAL" TYPE SENSORY NEURAL DEAFNESS (OR RETROCOCHLEAR DEAFNESS)		NOT PRESENT	VERY BAD	MARKED

FIG. 8—Typical findings in different types of deafness.
Legend ○ = Air conduction Right Ear
⌋ = Bone conduction Right Ear

Fig. 8 is a summary of expected results in the several types of deafness.

Testing of vestibular function is important. The great majority of cases complain merely of a sense of unsteadiness, especially on sudden movement, as in the two present cases, and only the minority have a true spinning vertigo.

Caloric tests are abnormal in nearly all cases so that a normal test is a point against the diagnosis of tumour and complete absence of response greatly favours it.

NEUROLOGICAL EXAMINATION

Examination of the cranial nerves should be done with some care. *Corneal sensation* is tested with a fine nylon thread which can be shortened or lengthened so that the pressure exerted by the tip of the thread when the thread begins to bend can be finely varied. Altered corneal sensation may be expected in about half the early cases.

Sensation of touch on the face can be tested with the same instrument, and will be altered in about 50 per cent, as also will pain as tested with a revolving pinwheel.

Tests of early involvement of the seventh nerve remain very difficult. An electrical taste stimulator has been devised and a raised threshold of 20 microamperes or more has been found in many cases on the side of the lesion. Of rather less value are the strips of filter paper hung over each lower eyelid for one minute to assess the quantity of tear secretion on either side.

Hitselberger has now described loss of sensation on the upper part of the posterior wall of the external canal near the drum—presumably a somatic sensory area of the 7th nerve as illustrated in geniculate herpes.

OTHER INVESTIGATIONS

Careful *radiograms of the internal auditory meati* are required, although a difference in diameter of the two canals of up to 2·5 mm may be normal. The *protein in the cerebrospinal fluid* will be raised in about 75 per cent of early

cases though often only slightly; 40 per cent may show a figure of over 100 mg/100 ml.

It is obvious that all the above tests form a valuable pattern of investigation which may lead to serious suspicion of tumour, but no one test is sufficiently reliable for final diagnosis. When these results lead to definite suspicion it is advisable to proceed to *positive contrast X-ray study of the cerebellopontine cisterns* and their extensions into the internal meati. The procedure can be a fairly uncomfortable one, but so far there have been no serious after effects. It will show up 95 per cent or more of these tumours, even small ones of 1 cm or so still within the canal.

Since this paper was first prepared a third but advanced case has been seen. He is a man of 50 who had been losing hearing in the left ear for 3 years, and had been told he had a nerve deafness for which nothing could be done. For the last 3 months he had noticed unsteadiness on standing up, and once fell over at a board-meeting on getting up to examine a document. He was otherwise well.

On examination he was severely deaf in the left ear. There was early papilloedema, slight nystagmus to both sides and a tendency to sway to the left, but no other cranial nerve defect.

He had large ventricles under very high pressure, relieved by Torkildsen's operation. Myodil studies showed gross shift of the aqueduct and 4th ventricle to the right, and suboccipital operation revealed a very large neurilemmoma which was partially removed.

Otological tests would have raised suspicion at a much earlier stage, and, even with the advent of internal hydrocephalus, 5th and 7th nerve function remained normal and neurological signs were few.

This paper is perhaps premature as two cases only have been treated by the new operative method. However, the encouraging results of early surgery by methods both old and new have prompted me to present to you these recent techniques for early diagnosis, and to emphasize that all cases of sensori-neural deafness need very careful investigation.

Acknowledgements

I would like to express my thanks for the advice of several otologists, Dr. Frank Ellis, Dr. John Tonkin, and also Dr. Bruce Benjamin, my otological partner in the Vertigo Clinic at Sydney Hospital.

I would also like to thank Mr. John Collins, the Medical Artist at Sydney Hospital, for his excellent illustrations.

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Discussion

Dr. Leonard Cox: I am shattered to hear the mortality reported. Most of ours have lived. The relation to paroxysmal vertigo is of interest. A series studied years ago contained no case of paroxysmal vertigo, yet we see as much paroxysmal vertigo as epilepsy.

Dr. Frank Morgan: This interesting field of otological and radiological diagnosis should eventually lead to earlier diagnosis. Many tumours seen are at an advanced stage with papilloedema and the tumour pole passing through the tentorium, hence operation is hazardous. Olivacrona's mortality for total removal was 15 per cent, whereas Cushing's series was for subtotal removal.

We may now get to the position of operating

in prophylactic fashion for a symptom, after which the patient will be no better. We do not know how many of these small tumours will eventually give trouble.

Dr. Anthony Fisher: James Bull considered that 25 per cent have normal plain radiograms and he believes House's criteria to be too wide. Dr. Fisher doubts the safety of putting myodil into the cisterns in the posterior fossa, and prefers gas. A tumour, 1 cm. in diameter, was shown by air in the ponto-medullary cisterns above the internal auditory meatus. He considers that a medially placed tumour may be missed by myodil.

ULTRASOUND IN NEUROLOGICAL DIAGNOSIS

ERIC DAVIS

Sydney

INTRODUCTION

There is now a formidable array of diagnostic aids available to the neurosurgeon and neurologist in the detection of intra-cranial expanding or space-occupying lesions. Such aids include air studies (by lumbar intra-thecal injection or by ventricular puncture) cerebral angiography, myodil ventriculography and scanning by means of radio-isotope techniques.

Once an expanding lesion is suspected diagnosis follows with nearly 100 per cent certainty. However, before embarking on time consuming neuro-radiological studies, which are not without minimal risk, it is advantageous to have diagnostic routine examinations which are without risk, which may be repeated any number of times and which may ensure a reliable primary diagnosis. Such are supplied by the techniques of elctro-encephalography and ultrasound echo-encephalography.

By virtue of its simplicity and rapidity of application echo-encephalography qualifies as a valuable supplementary diagnostic procedure in the elucidation of the following neurological problems, namely:

1. The detection of space-occupying lesions within the cerebral hemisphere.
2. Evaluation of patients suffering from cranio-cerebral trauma. It is of proven value in the detection of extra-cerebral haemorrhages and its use may cut down considerably the demand made on the X-ray department for cerebral angiography.
3. Evaluation of patients with cerebro-vascular disease and the differentiation of these from tumour cases.
4. Evaluation of a miscellaneous group of patients with diagnostic problems such as epilepsy, meningo-encephalitis, cerebral atrophy, organic mental disease, coma, etc.
5. Evaluation of certain acute problems which are an every day occurrence in a teaching hospital, viz.
   (a) Sub-dural haematoma versus cerebral contusion.
   (b) Sub-dural haematoma versus metabolic or toxic coma.
   (c) Hypertensive intra-cerebral haemorrhage versus sub-arachnoid haemorrhage.
   (d) Intra-cerebral space-occupying lesions versus acute cerebral infarction.

The practical application of ultrasound echo ranging to the intact skull as a diagnostic technique was first described by Leksell in 1956. He described an echo that documented the position of the intra-cerebral midline and showed that it could be measured to indicate whether it was in the middle of the intra-cranial cavity or shifted by expanding lesions.

Ultrasonic waves having frequencies above 20 kilocycles (20,000 cycles) per second, too high to be heard by the human ear, are changed by whatever material they strike. Although ultrasound follows the same physical laws as audible sound it has a greater tendency to beam and reflect (Ford and Ambrose). When beamed ultrasound is passed through body tissues it reflects from interfaces of differing acoustical impedances. Intracranial structures, although generally transparent and undifferentiated by the X-ray beam, do offer varying acoustical impedances to the ultrasound beam. Thus some of the sound is absorbed, some is reflected and some transmitted through the interface. In this technique of echo ranging use is made of the beam reflected at the interface.

INSTRUMENTATION AND TECHNIQUE

Echo scanning has now been carried out at Royal North Shore Hospital for 12 months and

the instrument used is a Portex (Smith) Diasonograph. Its transducer was selected to emit a 1·5 million cycle per second bundle of ultrasound, from the surface of the barium titanate crystal in the probe, for a duration of 1 μsecond. This is repeated 200 times a second. The source of the pulsed ultrasound is the crystal whose surface vibrates in response to a rapidly alternating current by virtue of the piezo-electric phenomenon.

The returning echoes are received by the crystal and displayed as right-angled deflections on the sweep of the cathode ray oscilloscope. An attached polaroid camera can be locked on the face of the cathode ray oscilloscope and permanent records of the display obtained within a matter of seconds.

In all cases paired recordings were obtained through the intact skull (without removing the hair) by the application of the probe to symmetrical points on opposite sides of the skull.

There is an optimal application area for the ultrasound probe. This is a farily concise spot just above the root of the ear in the vertical plane passing through the external auditory meatus. Air must be excluded by the application of a coupling medium such as glycerine, paraffin or a water base jelly. These were used successfully, but a water wetted surface may be just as satisfactory. With considerable patience and experience one can, by gentle rocking of the probe, locate and display the midline echo. Once this is obtained the probe must be held perfectly still whilst the display is photographed by the specially designed camera. The bony walls of the cranium are responsible for strong echos at the extremes of the cathode ray display. These are used to determine, by measurement on the scale, whether or not the midline echo is displaced.

A reading is taken from right to left and the echo display photographed. The probe is then placed on the left side of the skull, the image is inverted and the exposure completed on the same strip of film. Within 10 to 15 seconds the strip is removed and coated with special emulsoin. The ultrasonic echo-encephalogram is then ready for inspection and measurement.

Results of Investigation

Over the past 12 months more than 300 echo scans have been carried out in the Department of Electroencephalography. Of these, 141 patients were selected for analysis as confirmation of the midline was available subsequently either by cerebral angiography, air studies, operation or autopsy. For the purpose of analysis and documentation, five categories of patients were considered, namely:

1. Patients with subsequently proven cerebral tumours. There were 44 in this group. The position of the midline structures was correctly predicted in 39 cases, which represents an 89 per cent accuracy. The great majority of correct predictions indicated a shift to one or other side. In some there was no shift and this applied mainly to posterior fossa tumours. There were five false negatives. In one of these the patient had multiple secondaries. In several a glioma of the hemisphere infiltrated without displacement. In yet another, a patient with an enormous dermoid, the tumour occupied both hemispheres. A further eight patients with strongly suspected, but not proven, tumours also had echo scans. There was one false positive among these.

It may prove of interest to discuss the EEG findings in this group. Of the 44 patients with proven tumours there were 41 with supratentorial lesions and three with infra-tentorial lesions. Correct lateralization was obtained in 38 of the first group, which is in the order of 92 per cent. The EEG was unhelpful in the infra-tentorial tumours.

2. Patients with cerebro-vascular disease. The problems in this interesting category are manifold. The following common diagnostic dilemma are listed as follows:

(a) Acute cerebral infarction versus a space-occupying lesion.

(b) Acute dementia following falls and suspected head injuries in elderly people. The question usually asked is: "Are we dealing with a vascular lesion, with a space-occupying lesion or with a subdural haematoma?"

(c) The elucidation of such problems as hemiplegias, apraxias, aphasias, slowly progressive dementias and personality changes, and last but not least transient ischaemic attacks.

(d) Intra-cerebral haemorrhage due to ruptured aneurysm and to primary arterial rupture.

(e) Encephalopathy with coma and convulsions versus trauma.

(f) The detection of intra-cerebral haemorrhage in patients admitted in delirium or coma.

There were 35 patients submitted to echo scanning in this group. There were 32 correct predictions, giving an accuracy value of 91 per cent. There were three false positives, and in each case it was presumed that the shift displayed was due to acute cerebral oedema following infarction in the presence of proven arterial occlusions.

EEG examinations were carried out in this group and the findings are interesting. Nineteen patients had focal para-Sylvian slow wave activity of the type commonly ascribed to middle cerebral artery ischaemia. Eight had diffuse records and a further eight revealed mono-rhythmic frontal Delta activity either swinging or lateralizing. The author feels that, with the combined knowledge of elctro-encephalography and echo scan interpretation, much valuable information can be obtained, often reducing the need for neuro-radiological studies.

3. Patients suffering from cranio-cerebral trauma. There were 28 patients in this group. The midline echo was demonstrated and its position correctly predicted in 26 cases (92 per cent). There was one false negative in which a small posterior sub-dural haematoma was missed. There was one false positive, due probably to cerebral oedema. The problems included elucidation of a sub-dural haematoma in six cases, and of determining the presence or absence of a shift in patients suffering from traumatic encephalopathy and confusion. In this particular category and indeed in all the others, the combination of EEG and echo scanning is a valuable one. I have always used the two methods in combination to provide an answer.

As is well known the EEG is of no great help in chronic cases of cranio-cerebral trauma. Its value in acute situations is sometimes called into doubt, but there are certain situations which merit its use. In six patients with sub-dural haematoma the EEG failed in one case, but showed suppression of rhythms in five as it did in one case of extra-dural haematoma. Posterior temporo-occipital Delta activity, either bilateral or unilateral, is a feature of the EEG's in head injury cases and the EEG was lateralizing in seven cases.

4. The fourth group is a miscellaneous group and contains a number of interesting problems, for example a patient admitted in coma with or without convulsions and displaying focal neurological deficits. Another problem is the patient who is suffering from a suspected encephalitis or encephalopathy either viral, post-viral, toxic or metabolic. A tumour may be suspected, and here again an echo scan plus an EEG is of immense value. Other problems that have been encountered include sub-dural empyemas, abscesses and acute demyelinating disorders. There were 14 patients fully investigated in this group, with 12 correct predictions and two false positives. The prediction rate was 85 per cent correct. Two false positives have not been explained.

In this group the EEG was of value. In 11 cases the typical high amplitude bilateral Delta activity of acute encephalitis or encephalopathy was observed. Two cases showed non-specific changes and one a focal EEG.

5. The fifth group is one of 13 patients presenting with varying types of epilepsy and thought to be worthy of an echo scan because of the possibility of an underlying neoplasm. Thirteen were submitted to echo scanning plus the full gamut of neuro-radiological studies. Correct predictions were obtained in the 13 cases.

Whatever doubts one may have on the value of electroencephalography one must agree that its value reaches a peak in the diagnosis and investigation of epilepsy. In this final group the EEG demonstrated as one might have expected focal spike wave and other graphic elements of epilepsy in practically all these selected cases.

SUMMARY

The echo scan has been in use at Royal North Shore Hospital of Sydney for 18 months, and up to the present moment some 500 scans have been carried out. Whilst there are variables, particularly related to skull contour, on the whole this method of investigation has

proven of value and is now accepted as a routine in selected cases. When combined with electro-encephalography we have two complementary techniques which may reduce the work load on the Department of Radiology.

Discussion

Prof. A. Earl Walker: I was very much interested in this presentation of ultrasound. It is a technique which we have been using some years, and one which I think is very valuable, particularly in the acute emergencies —those patients who come into the hospital in a semi-comatose condition in which an accurate history is often difficult if not impossible to obtain—and we have found that it will give us enough initial information to carry out other diagnostic procedures which may enable us to localize the condition, particularly in patients with head injuries. In some patients with vascular insults this has saved us a great deal of time. We have operated several times on the basis of the echoencephalogram in suspected subdural and extradural haematomas, so that I think it is a real asset to the diagnostic armamentarium. I would like to ask Dr. Davis if he has used this technique in the operating room. We have been employing it now when the dura is about to be opened as a means of determining the precise location of the underlying tumour, and it does give the surgeon considerably more confidence, in cutting through an area of cortex that looks perfectly normal, to be assured that 2 cm below he will encounter a cyst or a solid tumour or something else, and I think with dural echograms one can be pretty certain of the location and of the extent of subcortical lesions.

Dr. Davis: We have not used the echo-scan yet in open surgery. Perhaps we'll be able to persuade our neurosurgical colleagues that this mightn't be a bad idea. I wonder, Dr. Walker, is there such a difference in the acoustic impedances in a tumour in the brain itself to be of any value. I know that if you have a fair amount of calcification in an intracranial tumour you will get an echo shift, but I was not aware that there was sufficient differences at the interface of tumours unless they are solid or cystic to be of any value.

Prof. Walker: The acoustical impedance varies a great deal with different types of tumours. With the cysts one can sometimes outline the two walls of the cyst. With meningiomas the tumour has in some cases a rather granular appearance, and the acoustical impedance of that is quite different from the adjacent brain tissue, and you can follow the extent of the tumour by the baseline of the CRO tracing. In intracerebral haemorrhages you very frequently find numerous pips on the baseline, presumably being the interfaces between clotted and non-clotted blood.

Dr. Millingen: In order to assess the value of echoencephalography, I wonder if Dr. Davis could tell us with what degree of confidence he would be quite happy about not proceeding to neuroradiology in the presence of a normal echo, because it seems to be a problem that those who are about to start this would like to know.

Dr. Davis: I don't think that I would be confident at all at the moment. I think the echo sound prediction ranges about 85-90 per cent. With increasing experience, Dr. Milligen, you could pick up most of your extracerebral haemorrhages; this is the acute ones that you are really interested in—subdural haematomas or epidural haemorrhages—with a reasonable degree of confidence, say, 90 per cent.

Prof. J. L. Lance: About 15 per cent of subdurals are said to be bilateral, and this would be the great problem for the echoencephalographer, just as it is for one who looks at a calcified pineal gland on a plain X-ray, so I think your 90 per cent would be of unilateral subdural haematoma, would it not ?

Dr. Davis: Yes, I think you are correct.

FOUR CASES OF SUBACUTE NECROTIZING ENCEPHALOMYELOPATHY IN CHILDHOOD (LEIGH'S SYNDROME)

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Leigh (1951) published a case of subacute necrotizing encephalomyelopathy in a 7-months-old boy. The infant had been normal until five and a half months of age when he ceased to cry, lay very still and slept for long periods, only waking when disturbed. He did not suck and had to be fed by spoon. The pupils were small with no reaction to light, or appreciation of light; ophthalmoscopy showed bilateral optic atrophy. The infant appeared to be deaf. The limbs were spastic, but tendon reflexes were not obtained. There were bilateral extensor plantar responses. The cerebrospinal fluid was normal. The child rapidly deteriorated and died 6 weeks from the onset of the illness. In the central nervous system the outstanding findings were dark grey-brown discolouration of part of the thalamus and substantia nigra on both sides. In the mid-brain similar discolouration involved the tegmentum, and in the pons the trigeminal motor nuclei were affected. The upper medulla showed a striking red-brown discolouration of the inferior olives and in the lower medulla the area involved was the central grey matter. The medial portion of the posterior columns of the cervical and thoracic spinal cord also showed a similar change, the lumbar segments being normal.

Microscopically in the affected areas all the small blood vessels appeared to be increased in number. Nerve cells in these areas were often severely damaged, but normal neurones were also present. Microglial cells were increased in number, especially at the periphery of the lesions. There was a considerable reduction in oligodendrocytes and a marked astroglial proliferation. There was a general loosening of the interstitial tissues. Leigh likened the histological findings to those found in Wernicke's syndrome although perivascular haemorrhages were not present and the mammillary bodies were not involved.

Since Leigh's report, 11 essentially similar cases have been published (Table 1). Feigin and Wolf (1954) published 3 cases, including a brother and sister who were children of a consanguinous marriage. They also considered that the condition resembled Wernicke's encephalopathy but found no evidence of dietary inadequacy and suggested that a congenital metabolic disorder was involved. They also pointed out that the lesion produced was essentially a demyelination with considerable sparing of axons.

Richter (1957) published 3 similar cases and noted that in all 3 the optic nerves, chiasm and tracts showed demyelination. In one patient the caudate nuclei and lentiform nuclei were also affected. The maternal uncle of one of the patients died of a similar illness at a similar age, but no autopsy was available. Ford (1960) mentioned a further case. Reye (1960) published 4 cases and in one instance a sibling had died of a similar illness, but no autopsy report was available. Reye also found loss of myelin in peripheral nerves.

In the last four years 4 similar cases have come to our attention at the Royal Children's Hospital. Only the salient features of each will be mentioned (see Fig. 1).

TABLE 1

Reported Cases of Subacute Necrotizing Encephalomyelopathy of Childhood.

Author	*Case no.*	*Sex*	*Age at onset of symptoms (months)*	*Age at death (months)*	*Comment*
Leigh (1951)	1	M	5·5	7	—
Feigin & Wolf (1954)	1	M	1·5	12	—
	2	M	2	21	—
	3	F	6	48	Sister of Case 3
Richter (1957)	1	F	1·5	16	
	2	F	3	4·5	
	3	M	From birth	13	Maternal uncle died of similar illness
Ford (1960)	1	F	2	2·5	—
Reye (1960)	1	F	15	23	Sister died with similar illness
	2	M	16	40	—
	3	M	13	24	—
	4	M	24	27	Retarded development
Anderson This series	1	F	8	25	Retarded development
	2	F	6	15	—
	3	F	15	26	Retarded development
	4	M	85	110	Epilepsy for 3 years

CASE I (J.B.), female, died at the age of 2 years 1 month. Distress was noted during delivery, which was 3 weeks after the expected date. She was slow to revive and at 2 months of age she presented with generalized seborrhoeic dermatitis which responded to treatment but never entirely cleared up.

Her progress was retarded; she was never able to sit up or stand and never formed words. At 7 months of age she commenced to have turns in which she became pale and her arms and legs twitched. These were controlled with small doses of phenobarbital. At 8 months of age she appeared to see and hear, but after this time both functions deteriorated. Optic atrophy was present and the pupils reacted sluggishly to light. Three weeks prior to her death she refused food and fluid, and lost weight. In her final admission she required pharyngeal suction, developed pneumonia and respirations became irregular. The cerebrospinal fluid was normal and amino acids were present in the urine.

Autopsy findings: Bronchopneumonia was present. In the brain the lateral and third ventricles were mildly dilated and ectopic grey matter was seen in the periventricular white matter. Grey-brown discolouration and softening was noted in the subthalamic nucleus and the substantia nigra on both sides. The central grey and white matter of the mid-brain, pons and medulla adjacent to the aqueduct and fourth ventricle showed similar discolouration. Microscopic examination of the lesions showed an apparent increase in small vessels, all of which were congested. There was looseness of the texture associated with complete loss of myelin and mild astrogliosis. Microglial cells containing neutral fat were plentiful at the edge of the lesions. Neurones in the affected areas, on the whole, appeared normal and axons were plentiful. The optic nerves, chiasm and tracts showed demyelination.

CASE II (L.D.), female, died at 15 months of age. Pregnancy and delivery were normal. Progress was normal until 6 months of age, when she developed bronchitis and showed twitching for 48 hours. She recovered, but did not play with toys as well as before. Three weeks later she developed ear infection and with this commenced to have tonic spasms. These cleared but afterwards she would just lie in bed able to see toys but not able to grasp them. At this time she began to vomit once or twice per day. When examined at this time she showed spasticity of limbs and inability to hold up her head. Cerebrospinal fluid was normal. Her condition thereafter slowly deteriorated and she was subject to recurrent attacks of bronchitis. Finally she developed a divergent squint

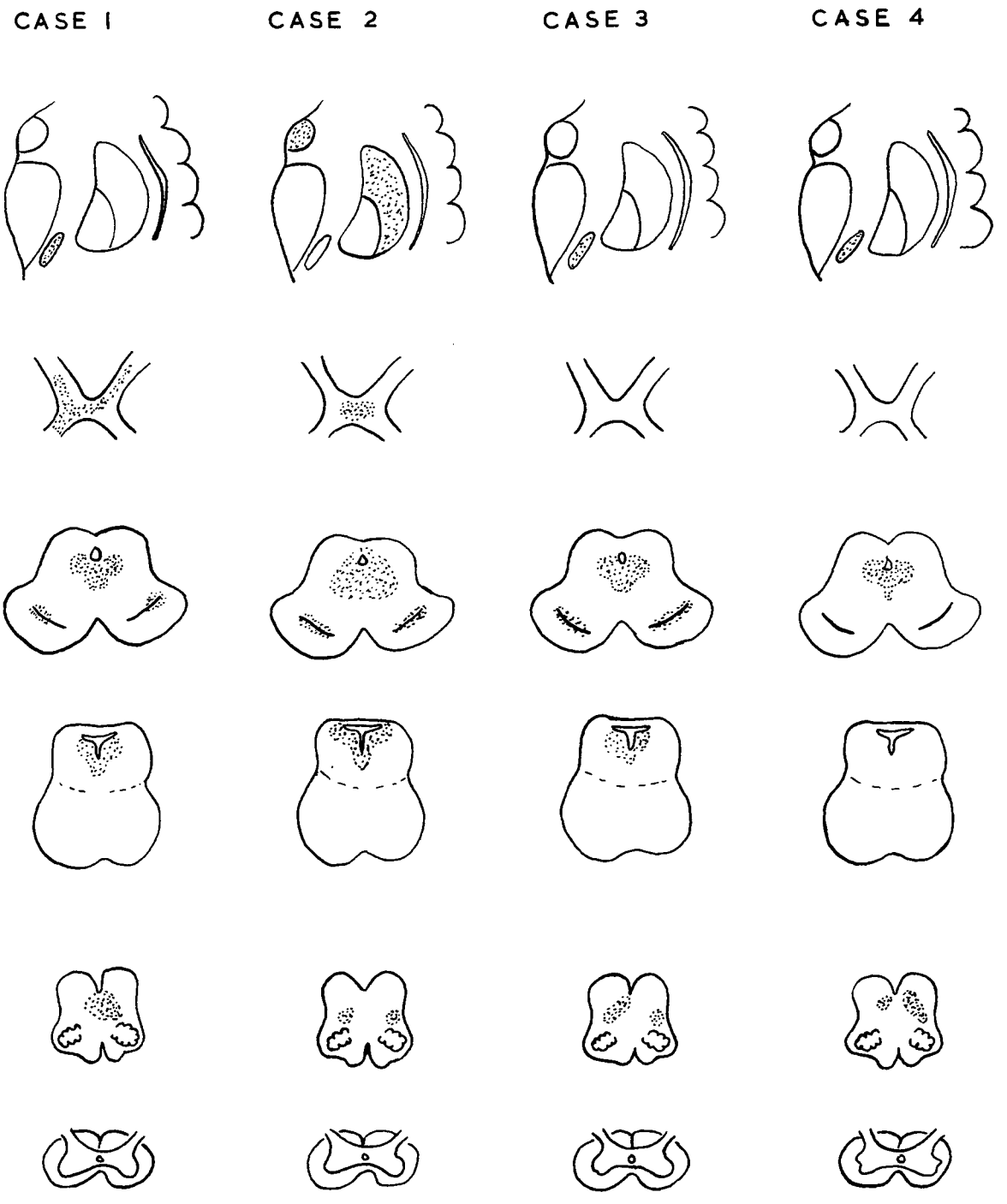

FIG. 1—Diagram showing distribution of lesions in basal ganglia, optic nerves, mid brain, pons, medulla and spinal cord of 4 cases reported in the present series (see text). The lesions are indicated by the shaded areas.

and lapsed into coma. Her respirations became irregular. Plantar responses were extensor and tendon reflexes were absent. Prior to her death her temperature dropped to 31°C, and her respirations were gasping.

Autopsy findings: Apart from bronchitis, abnormal findings were confined to the nervous system. Grey-brown softening was found in the caudate nucleus and the putamen bilaterally. Similar changes were found in the tegmentum of the mid-brain and pons and in the medulla two small areas of softening were present dorsal to the inferior olives. Microscopically the lesions were similar to those in Case I, and a similar histological change was seen in the central portion of the optic chiasm.

CASE III (K.B.), female, died at the age of 2 years 2 months.

Pregnancy and delivery were normal. She sat at 6 months of age but presented to hospital at 14 months of age with failure to progress. At this stage she had not crawled or walked, or said any words, and there was difficulty with swallowing of solid foods. On examination, there was a pendular nystagmus and general hypotonia. At 18 months of age she was still hypotonic, but was able to walk with support and say a few words. The nystagmus originally noted was not present.

A few days before her death she refused food, and when admitted to hospital was semicomatose and flaccid. Her respirations were shallow and irregular and there was twitching of the right side of her face and right arm.

Autopsy findings: A perforated gastric ulcer and bronchopneumonia were present. In the central nervous system a small vascular malformation was present in the white matter of two gyri in the right frontal lobe. Grey-brown discolouration of the subthalamic nucleus and substantia nigra was present, bilaterally. The tegmental areas of the mid-brain and pons and the left middle cerebellar peduncle were similarly affected. In the medulla, bilateral lesions were found dorsal to the inferior olivary nuclei. Microscopically, the lesions showed similar changes to those described in Case I.

CASE IV (G.W.), male, died at the age of 9 years 2 months.

He first presented at the age of 7 years 2 months with one month's history of progressive clumsiness in walking and handling of objects. It was said that he was always a restless child, and frequently woke with shaking or jerking movements in the hands and legs. His progress in schooling was average. On examination, he was ataxic, tendon reflexes were hyperactive and the plantar responses extensor. The cerebrospinal fluid was normal. The urinary chromatogram showed a mildly elevated excretion of amino acids. He was treated with dilantin and improved for several months, but at the age of 8 years 11 months was admitted following rapid deterioration giving rise to severe ataxia and twitching in the arms and legs. On examination there was frequent twitching in the left arm; inability to walk and pallor of the optic discs. An air encephalogram showed mild cerebral atrophy. He was discharged from hospital two weeks later but was re-admitted two days before death with a history of gasping respirations and pooling of pharyngeal secretions and inability to walk more than a few yards. For two days before admission he had complained of pain in the abdomen and had vomited and for one day was unable to pass urine. On examination he had bilateral ptosis of the eyelids, external squint, right palatal paralysis, active deep reflexes and extensor plantar responses. His bladder was distended. He rapidly deteriorated, becoming hypotensive and flaccid in the left limbs. Immediately prior to death, twitching was observed in the right limbs.

Autopsy findings: There were several shallow acute ulcers in the stomach and bronchopneumonia was present. In the nervous system a small vascular malformation was found in the white matter of the left frontal cortex. Grey-brown softening was present in the tegmentum of the mid-brain and in the dorsal half of the medulla. The substantia nigra nuclei were pale. Microscopically the softened areas showed demyelination and increased vascularity as described with Case I. The subthalamic nucleus, substantia nigra, inferior olives and dentate nuclei all showed gliosis associated with a mild loss of neurones.

CONCLUSION

The four cases described conform to the pattern shown in the 12 previously published cases. Microscopically the lesions show demyelination, mild to moderate neuronal damage, and, perhaps the most outstanding feature, an intense vascularity. The lesions have been likened to those seen in Wernicke's encephalopathy although they are not so florid, without haemorrhages, and show differences in distribution (Table 2). For example, the mamillary bodies, always affected in Wernicke's encephalopathy, were not involved in any of the cases so far described, and other areas such as the putamen and optic nerves, which are frequently affected in this condition, are not damaged in Wernicke's syndrome.

There is, however, enough similarity in distribution to raise the possibility that we are dealing here with a deficiency syndrome (al-

TABLE 2

MAIN SITES OF LESIONS IN 16 CASES

Site	*No. of Cases*
Mid brain-tegmentum	15
Medulla	12
Substantia nigra	10
Pons	9
Spinal cord	6
Putamen	6
Subthalamic nucleus	5
Thalamus	4
Caudate nucleus	3
Hypothalamus	3
Peripheral nerves	3
Globus pallidus	1

though in none of the cases has there been any evidence of this). In the majority of cases, symptoms date from the early months of life, and this feature coupled with evidence of a familial incidence in one reported case and probably present in two others, is suggestive of an inborn error in metabolism.

In a number of the patients the illness has followed an intermittent course, and this asso-caited with the finding of lesions of different ages (Reye, 1960), raises the thought that some extrinsic factor may be activating the process.

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DISCUSSION

Dr. Turner: Were the lesions in the optic nerves local or associated with retinal pathology, and what was the nature of the chiasmal lesions?

Dr. Anderson: In no case was the retina examined pathologically, but clinically optic atrophy was noted. In two of the cases lesions in the optic nerves and chiasm were associated with an increase in the number of blood vessels which were congested. In another case demyelination of the optic nerves was associated with gliosis without increased vascularity.

Dr. Eadie: Was there only demyelination or also axonal and neuronal damage?

Dr. Anderson: Axons were largely normal and neurones appeared plentiful and healthy.

Dr. Allsop: Were pyruvate estimations done?

Dr. Anderson: Yes, and the levels were normal. In two cases amino acids were increased in the urine, but the significance of this finding is not clear.

Dr. Davis: Any possibility of intranatal factors?

Dr. Anderson: In one case placental insufficiency was considered to have been present and the child was born prematurely.

Dr. Rail: The duration of illness in Wernicke's encephalopathy is short, but the cases you have presented go on for months. This suggests an inborn error of metabolism rather than a nutritional deficiency.

Dr. Anderson: All died of an exacerbation of their illness producing respiratory distress and lesions seen at autopsy vary in age, suggesting an intermittent course. It may be that an inborn error of metabolism is present and an exogenous factor initiates the disease process.

SOME ASPECTS OF THE NEURON-NEUROGLIA RELATIONSHIP

C. P. WENDELL-SMITH*

Sydney

In 1966, 120 years after Rudolf Virchow first recognized that neuroglia was a non-neuronal component of nervous tissue, our knowledge of its function, and even some aspects of its structure, remains woefully incomplete. That neuroglia might be something more than "nerve glue" or packing material was suggested at the beginning of the century by Holmgren. He thought that glial cells had a nutritive function and called them trophocytes. At the beginning of the century, metallic impregnation methods were in their infancy. Their subsequent development and exploitation by Ramon y Cajal and del Rio-Hortega led to the recognition of three types of neuroglial cell: astrocytes, oligodendrocytes and microglial cells (Fig. 1). To these we may add the capsule cells of ganglia and the Schwann cells of peripheral nerve. I

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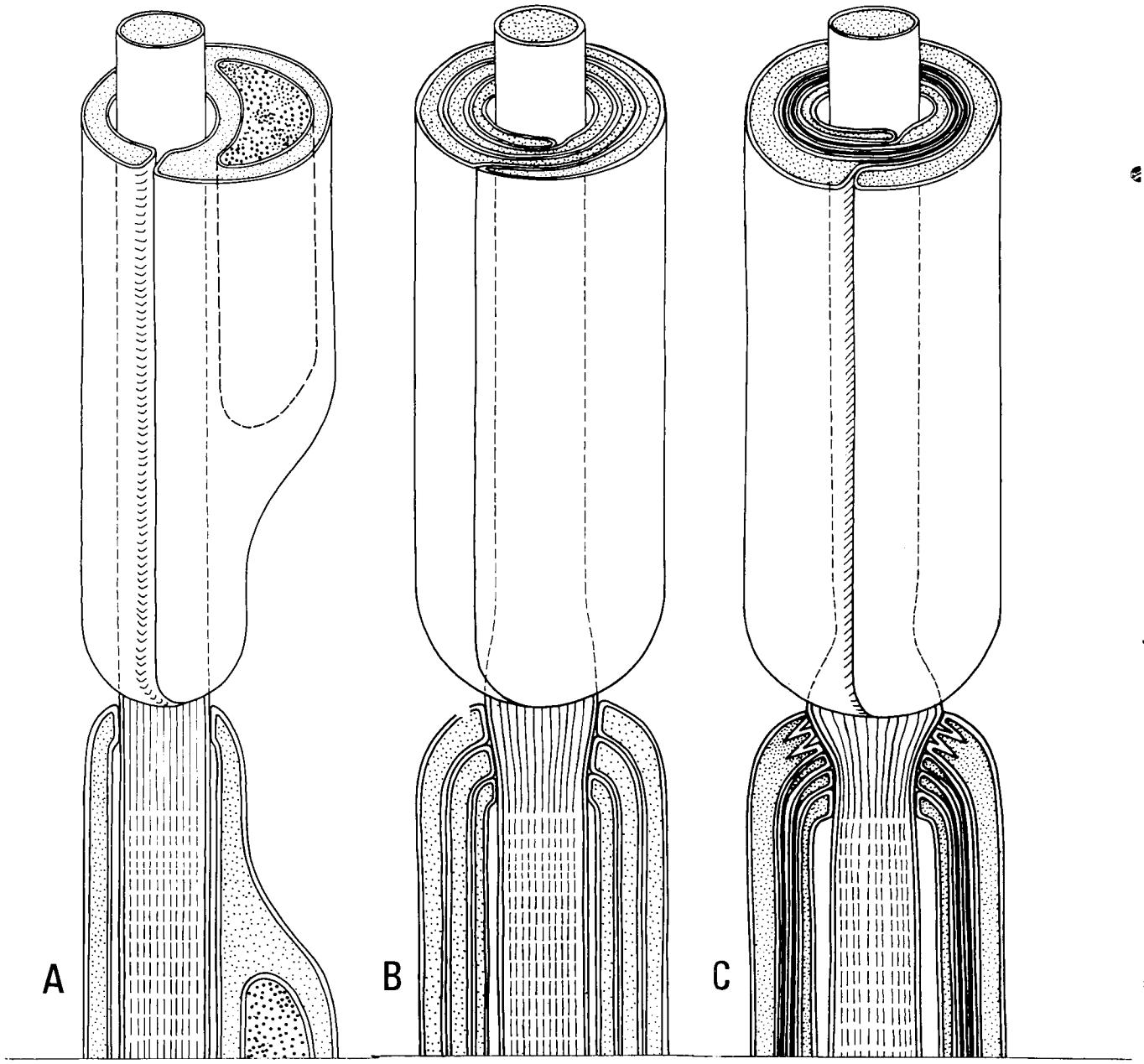
FIG. 1—An electron micrograph of part of a septum from cat optic nerve illustrating the electron microscopical substrate of the microglia. At the top of the field is a blood vessel. Its lumen (1) is bounded by an endothelial cell (nucleus at E). Outside the endothelial cell is a basement membrane (b) with a pericyte (nucleus at P) embedded in it. Basement membrane (b) also separates the pericyte from collagen fibres (c) and a fibroblast (nucleus at F). Note that the pericyte is wholly surrounded by basement membrane. Pericytes like this stain by del Rio Hortega's method and are identified as microglia.


A
B
C

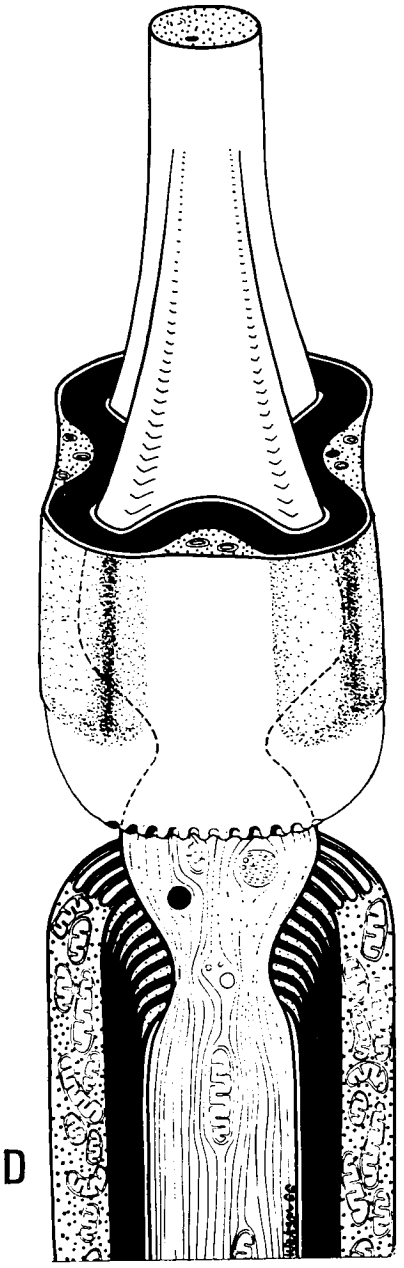


FIG. 2—Diagrams A, B and C represent successive stages in the myelination of a fibre in the peripheral nervous system; Diagram D represents, at lower magnification, a well-myelinated fibre. In each case the junction between two Schwann cell territories is represented: above the junction is a stereogram of an axon and its sheath; below is a longitudinal section of similar structures. In A, Schwann cells have lined up along the axon and engulfed it. At first, a single Schwann cell may engulf several axons (this one-to-many relationship persists in non-myelinated nerve), but, at the onset of myelination, the Schwann cells multiply and the one-to-one relationship illustrated is established for each myelinating segment. In B, the Schwann cells have wrapped around the axon like a Swiss roll and formed "loose" myelin (the myelinating segments are assumed to have elongated and the Schwann cell nuclei, represented in A, to have moved beyond the confines of the diagram). In C, the cytoplasmic surfaces of spiralling Schwann cell membranes have fused and formed dense lines which with the less dense intermediate lines comprise "compact" myelin. When sufficient layers are present, compact myelin becomes demonstrable by light microscopy. The junction between two Schwann cell territories can then be called a node of Ranvier. Near the node the cytoplasmic surfaces do not fuse, but successively peel off the inner surface of the compact myelin and abut against the axonal membrane. Cytoplasm also persists as more or less complete collars around the axon and around the compact myelin; the latter collar extends finger-like processes which may mesh with those of the next Schwann cell in series and abut against the axonal membrane. In D, which represents a well-myelinated fibre at lower magnification, the cylindrical axon is seen to become fluted and flanged as the node is approached. The compact myelin sheath, represented solid black at this magnification, is created to conform; Schwann cell cytoplasm forms a series of columns lying in channels on the fluted paranodal myelin sheath; the columns are packed with mitochondria and merge to form a complete cytoplasmic collar at the node, beyond the compact myelin; the collar extends finger-like processes, with a distinct internal organization, to the axonal membrane.

propose to refer briefly to certain concepts developed by other workers and then to consider those emerging from the work of our group at the University of New South Wales, headed by Professor M. J. Blunt and including Mr. F. Baldwin and Mr. P. B. Paisley.

Light and electron microscope studies have revealed intimate cell contacts and membrane relationships between neurons and neuroglia (Peters, 1962). There is also evidence that the number of neuroglial cells surrounding a neuronal perikaryon is directly related to the functional load it bears. For example, the number of glial cells per nerve cell (glia index) depends on brain size (Hawkins and Olszewski, 1957), and axonal length (Friede and van Houten, 1962); the glia index increases with neuronal hyperactivity (Kulenkampff, 1952; Kulenkampff and Wüstenfeld, 1954) and stimulation (Kuntz and Sulkin, 1947a, 1947b). Microchemical studies also demonstrate the complementarity of perikarya and their satellite cells. For example, vestibular stimulation produces an increase in respiratory enzyme activity and ribonucleic acid (RNA) content, and a decrease in anaerobic glycolysis, in the neurons of the lateral vestibular nucleus; the glial cells respond with inverse changes—a decrease in respiratory enzyme activity and RNA content, and in increase in anaerobic glycolysis (Hamberger and Hyden, 1963). It has thus been suggested that the perikaryon and its satellite cells form a metabolically-linked cytophysiological unit. The analysis of this linkage has been hampered by the use of mixed samples of oligodendrocytes and astrocytes in these experiments (Hamberger, 1963).

The problem in peripheral nerve might appear simpler as only one type of cell is concerned. It is not, in fact, simple because of the several roles which the Schwann cell may have to play. Its role in myelin formation and maintenance is now well established (Geren, 1954). Schwann cells line up along axons at intervals of approximately 250 microns (Fig. 2A). A Schwann cell wraps round an axon like a Swiss roll (Fig. 2B). The Schwann cell membranes then fuse to form compact myelin (Fig. 2C), but leave more or less complete inner and outer collars of Schwann cell cytoplasm surrounding the axon and compact myelin respectively. Near the end of each Schwann cell's territory its membranes do not fuse but successively peel off the inner surface of the compact myelin and abut against the axonal membrane. At the node the outer cytoplasmic collar extends finger-like processes which may mesh with those of the next Schwann cell in series, and abut against the axonal membrane. In well-myelinated fibres (Fig. 2D) the Schwann cell cytoplasm forms columns lying in channels on the fluted paranodal myelin sheath (Williams and Landon, 1963). These columns are packed with mitochondria, perhaps sixty times as many as in the axoplasm of the region. At the node (Landon and Williams, 1963) the columns merge to form a collar which extends highly organized nodal processes to the axonal membrane. This dense population of mitochondria is probably an important source of energy-rich compounds to be transported to the axon via the nodal processes. They may provide the energy to drive, for example, the ionic pump. Thus in peripheral nerve the Schwann cell is both sheath-supporting and node-supporting.

In the central nervous system, with its several neuroglial cell types, we might expect to find a division of labour and different cell types filling these different roles. From our work on the optic nerve head of the cat we believe this is the case. As in man, the cat optic nerve has a lamina cribrosa. The laminar and prelaminar parts of the nerve contain only astrocytes; the postlaminar part contains both astrocytes and oligodendrocytes (Blunt, Wendell-Smith and Baldwin, 1965). This distribution has permitted identification and ultrastructural characterization of these cell types (Wendell-Smith, Blunt and Baldwin, 1966). Astrocytes are characterized by light amorphous matrices of their cytoplasm and nucleoplasm, by occasional scattered, irregular, dense particles identified as glycogen and/or by long fine filaments which extend into their processes. Oligodendrocytes are characterized by dense amorphous matrices of their cytoplasm and nucleoplasm and by canaliculi which extend into their processes. Using these mutually exclusive criteria (Fig. 3) all neuroglial cells of the adult cat optic nerve fall into one or other category. Thus much for the morphology of the astrocyte and oligodendrocyte, but what of their separate functions? For elucidation we seek the aid of histochemistry. We have studied

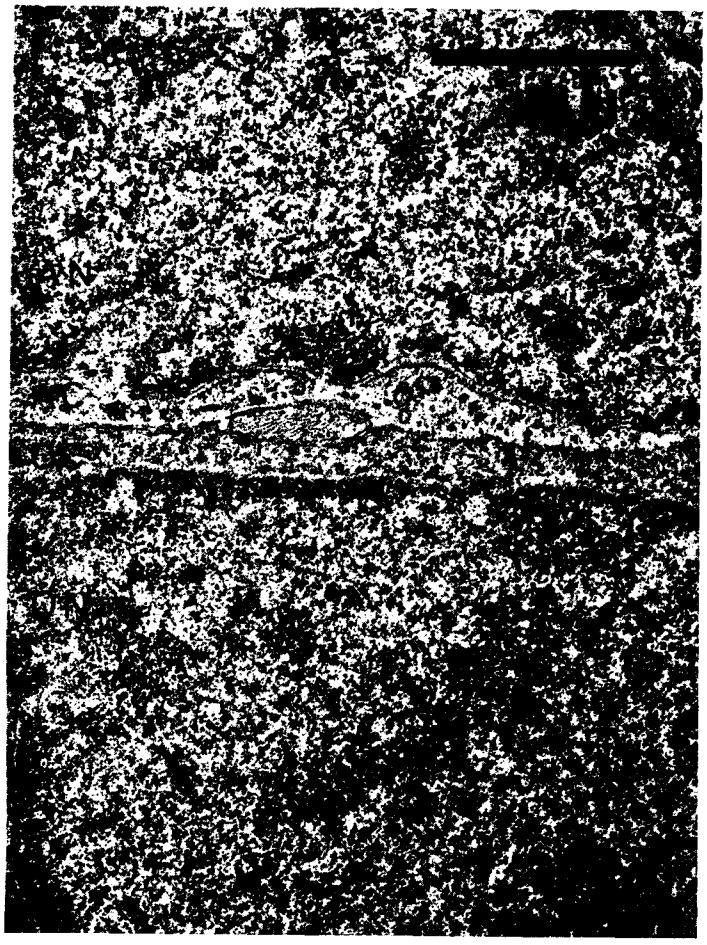


FIG. 3—An electron micrograph of parts of two adjacent macroglial cells illustrating the ultrastructural differences between astrocytes and oligodendrocytes. At the top of the field is an astrocyte nucleus (AN): note that this has a less dense matrix than that of the oligodendrocyte (ON). A nuclear membrane separates the astrocyte nucleus from its cytoplasm (AC) which in its turn is separated by the surface membranes of the two cells from oligodendrocyte cytoplasm (OC): note that the latter has a more dense matrix than that of the astrocyte. Both types of cytoplasm contain particles which are small and regularly-sized, and are identified as ribonucleoprotein; the astrocyte has in addition occasional, scattered, denser, larger and less regularly-sized particles identified as glycogen. The profile near the centre of the micrograph is an oligodendrocyte process exhibiting characteristic canaliculi.

a number of enzyme systems in the glycolytic pathway (Blunt, Wendell-Smith, Paisley and Baldwin, 1966). I would remind you that the early steps in this pathway take place in general cytoplasm and the later steps, particularly the citric acid cycle, in mitochondria. The cytoplasmic steps give an overall energy yield of only 2 high energy phosphate groups ($\sim$P) whereas the mitochondrial steps yield 36-38 $\sim$P. Microscope sections are incubated with a selected substrate (e.g. succinate) and a soluble tetrazolium salt. If the appropriate enzyme system (e.g. succinic dehydrogenase) is active the substrate is oxidized with the release of electrons. The tetrazolium salt intercepts the electrons and is reduced to an insoluble formazan product which is precipitated at the site of activity. Consider now the optic nerve from the ultrastructural and histochemical points of view (Fig. 4). The prelaminar field contains

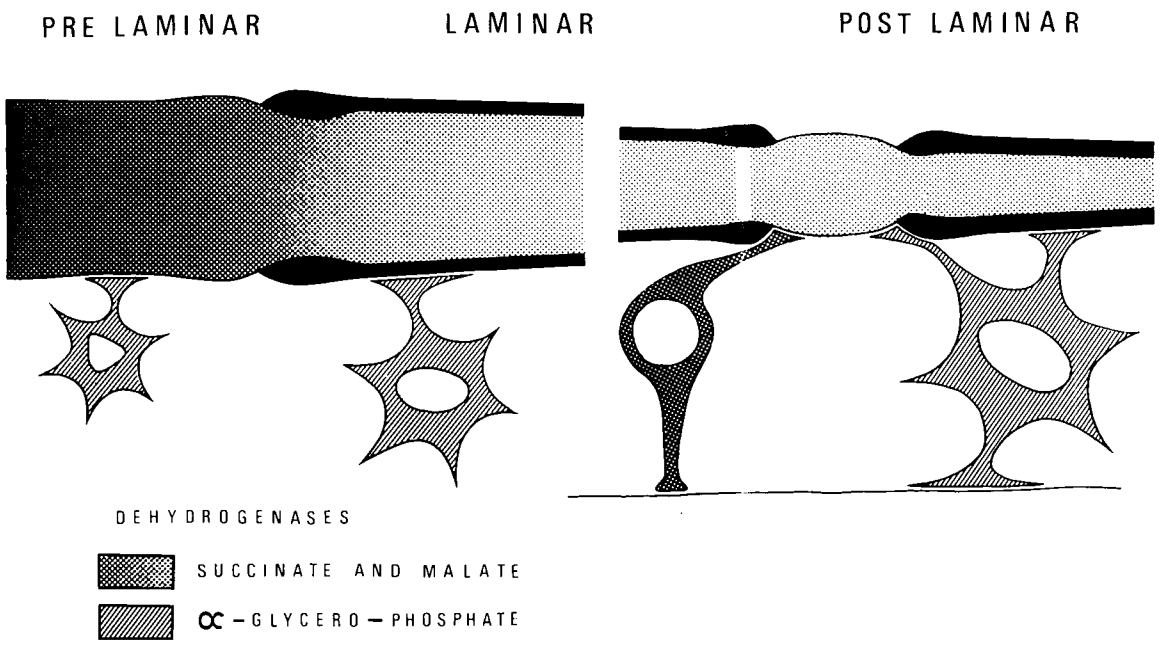


FIG. 4—A diagram summarizing the results of ultrastructural and histochemical studies on cat optic nerve. The prelaminar axons are non-myelinated and rich in mitochondria respiratory enzymes such as succinate and malate dehydrogenase; the laminar axons are non-myelinated or finely-myelinated and when traced back show a falling off in respiratory enzyme activity; the postlaminar axons are well-myelinated and show low respiratory enzyme activity. Astrocytes of somewhat differing size and form are found in the three regions, but all are strongly reactive for the enzyme alpha-glycerophosphate dehydrogenase and may be concerned with the isolation and insulation of axons. Oligodendrocytes are strongly reactive for the respiratory enzymes and are found only in the postlaminar region where they be node-supporting energy donors.

non-myelinated axons and small astrocytes: the axons contain many mitochondria—the glia cells few. The laminar field contains both non-myelinated and finely-myelinated axons with many mitochondria and, as we have seen, astrocytes with few. Examined for mitochondrial respiratory enzymes, the axons of the prelaminar and laminar regions show high reactivity, but the astrocytes and postlaminar axons show low activity. Postlaminar axons, you will recall, are well myelinated and contain few mitochondria. Respiratory enzyme activity in the postlaminar region is high in the oligodendrocytes. These results suggest that the citric acid cycle, with its 36-38 $\sim$ P yield, operates primarily within the axon of the non-myelinated part of the cat optic nerve and within the oligodendrocytes of the myelinated part. They favour the concept of the oligodendrocyte as an energy donor (Hyden and Pigon, 1960), specifically to the axon at the central node of Ranvier, and hence assign to it a node-supporting role. The relationship between axons and astrocytes in the cat optic nerve is less clear. In the prelaminar region they isolate the non-myelinated axons from each other. In the laminar region some axons are finely-myelinated and since the related cells are all astrocytes, they must have a sheath-supporting role at this site. Astrocytes in all regions react strongly for alpha-glycerophosphate dehydrogenase, which indicates either lipid production (possibly phospholipid membrane formation) or lipid breakdown (energy production).

Taking into account observations of other workers on the relationship between neuronal perikarya and macroglia (e.g. Hyden and Pigon, 1960; Peters and Palay, 1965) we can

generalize and hypothesize that the oligodendrocyte is concerned with the energetic support of the neuron and the astrocyte with its isolation and insulation, morphological and ionic (Wendell-Smith and Blunt, 1965). We can also conclude that the neurons and the macroglia form a cytophysiological unit and agree with Ramon y Cajal that "they are mutually serviceable and there (is) established between them something like a symbiosis, comparable to the well-known symbiosis of fungi and algae".

ACKNOWLEDGEMENTS

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DISCUSSION

Dr. Game: Thank you, Professor Wendell-Smith, for your further contribution to this interesting subject, the neuroglia, one which is obvoiusly coming to the fore. The paper is now open for discussion.

Dr. Lamoureux: Professor Wendell-Smith, can you explain how the myelin is formed in central nervous tissue rather than peripheral nervous tissue?

Prof. Wendell-Smith: In the central nervous system (Fig. 5), the myelin-forming neuroglial cell extends several processes which wrap around individual axons and form myelin sheaths generally similar to those of the peripheral nervous system. In the central nervous system, however, the cytoplasm outside each compact myelin sheath is limited to a tongue or lip where the neuroglial process reaches it; elsewhere the outermost lamella of compact myelin is exposed and can fuse with neighbouring sheaths, giving the close-packed organization characteristic of white matter. In discussing the cell type responsible, we should recognize that the cell concerned with only a very slow rate of myelin turnover in the adult

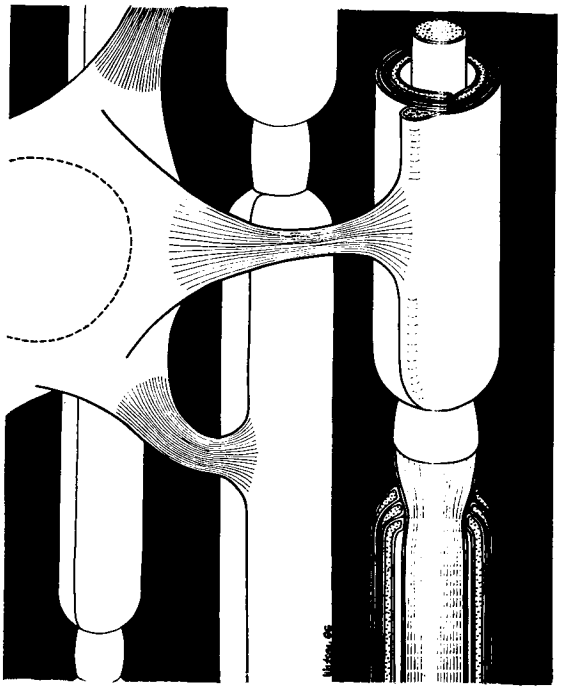

FIG. 5—A diagram representing a stage in the myelination of a fibre in the central nervous system corresponding to the stage represented in Fig. 1C for the peripheral nervous system. Note that instead of the one-to-one relationship between the Schwann cell and myelinating axon in a segment of peripheral nerve fibre, there is a one-to-many relationship between the neuroglial cell and myelinating axons in the central nervous system: the neuroglial cell extends processes and forms myelin around several axons. Note again that while there is a more or less complete cytoplasmic collar between the compact myelin and the axon, there is only a cytoplasmic tongue outside the compact myelin. Compared with the peripheral nervous system, there is a wider nodal gap but no specialized paranodal apparatus and no organized nodal processes.

is likely to look different from the same cell earlier in its life when it was concerned with active myelin formation. The myelin-forming cell is not an adult astrocyte, nor is it an adult oligodendrocyte, but it has some of the characteristics of both: it is a bivalent cell with dense amorphous matrices on the one hand and long fine filaments on the other.

Dr. Lamoureux: How does this cell differ from the Schwann cell?

Prof. Wendell-Smith: In the possession of long fine filaments and in its transient nature: the formative bivalent cell presumably "modulates" and is transformed into a definitive astrocyte, whereas the Schwann cell is both formative and definitive.

Dr. Ross Anderson: Have you any information on the mitochondrial content of axons in an area of pathological demyelination? There is, of course, evidence that axons will conduct through a demyelinated area.

Prof. Wendell-Smith: We have no direct information. Whereas in well-myelinated areas many more mitochondria are found in satellite cells (oligodendrocytes or Schwann cells) than in axons, in finely-myelinated and non-myelinated areas we have found more mitochondria in axons than in satellite cells. Whether this would be so in a demyelinated area I do not know.

Dr. Rail: Am I to understand that all myelin is intracellular? Is all the myelin sheath contained within the cell, or is there additional supporting material outside the cell?

Prof. Wendell-Smith: The myelin sheath and axon are intracellular in the same sense that

the intestines are intraperitoneal: the intestines are virtually surrounded by peritoneal cavity but are connected with the extraperitoneal tissues by a fused double membrane—the mesentery; the peripheral axon and myelin sheath are virtually surrounded by Schwann cell cytoplasm (Fig. 2C) but are connected with the extracellular space by a fused double membrane—the mesaxon. The terminology of peripheral nerve sheaths is confused (J. Z. Young, *Physiol. Rev.*, 22, 318-374, 1942), and terms like 'neurilemma" and "Schwann sheath" which have been used in different senses by different authors are better avoided. The outer collar of Schwann cell cytoplasm was formerly believed to comprise the whole cell of Schwann. Outside it is a basement membrane and outside this again is a network of small collagen fibres —the inner endoneurium or sheath of Plenk and Laidlaw. The outer endoneurium or sheath of Key and Retzius consists of more compact longitudinal bundles of collagen fibres.

THE ORIGIN OF BRAIN MACROPHAGES IN THE RAT

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A variety of opinions has been expressed concerning the origin of brain macrophages (compound granular corpuscles). Russell (1962) in an account of historical aspects revealed that early histologists considered that the origin of brain macrophages was from blood leucocytes; later Nissl and Alzheimer suggested a vascular adventitial cell origin. In the 1920's del Rio Hortega, who had identified microglial cells as being of mesodermal origin, considered these cells to be the precursors of brain macrophages. The opinion of del Rio Hortega has held sway until recent years, when the question of the origin of brain macrophages has been reopened by investigation with new methods. Koningsmark and Sidman (1963), using tritiated thiamidine labelling, estimated that approximately 65 per cent of brain macrophages appearing in stab wounds of the brains of mice were derived from blood monocytes. However, Cammermeyer (1963) in discussion of this paper was of the opinion that the stab wound method of producing brain damage, with its damage to blood vessels and discontinuity of tissue, produced complex reactive changes and made evaluation of experiment difficult. At the same meeting Kosunen and Waksman (1963) presented findings in a different type of experiment which overcame some of Cammermeyer's criticism. Using trtiated thymidine labelling they found that a high percentage of labelled macrophages appeared in lesions of experimental allergic encephalomyelitis in rats. They considered also that they could follow the development of these macrophages from monocytes closely resembling those in the blood stream, and were of the opinion that practically all of the macrophages appearing in the perivascular lesions in this condition were derived from circulating monocytes.

Hain (1963), reporting the effects of whole body irradiation in mice, stated that with only the head shielded the number of macrophages appearing in brain stab wounds was the same as in controls. In a second series, where only the head was irradiated, the macrophages response was reduced to 30 per cent of the control level. His interpretation of these results was that local cells in the brain, not circulating cells, made the major contribution to the brain macrophage population.

More recently Maxwell and Kruger (1965) in an electron microscopic study of heavy particle irradiation of rat brains, have thrown doubt on the concept of the microglial cell. They were unable to find a cell separate from the blood vessel walls which could with certainty be called a microglial cell. In their opinion macrophages in the irradiation lesions could well be derived from blood vessel pericytes.

With these experimental results in mind we have attempted, using another method, to show whether or not circulating mononuclear cells make a contribution to the brain macrophage population of experimental cold injury in rats. The technique employed was to label circulating mononuclear cells with carbon particles visible with light microscopy, and to examine the brain lesions for labelled cells.

METHOD

Six 150 G rats were used. Circulating mononuclear cells were labelled with carbon using a technique developed by Hurley, Ryan and Friedman (1966). Each rat was injected intravenously, twice daily, for five days with carbon

suspension (Günther Wagner, Batch C11/1431a, Hanover, Germany). Each injection dose equalled 0·1 ml. of carbon suspension per 100 G of animal. Three days after the end of the injections the only labelled cells seen on smear of tail blood were mononuclear cells (this includes all cells with a single nucleus ranging from small lymphocytes to monocytes). The percentage labelling varied from 10 to 18 per cent (see Table 1).

TABLE 1

Animal No.	*% labelling of blood mononuclear cells*		*Time killed (days after injury)*	*% labelling of macrophage cells in brain lesion*
	At time of injury	*At death*		
1	12	18	1	not estimated
2	18	14	2	8
3	18	11	3	8
4	12	14	4	4·5
5	18	8	7	not estimated
6	10	8	7	not estimated

Showing % carbon labelling of blood mononuclear cells in rats three days after a five day course of intravenous carbon suspension, and carbon labelling of blood mononuclears and brain macrophages when the animals were sacrificed.

At this stage cold injury was produced unilaterally in the frontal cortex of each rat, using a modification of the technique described by Bakay and Hague (1964). Each rat was anaesthetized with ether, the skull exposed and a circular piece of bone, 5 mm. diameter, removed with a dental drill and forceps. The dura mater was left intact and undamaged and was covered with a thin polythene sheet.

The source of cold was a freezing mixture of dry ice and ethanol contained in a metal funnel, the stem of which had been cut short and sealed with a flat copper disc 4 mm. diameter. The sealed stem of the funnel was applied to the protected dura for three minutes. The plastic sheet was easily removed from the frozen dura and no adhesions formed between the dura and the leptomeninges. The scalp wounds were closed with metal clips. The animals recovered from anaesthesia and showed no abnormality.

The animals were sacrificed one to seven days after the cold injury. Just prior to the death of the animals further smears of tail blood were examined for carbon labelling (see Table 1). Immediately after death the brains were removed and placed in 10 per cent formal saline. Paraffin sections cut at 7μ thickness were stained with haematoxylin and eosin, and neutral red and tartrazine, the latter staining giving excellent contrast to carbon in labelled cells. Frozen sections were also examined for the presence of sudanophilic lipid in cells.

Labelling estimates were arrived at by counting at least 200 mononuclear cells in random high power fields of blood smears and at least 200 macrophages in brain lesions.

RESULTS

(See Table 1)

The lesions produced were fairly uniform in size. At the surface the saucer-shaped lesions measured 4 mm. in diameter and reached depths of 1 to 1·5 mm. centrally.

Rat 1—One day after injury

Carbon labelled cells in blood vessels were more numerous in the region of injury. In the area of damage there were a few small haemorrhages and a mild polymorph cell accumulation. A few labelled mononuclear cells were seen in the regions of haemorrhage. Beyond the area of damage no labelled cells were seen outside vessels.

Rat 2—Two days after injury

In the area of damage a large number of mononuclear cells and a few polymorphs were seen. The mononuclear cells were especially numerous around blood vessels in the base of the lesion. Some of the mononuclear cells contained lipid. A few binucleate macrophages and an occasional mitosis were noted.

At the time of death blood smears showed that 14 per cent of mononuclear cells were labelled. In the brain lesion 8 per cent of macrophage cells were labelled.

Rat 3—Three days after injury

The main cell type in the lesion was a mononuclear cell. The cytoplasm of the cells farthest from blood vessels showed the greatest amount of swelling with lipid.

At the time of death blood smears showed that 11 per cent of mononuclear cells were labelled. In the brain lesion 8 per cent of macrophages were labelled.

Rat 4—Four days after injury

Similar appearances to those seen in rat number 3. In blood smears at time of death 14 per cent of mononuclears were labelled whilst in the brain lesion 4·5 per cent of macrophages were labelled.

Rat 5, Rat 6—Seven days after injury

There was liquefaction of the damaged area with loss of a large amount of the damaged tissue. Some astrocytic cell proliferation and early connective tissue formation was evident. Adjacent to the area of damage perivascular cuffing with small round cells was present. Some of the cells in these cuffs were labelled.

The lesions were unsatisfactory for the estimation of labelled cells. The blood smears of both animals at the time of death showed that 8 per cent of mononuclear cells were labelled.

Discussion

The results of this experiment indicate clearly that labelled circulating mononuclear cells make a contribution to the macrophage population of experimentally induced cold injury in the rat brain. Intravenous carbon of the type used is cleared rapidly from the blood stream by polymorphs, mononuclear cells and phagocytic cells of the reticulo-endothelial system which remain labelled and there is no significant labelling of other cells. When the blood was examined three days after the last carbon injection polymorph labelling was zero with light microscopy. This fact eliminates the possibility that labelled polymorphs might have carried carbon to the lesion, disintegrated and the debris be phagocytosed by local cells which themselves would then be labelled.

Of course what one would like to know is the percentage of brain macrophages which were derived from the blood stream. It might be argued that since equivalent percentages of labelled cells were seen in blood smears and brain lesions, allowing for some deficiency in counting of 7μ brain sections, that the vast majority of macrophages came from the blood.

However, in the counting of blood smears all mononuclear cells were counted, many of which will be lymphocytes with no ability for phagocytosis. If this was so then it might be expected that the brain lesions would show a higher than blood percentage of labelled cells. Against this is the possibility that labelled cells in the blood stream might not be as able as unlabelled phagocytic cells to get through blood vessel walls; if so the percentage labelling of macrophages in the brain lesions would be less than expected even if they all arose from the blood stream.

In conclusion, all that can be said from this experiment is that at least a proportion of brain macrophages was derived from circulating mononuclear cells.

Summary

Circulating mononuclear cells were labelled with carbon visible with light microscopy. Following cold injury of the brain carbon labelled macrophages appeared in the lesions. It is suggested that these labelled macrophages were derived from the blood mononuclear cells.

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Discussion

Dr. B. A. Kakulas: I would like to congratulate Dr. Anderson on a fine piece of work confirming the results of Konigsmark and Waksman, and Sydenham, on the

origin of brain macrophages. Although we shouldn't detract from the observations of del Rio Hortega in his identification of the microgliacyte as being mesodermal in origin, there are a couple of points requiring revision in his hypothesis. The first, as indicated, was that in the resting or normal brain the classic descriptions and morphologic representations of microgliacytes by silver impregnation techniques are probably endothelial cells, and that no definite macrophage nor microgliacyte exist in the normal brain, and that these such cells are purely observed in lesions. The other point requiring revision is Hortega's postulate that these macrophages or microgliacytes enter the nervous system at about the time of birth. I believe that the small cells at the base of the brain, which Hortega observed, were really developing neuroblasts; and I think it is quite clear to anybody who has looked at tissue from foetal brains where there are pathological reactions that macrophages are a prominent component of these lesions. The question of the origin of all macrophages in cerebral lesions is at present unanswered. I think it is reasonable still to maintain that there is a cell in the subarachnoid space, an arachnoidal cell, which probably has macrophage abilities. Whether or not the adventitial cell in the blood vessel has this in the normal state, or whether the cells here are present and react in the same way as reticulo-endothelial cells do in other parts of the body, must remain an open question. I think we all agree that certain lesions, mainly tumours, do provide information on this fact, or suggest that the representation from the lymphatic system in the nervous system is in the Virchow-Robin space. I would like to ask Dr. Anderson if he had any information of the migration of these macrophages back into the blood vessels, which I feel would be a logical follow-on from his work.

Dr. Anderson: No, I have not any good information. There is in another field—Dr. Cliff in Canberra has looked at macrophages in ear chamber work, and produced carbon labelling of the macrophages, and has been able to watch a particular macrophage for 50 or 60 days, and the macrophage has not moved except in pulsatile fashion. It has not moved back to the blood vessel. This is very interesting because it is suggested that these cells have only a short life span, and you might expect that perhaps the cell dies and another cell mops up the carbon and so on, but Cliff's work, I think, is a little against this. Obviously, these cells must remove material back to the blood stream—the material disappears after a while and it goes somewhere, but I do not know of any method of showing just how this happens.

Dr. Lamoureux: What is your definition of a macrophage. Is that an histiocyte?

Dr. Anderson: The definition of a macrophage, I think, must depend on the inclusion of largely lipoid material in this site, whether it be haemosiderin or carbon. It must phagocytose material, in this sense.

Dr. Lamoureux: The gial cells have this function too.

Dr. Anderson: Yes, that is true. In haemorrhages in the brain, and so on, you can show that even nerve cells can phagocytose haemosiderin. I think one has got to accept that, but I think that these cells that we are talking about are almost certainly of the monocyte derivative type and not related to astrocytes or to neurones, or to oligodendroglial cells, which have only a very limited capacity for phagocytosis.

Prof. Wendell-Smith: I would like to take issue with the first speaker here and support Dr. Anderson in his concept of the normal microglia being pericytes, rather than endothelial cells as Dr. Kakulas suggested. The whole question of what constitutes normal microglia is a very difficult one. Electron microscopists have usually identified as microglia a cell type characterized by its small size, great electron density and indented outline. This identification has recently been questioned by Maxwell and Kruger (*Exp. Neurol.*, 12, 35-54, 1965) and by us (Wendell-Smith, C. P., Blunt, M. J., and Baldwin, F., *J. comp. Neurol.*, 127, 1966, in press): although we differ on the identification of the electron-dense cells, we are agreed that at least many of the cells stained by del Rio Hortega's method for microglia are pericytes. In our preparations of normal cat optic nerve, the microglia of light microscopy are found only in the septa in a perivascular position. Fig. 1 of the preceding paper is an

electron micrograph of part of such a septum: the lumen of a blood vessel is seen; it is surrounded by an endothelial cell with a flattened nucleus; a pericyte occupies the centre of the field and is separated from the endothelial cell on the one hand and from collagen fibres and a fibroblast on the other hand by a basement membrane. The morphology, distribution and properties of pericytes are consistent with their identification as microglia.

Dr. Kakulas: I would like to thank Professor Wendell-Smith for his remarks. I think he will agree that with the conventional light microscopic preparations it would be difficult to distinguish the endothelial cell from the pericyte, and that the point is as I made it.

SOME ASPECTS OF THE DEVELOPMENT OF KNOWLEDGE OF THE PINEAL BODY

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The pineal body of mammals has been known to anatomists and biologists in general since early times and its function, about which there is still doubt, has from the beginning been the subject of considerable interest and speculation. The earliest reference to it in existence was made about A.D. 173 by Galen in his *De usu partium*, and it is clear from this text that its presence was already well known. In this work, written in Greek, the author referred to the pineal body as the konarion, which literally means pine-nut, pointing out it was given this name on account of its shape being reminiscent of a pine cone. This term was subsequently latinized to "conarium", a name still used by some.

The account provided by Galen in the second century not only holds interest, because of his position in the history of medical knowledge, but has an especial value, since it states the position at the time of commencement of the documented study of this structure. The following translation has been made from the Daremberg (1854) edition of the *Anatomical, Physiological and Medical Works of Galen.*

"As for this conoid gland which resembles a pine cone and which fills up the bifurcation of the large vein (veins of Galen) from which are derived almost all the choroid plexus of the anterior ventricles, I believe that it exists for the same useful purpose as those glands charged with consolidating the bifurcations of veins. In fact, the position of the conarium is, in all respects, the same as that of analogous glands whose apex supports the parts of the vein at the point of bifurcation, while all the rest of the gland becomes more voluminous according as the vessels proceeding from the bifurcation move further away, and accompanies them as long as they remain suspended. As soon as these veins are supported on the body of the encephalon itself, the conarium abandons them. The body of the encephalon at this point becomes a support for the conarium itself and simultaneously for the veins."

Thus it can be seen that Galen regarded the pineal body as a gland serving to support vessels, a belief re-expressed much later by Vesalius (1543) in *De humani corporis fabrica.* The quotation used here is taken from the translation of the seventh book of the *Fabrica* prepared by Charles Singer (1952).

"I believe that, like many other small glands, this has been made as a support for some vessel (such as that) which enters the third ventricle (internal cerebral vein) and, immediately after its origin, is divided into plentiful branches which are woven closely together by a membrane supporting it—lest the passage which lies at its origin and immediately at its entrance be blocked and so prevent the animal spirit from being carried out of the third ventricle into the fourth."

A century later, Descartes (1649) put forward his view that the pineal body was an organ of psychic life and the site of the soul in the following words:

"The reason which convinces me that the soul can have no other place in the entire body but this gland, where it directly exercises its function, is that I consider that other parts of our brain are all double, as we also have two eyes, two hands, two ears, and finally all organs of our exterior senses are double; and inasmuch as we have but one single and simple thought of the same thing at the same time, there must

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necessarily be some place where the two images which arrive through the two eyes, or the two impressions of one single object which reach us through the double organs of other senses, could meet and join before they reach the soul, so as not to represent two objects instead of one. And it is easily conceivable that these images or other impressions assemble in this gland, through the medium of the spirits which fill the cavities of the brain; indeed there is no other place in the body where they could be so united, save this gland."

Although, in the *Ethica* published after his death in 1677 Spinoza referred to the Cartesian view as "a hypothesis which gives to things obscure the quality of being even more obscure" (Hypothesin . . . omni occulta qualitate occultiorem"), it did represent an attempt to localize psychic phenomena in the central nervous system and influenced thought concerning the pineal body for a long time. As a consequence an attempt was made to explain mental illnesses upon the basis of alterations of this body. In this connection a great deal of attention was given to the study of the pineal concretions which are such a noticeable feature in so many specimens of the organ, and Guenz, for example, in 1753 published a work titled *De lapillis glandulae pinealis in quinque mente alienatis* ("On the concretions of the pineal gland in five cases of mental illness"). However, as a result of detailed studies, Soemerring (1785) in his *Dissertatio de acervulo cerebri* removed false ideas concerning the occurrence of pineal concretions when he found that they occur in healthy humans of both sexes and all ages. Burdach (1826) assumed that the pineal "affects more the general mood of brain activity" rather than performing a more specific function.

Many observations relating to the structural character of the pineal body were made before its microscopic anatomy became well known. Gall and Spurzheim (1810), for instance, considered the organ to be a ganglion of the nervous system and stated in reference not only to the pineal body but the hypophysis cerebri as well:

"Nowadays it is generally agreed that these two parts are not glands. Their integral particles are regarded as being analogous with all those of the mass of the brain, and it is believed that similarly to the brain they are composed of grey matter and white matter. In conformity with the principle which we have established, these two parts must be considered as true ganglia where particular nerve strands originate."

The principle alluded to was the doctrine that nerve bundles emerged from grey matter which they had established from gross observations: in fact, methods of histological technique were of necessity well advanced before any great deal of attention could be given the minute structure of the pineal body and it was not until the commencement of the second half of the nineteenth century that Koelliker (1850) recorded for the first time the presence of nerve fibres within the pineal body determined as a result of microscopical examination.

The conception of the ganglionic nature of the pineal body persisted for a considerable time. Thus, Meynert (1872) believed it to be a ganglion of the nervous system and included it in the system of nervous bodies which he held gave rise to the fibres of the tegmentum of the cerebral peduncle. In 1879, however, Koelliker felt justified in saying "the hypophysis and pineal are two physiologically incomprehensible and undoubtedly insignificant organs". The origin of such a view concerning the pineal body arose from the circumstance that it could not be satisfactorily included, on the basis of microscopical investigations, in the organic systems known at that time. However, by 1890 histological procedures had advanced to the stage where all the tissues and parts of the body were readily accessible to examination under the microscope, and Dallinger (1891) was able to comment:

"The cutting, staining, and mounting of the most delicate organic tissues in almost every conceivable state has thrown light upon histological and pathological matters, the present and prospective value of which we can scarcely estimate too highly; while some of the profoundest and most interesting questions of biology are opening themselves to renewed research by its means."

The need for a procedure that would allow a more complete analysis of the finer structural interrelations between nerve bundles and fibres on the one hand and nerve cells on the other was met by the use of the silver-impregnation

technique, originally described by Golgi (1873) but not generally known until 1887 when Ramon y Cajal recognized its value.

Through the Golgi method, Ramon y Cajal was able to furnish, in greater detail than before, many valuable descriptions of different parts of the nervous system. In the French edition (1911) of his classical work dealing with the histology of the nervous system, Ramon y Cajal stated:

"A few years ago the opinion was still currently held that the pineal gland of lower vertebrates, and more particularly of reptiles, is nothing more than an unpaired visual organ, the parietal eye, which was believed to be atrophied in mammals and birds. This, at least, is what seemed to result from the work of Graaf and B. Spencer. This opinion, refuted by the work of Beraneck and others, is today tending to disappear. Moreover, the conarium of mammals, in our opinion and with absolute certainty, has absolutely nothing in common with that of reptiles. On the one hand, it possesses none of the anatomical characteristics of an eye, and on the other hand, it does not receive any fibre either from the optic nerve or from the brain; finally, a detail which is also quite decisive, far from tending to disappear like a vestigial organ, in man it attains greater size than in small mammals and birds. The great development of its sympathetic plexus tells us, moreover, that indubitably the epiphysis is purely and simply a blood-vascular gland."

However, the related problems of the nature and morphological characteristics of the cell components of the pineal body remained subjects of controversy. Considerable confusion as to whether the cells composing the principal part of its parenchyma were nervous, neuroglial or specific prevailed until the classic work of Rio-Hortega (1923) who developed a technique involving impregnation with silver carbonate, which allowed the fundamental tissue of the parenchyma to be electively distinguished. By this means he was able to show for the first time, and in a very striking manner, the manifold forms exhibited by the cells of this tissue in the human which he termed parenchymatous cells. After thoughtful and exhaustive analysis, Rio-Hortega concluded that the parenchymatous cells were neither nervous nor neuroglial but a distinct cell type proper to the pineal body, but was not prepared to venture any theory as to their probable function.

At the present time there is no generally accepted view as to the physiological status of the pineal body. For many years attempts have been made to ascribe an endocrine function to it, but in the main these have led to very conflicting results. However, at least one aspect of its activity has been uncovered in recent years as it has been shown to contain a group of substances related to indole, and these have been referred to tentatively as the "indole hormones" (McIsaac, 1962). These substances first attracted attention in 1958 when Lerner and his co-workers extracted N-acetyl-5-methoxytryptamine from bovine pineals and gave it the name melatonin since they found that it had an effect upon frog melanocytes which caused lightening of skin colour. It is now known that melatonin can be synthesized from serotonin (Axelrod and Weissbach, 1960) which has been discovered in a number of mammalian pineal bodies, and has been found to be particularly abundant in that of the rat (Quay and Halevy, 1962). Bertler, Falck and Owman (1963) have since demonstrated, by means of a fluorescence technique, the presence of serotonin in both the nerve fibres and the parenchymatous cells of the rat pineal body, and Owman (1964) has suggested that the intrapineal nerve fibres take up serotonin from the parenchyma of the organ and store it. This view is particularly interesting in the light of the claim made by Robertis (1962) for the inclusion of the pineal body among the organs of neuro-secretion. Moreover, it has been shown that in the rat, the pineal content of serotonin is influenced by the photo-period and accords with the concept of a circadian rhythm being at a maximum after eight hours of light and a minimum after four hours of dark (Quay, 1963). Fiske (1964) has shown that this rhythm is abolished by removal of the superior cervical ganglia. Also Wurtmann, Axelrod and Fischer (1964) have shown that in the same animal the sympathetic nervous system is directly involved in the synthesis of melatonin and that its action, which is dependent upon information concerning environmental lighting received from the eyes, is exerted through the superior cervical ganglia from which the fibres of the nervi conarii, the major nerves of supply

to the pineal body of the rat, have been traced by experimental means (Kappers, 1960).

It may be that these interesting findings will be of great significance in finally elucidating the rôle played by the pineal body in the economy of vertebrate organisms. Perhaps it is reasonable to hope that it will not be much longer before this organ ceases to be the enigma it has been for so many years.

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DISCUSSION

The President: This is the third of a series of papers Dr. Kenny has given us on the pineal. In our present state of knowledge his work seems to have no application at all. This is pure research, reminiscent of the *Spirit of Research*, a book by Professor Brailsford Robertson. A few days ago we heard of new knowledge of the thymus, suggesting that this gland has an important role in immunity. In the past the thymus, like the pineal, has been thought to be of little importance.

We are fortunate in having in our audience the Professor of Pathology of the Royal Australasian College of Surgeons, Professor Gault. He also is interested in the pineal, and we should be most grateful to hear his comments.

Professor E. W. Gault congratulated Dr. Kenny on his learned and interesting paper. He was interested to find that such a careful survey had ended on a note of uncertainty as to the exact rôle of the pineal in the physiological processes of the body. He recalled that several years ago, when discussing the recently discovered hormone, melatonin, with a colleague the latter had commented: "We have had an exciting year. The thymus has now been given a function and the pineal has produced a hormone." This made Professor Gault speculate on the similarities between these two bodies about which so little has been known until recently. Firstly, they were both structures which steadily increased in size until puberty, but then tended to atrophy. Secondly, both developed calcified bodies quite early in life. Thirdly, they both produced hormones and had specialized cells in their structure. Fourthly, they were both centrally situated, and teratoma developed in both the thymus and the pineal.

If someone could give to the pineal the same enthusiastic study and research which Sir Macfarlane Burnet had given to the thymus, its function may well serve a more useful purpose than as a calcified marker in the brain to indicate displacement by pathological lesions in its vicinity. Dr. Kenny's paper would stimulate thought and study towards this goal.

Dr. Lance: Dr. Kenny, what is the distribution of calcium in a calcified pineal gland? Is it diffuse or in the form of a localized concretion? Also, are the cells of a calcified pineal gland degenerate and why may calcification extend to the habenular trigone?

Dr. Kenny: Microscopically, the calcareous material may be distributed in both ways—either in the form of concretions scattered throughout the organ or less frequently, as a single localized mass. Some of these concretions are too small to be detected radiographically, and commonly only a single mass may be apparent radiologically. The occurrence of calcification does not indicate degeneration of all the parenchymatous cells; many may still remain active. Why the concretions may also occur in the habenular region is not clear at the present time.

IMMUNOLOGICAL STUDIES IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS AND IN MULTIPLE SCLEROSIS WITH SPECIAL REFERENCE TO PATHOGENESIS AND DIAGNOSIS

GILLES LAMOUREUX*

SUMMARY

A brief immunological comparison between simian allergic encephalomyelitis (EAE) and human multiple sclerosis (MS) is presented. Findings revealed by electrophoresis and immunoelectrophoresis of CSF in both diseases and tests for cytotoxicity of serum and CSF for cultured nervous tissue, not only implicate some immunological disturbances in MS but more closely relates EAE to MS.

Evidence is also presented that electrophoresis, immunoelectrophoresis and tests for cytotoxicity in cultured nervous tissue may help the neurologist in a diagnosis of MS.

INTRODUCTION

Experimental allergic encephalomyelitis (EAE) is an experimental model of multiple sclerosis and certain other demyelinating diseases. It is produced in animals by injection, usually with adjuvants, of heterologous, homologous and autologous nervous tissue and by encephalitogenic protein (molecular weight, M.W., 12,000) and polypeptides (M.W. around 3,400) extracted from myelin of various animal species.

EAE is regarded as an experimental autoimmune disease for the following reasons: (1) It can be produced by immunizing an animal with its own nervous tissue. (2) It has been produced in a non-immunized animal by transfer of lymph node cells from an immunized donor. (3) It is accompanied by the production of humoral antibodies which however have no correlation with the severity of the disease, by delayed hypersensitivity skin reactions, lymphoid cell infiltration in the central nervous tissue, and a gradual increase in concentration of immunoglobulins in the serum and cerebrospinal fluid. (4) EAE is prevented if animals are made tolerant to adult nervous tissue in the neonatal period, or immunized as adults with minute amount of encephalitogenic peptides or proteins. (5) EAE cannot be induced in animals which are immunologically deficient, as after neonatal thymectomy. (6) EAE is delayed by treatment with irradiation or immuno-suppressive drugs such as 6-mercaptopurine, azathioprine, cyclophosphamide, or corticosteroids, and all such drugs are most effective when given soon after immunization with the encephalitogen.

Both adjuvants and encephalitogenic substances may have a dual function; in certain circumstances they produce disease and in others they give protection. The mechanism for this form of enhancement and/or protection, and/or tolerance, and/or desensitization or whatever it is, is still unknown. However, because of this consistent protection effect obtained with encephalitogens (Ferraro and Cazzullo, 1949; Kies *et al.*, 1960) or with adjuvant (Kies and Alvord, 1958; Svet-Maldovsky *et al.*, 1960; Cunningham and Field, 1965) in certain defined experimental conditions, Cunningham and Field (1965) suggested that these substances (adjuvants, encephalitogens, or both) could be used "to prevent or to reduce episodes" of multiple sclerosis.

The present work describes immunological comparisons between EAE and human multiple sclerosis (MS) and describes some laboratory techniques such as electrophoresis and immuno-

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electrophoresis of the cerebrospinal fluid (CSF) and tests for cytotoxicity of serum and CSF, which may help the neurologist in the diagnosis of multiple sclerosis.

Materials and Methods

Serum and cerebrospinal fluid was obtained from rhesus monkeys in which EAE was induced by immunization with whole rabbit nervous tissue, and from patients with multiple sclerosis at different stages of the disease.

These were analysed by electrophoresis on cellulose acetate strips and by gel immunoelectrophoresis, and studied for cytotoxic activity on cultured nervous tissue. The techniques and the selection of patients were as described by Lamoureux, Boulay and Borduas (1966). One group of seven patients with acute MS had been treated with ACTH. None of these patients, however, received more than three injections of 40 units of ACTH when the samples of serum and CSF were obtained.

Studies on cytotoxicity were performed by exposing cultured nervous tissue with 1 per cent fresh human serum as a source of complement to the test sample of serum or CSF. Damage to glial cells, myelin and neurons was assessed visually after 18 to 24 hours of contact, using the criteria of damage described by Lamoureux *et al.* (1966). The degree of damage was expressed by a semi-quantitative cytotoxic index which was derived from a summation of degrees of damage, graded from 0 to 4+, to glial cells, to myelin, and to neurons, and from the amount of debris present, also graded from 0 to 4+. The cytotoxic index could therefore range from 0 to 16. The cytotoxic index obtained with 31 sera and CSF from normal rhesus monkeys and with 28 normal human sera and CSF was always below four.

Results

Similarity of histopathological lesions in MS and EAE

Many workers have recognized a similarity between the neurological lesions of MS and EAE. In particular, both diseases show perivascular and often diffuse infiltration with histiocytes, lymphocytes and plasma cells in the central nervous system. The pattern of demyelination in EAE varies from species to species,

Table 1

Monkeys	*CSF γ globulins*		*Sera γ globulins*	
	1st d.	*Day of Death*	*1st d.*	*Day of Death*
570	13·6	29·0	13·8	21·5
566	17·2	24·0	14·5	20·0
634	17·0	22·2	21·0	31·6
611	14·5	17·4	14·8	19·6
568	13·3	—	10·8	17·7
564	17·0	Killed at 253 days 22·0	17·7	18·9
569	10·1	14·2	10·9	15·0

Percentage of gamma globulin in serum and CSF of seven monkeys before immunization (1st d.) with rabbit nervous tissue and at day of death. Monkeys no. 564 and 569 did not develop EAE —their serum and CSF was not toxic but showed an increase of gamma globulin.

Table 2

Diagnosis	*No. of cases*	*Gamma globulin percent*	
		Mean	*Range*
Normal	24	7·1 ± 2·8	2·7–9·6
Acute MS	15	20·1 $p < 0.001$	15·4–30·8
Remission and chronic MS	17	14·4	8·8–19·3
Acute ACTH treated MS	7	6·6	5·4–11·2
Doubtful MS	7	10·6	–
Other neurological diseases	28	7·3	3·1–13·8

Percentage of gamma globulin in CSF in multiple sclerosis. The figures represent the mean percentage of gamma globulin for the six diagnostic groups. The difference in the gamma globulin value between cases of acute MS and controls (normal) was highly significant ($P < 0.001$).

but in higher animals such as monkeys, as compared with the rat, guinea pig and rabbit, the lesions more closely resemble the early "plaques" seen in human multiple sclerosis.

Immunoglobulins in CSF of rhesus monkeys with EAE and humans with MS

On electrophoresis the serum and CSF from seven rhesus monkeys immunized with rabbit nervous tissue showed an increase of immunoglobulins particularly the gamma globulin (Table 1). The rise was progressive and reached a maximum value at the time of death of the animals. In the CSF there was an increase in the gamma globulin ranging from 2·9 per cent to 15·4 per cent of the total CSF proteins and in the serum, ranging from 1·2 per cent to 10·6 per cent of the total serum proteins.

Similarly, 15 out of 15 patients in the acute phase of MS showed an increase in the percentage of gamma globulin in their CSF: the minimum value obtained for these 15 cases was greater than three standard deviations from the mean (7·1 per cent) for our control population, and the range was from 15·4 per cent to 30·8 per cent of the total CSF proteins (Table 2). In a group of 17 patients in remission or in the chronic stage of MS, the gamma globulin level was also elevated, the mean being 14·4 per cent of the total CSF proteins. In 7 cases of MS patients in the acute phase of disease but treated with ACTH, the CSF gamma globulin was normal, the mean for this group being 6·6 per cent of the total CSF proteins.

TABLE 3

Monkeys		*Cytotoxic Index*
With EAE (6)	Sera	14·7
	CSF	11·3
Vaccinated with Salk Polio Vaccine (7)	Sera	4·0
	CSF	2·0
Normal (31)	Sera	1·5
	CSF	1·3

Mean cytotoxic index of sera and CSF for 6 monkeys with EAE, 7 monkeys vaccinated with Salk poliomyelitis vaccine and samples from 31 normal monkeys. The mean cytotoxic index obtained with sera and CSF from monkeys with EAE was considerably higher than the mean cytotoxic index for the other two groups.

TABLE 4

Diagnosis	*No. of Toxic Sera*	*Mean Cytotoxic Index of Serum*	*No. of Toxic CSF*	*Mean Cytotoxic Index of CSF*
Normal Subjects	4/24	2·1	3/23	1·6
Acute MS	14/15	11·5	14/15	10·4
Remission and Chronic MS	12/16	7·2	11/14	9·2
Acute ACTH treated MS	2/7	3·3	5/7	8·3
Doubtful MS	3/6	6·1	5/7	7·0
Other Neurological Diseases	7/28	4·4	8/28	3·8

Mean cytotoxic index of serum and CSF for six diagnostic groups: the cytotoxic index was highest in patients in the acute phase of MS.

Toxicity of serum and CSF from monkeys with EAE and MS patients for cultured nervous tissue

Both serum and CSF from rhesus monkeys with EAE were toxic for cultured nervous tissue: the mean cytotoxic index for 6 paralysed monkeys was 14·7 for the serum and 11·3 for the CSF (Table 3), this being well above the cytotoxic index found in 31 normal sera (1·5) and 31 normal CSF (1·3).

The mean cytotoxic index obtained for 15 patients in the acute phase of MS was 11·5 for serum, and 10·4 for CSF (Table 4). It is significant that the mean cytotoxic index was much lower in remission and in the chronic phase of MS, being 7·2 for serum and 9·2 for CSF. In seven cases of MS in the acute phase of the disease and treated with ACTH, the cytotoxic index was negative (< 4) with serum but positive (> 4) with CSF. It is by no means certain that the cytotoxic factor present in the CSF is an immunoglobulin even though the cytotoxic index correlates well with the level of gamma globulin in both diseases.

Immunoelectrophoresis of CSF in MS

Figure 1 shows an immunoelectrophoresis of a normal human CSF and of a normal human

serum. The immunoglobulin G line (arrow) has a normal appearance. Figure 2 shows an immunoelectrophoresis of a CSF from a patient in acute phase of MS: the immunoglobulin G line is about three times longer and is prolonged toward the anode, representing an abnormal fast

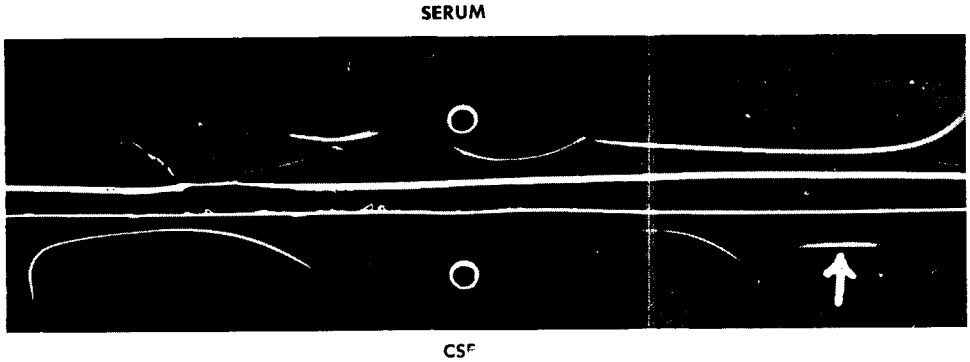

FIG. 1—Immunoelectrophoresis of normal CSF. The upper well contained normal serum and the lower well normal CSF, and the trough contained a rabbit antiserum to whole human serum protein. The IgG line of normal CSF (arrow) is shorter than that of serum.

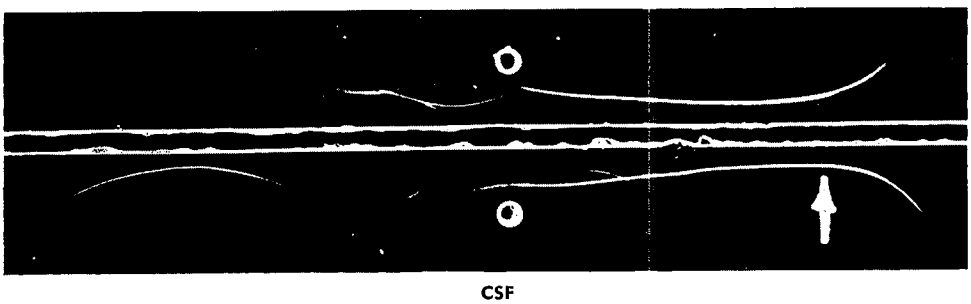

FIG. 2—Immunoelectrophoresis of CSF in acute multiple sclerosis (MS). The conditions were as for Fig. 1 except that the lower well contained CSF from a patient with MS. The IgG line (arrow) is extended toward the anode and is termed a fast gamma globulin.

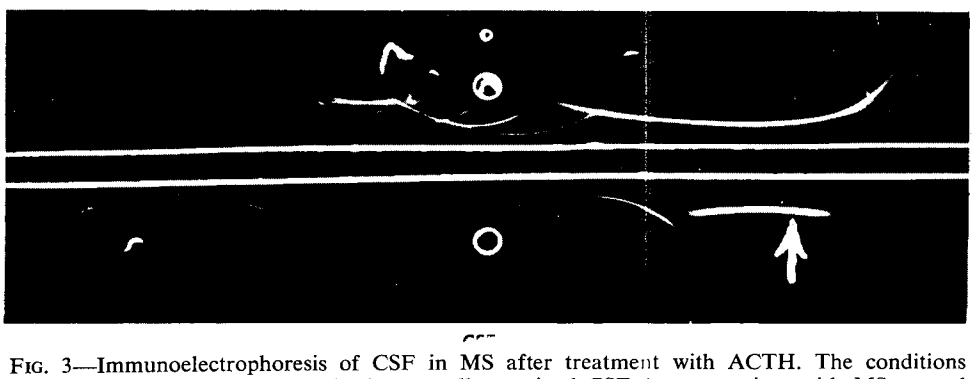

FIG. 3—Immunoelectrophoresis of CSF in MS after treatment with ACTH. The conditions were as for Fig. 1 except that the lower well contained CSF from a patient with MS treated with ACTH. The IgG line (arrow) has a normal appearance.

gamma globulin component. This pattern was found in 50 per cent of our patients in the acute phase of MS and in 33 per cent of cases of MS in remission or in the chronic phase of the disease. Figure 3 shows that in ACTH-treated patients the immunoglobulin line has a normal appearance.

Further studies with specific anti-human immunoglobulin sera showed that 50 per cent of MS patients in remission and in the chronic phase of MS have an increase of immunoglobulin A (IgA) in their CSF, while only 20 per cent of the patients in acute phase of MS showed this type of immunoglobulin. Immunoglobulin M (IgM) was found in 16 per cent of patients in acute phase of MS. The concentration of CSF we used (100-200 times) revealed that immunoglobulins A and M were present in less than 5 per cent of our controls (24 normal CSF and 28 CSF from other neurological diseases).

Discussion

Multiple sclerosis is considered to be closely related to allergic encephalomyelitis for the following reasons: (1) There are similar histopathological lesions in the nervous tissue. (2) Injection of nervous tissue in man whether for rabies vaccination (Shiraki and Otani, 1959) or for other therapeutic purposes (Jellinger and Seitelberger, 1958) produces plaque-like lesions indistinguishable from the lesions found in multiple sclerosis. (3) Serum and CSF in both diseases contain cytotoxic factor for cultured nervous tissue, and the degree of cytotoxicity correlates well with the increase in gamma globulin in the CSF. (4) It is virtually certain that in EAE an immunological mechanism, whatever it is, is responsible for the production of lesions, and the present immunological findings suggest that there are immunological factors involved in multiple sclerosis. However, it is uncertain whether these abnormalities are primary or secondary in the development of MS, whether they act as pathogenic or protective agents, or are simply the result of some other secondary factor. We believe that further work on EAE will solve some of these controversial questions. One problem however remains, and this is the explanation for the chronicity and the relapsing course of multiple sclerosis; this is produced only with great difficulty in animals.

The great variety of factors and agents implicated as being the cause of multiple sclerosis since Charcot, led us to believe that no specific agent is involved in the initiation of the disease. This rather suggests that some common denominator may be found to relate all MS cases. If one agrees that somewhere in the development of MS immunological features appear, one can perhaps speculate on the role of these immunological abnormalities in the pathogenesis of multiple sclerosis. I would believe that any virus, rickettsia, bacteria, trauma or any other process capable of either altering myelin encephalitogens (such as the encephalitogenic protein or peptide) or causing its liberation in the organism may sensitize the body. Once autosensitized by its own encephalitogens which could have the function of a common denominator, the body responds by defence mechanisms which are of different types, (a) humoral immunoglobulins, (b) cellular hypersensitivity mechanisms like those involved in the rejection of grafts. Whether or not one of these two types of response can produce the lesions found in the target organ, remains to be answered.

If it were accepted that the pathogenesis of EAE has something similar to that of multiple sclerosis, then certain conclusions could be drawn. In EAE the level of humoral antibodies does not correlate with the severity of disease (Hill, 1949; Thomas *et al.*, 1950; Lumsden, *et al.*, 1950), and it seems that the higher is the titre of humoral antibodies the better would be the protection of animals (Paterson and Harwin, 1963; Paterson, Jacobs and Coia, 1965). On the other hand the delayed hypersensitivity skin reaction produced by the sensitized cells in contact with the encephalitogens correlates well with the disease severity (Waksman and Morrison, 1951; Waksman, 1956 and 1959; Shaw *et al.*, 1965). This strongly suggests that in EAE and perhaps also in other autoimmune diseases) allergic thyroiditis, orchitis, uveitis, etc.) sensitized cells produce in the target organ a *chronic hypersensitivity-type reaction* which causes the histiopathological lesions. If this is so the lymphocytes and histiocytes found in the central nervous tissue may be the autodestructive agents of the nervous tissue. Continuing this speculation some of the autoantibodies would act as protective agents

trying to minimize the damage caused by the autodestructive cells. They may be synthetized *in situ* as evidences the presence of the plasma cells in the cellular infiltration. The disease would then be the result of a *"balance of power"* between the autoantibodies acting as protective agents on one hand, and the autodestructive cells on the other hand. The severity of the disease then depends on this *"balance of power"* toward the protective side or the pathogenic side and all intermediate forms are possible.

The practical aspect of the present work relates to the laboratory diagnosis of multiple sclerosis by means of electrophoresis and immunoelectrophoresis of CSF and by the use of cultured nervous tissue. Features suggestive of multiple sclerosis on electrophoresis of CSF include a raised CSF gamma globulin, a low beta-gamma globulin ratio and in the acute phase a decrease of alpha 1 globulin. On immunoelectrophoresis there is a fast IgG causing an extension of the IgG line towards the anode, and an IgA in the chronic phase of the disease (Table 5).

TABLE 5

Electrophoresis:
↑ of Ig.
↓ of α 1 in acute phase of MS.
↓ of B/Y globulin ratio.

Immunoelectrophoresis:
↑ of IgG in 50% of acute MS. cases.
↑ of IgA in 20%-50% of all MS. cases.
↑ of IgM in 16% of acute MS. cases.

Cytotoxicity for cultured nervous tissue +ve in:
Acute phase of MS.
Remission phase of MS.

This table summarizes the findings revealed by electrophoresis, immunoelectrophoresis and cultured nervous tissue favouring the diagnosis of MS.

↑ = increased; ↓ = decreased.

The cytotoxicity of serum and CSF from MS patients for cultured nervous tissue, even if not specific for MS, may greatly help in the diagnosis of the disease, and it would be quite practical for hospital laboratories to establish facilities for the culture of nerve cells.

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DISCUSSION

A./Prof. J. W. Lance: Dr. Lamoureux, could you give us any information about the correlation of the Lange curve with the changes in globulin and the various fractions which you have determined in cases with multiple sclerosis?

Dr. Lamoureux: I have not made any correlation between changes in globulin fractions and the colloidal gold curve.

Dr. B. A. Kakulas: I would like to ask Dr. Lamoureux if he has isolated the factor in the serum which is cytotoxic. Is it an IgG (7S gamma globulin)?

Dr. Lamoureux: I did not. However, may I recall that in 1963 Bornstein, from the Mt. Sinai Hospital in New York, reported the extraction of a globulin fraction from the serum of animals with EAE and also from the serum of patients with multiple sclerosis capable of causing destruction of the cultured nerve cells. This factor is also absorbed from the cytotoxic serum specifically by nervous tissue and not by other tissue. It is then possible that the immunoglobulins may have something to do with the cytotoxic factor.

Dr. Lorenz: I would like to ask Dr. Lamoureux about his controls—whether he used patients with acute or chronic conditions, the same as he described in multiple sclerosis cases, and whether he has chosen cases with a lot of destruction of nervous tissue and so forth?

Dr. Lamoureux: Our controls were sera from 24 normal subjects and cerebrospinal fluid from 24 patients obtained during myelography or pneumoencephalography. These patients were thought to have no organic intrinsic nervous disease; the diagnosis included neurosis, headache and herniated disc. The other control group included 28 patients: 15 with organic neurological diseases other than multiple sclerosis, 5 with organic neurological diseases of uncertain diagnosis and 8 with acute psychosis. We have not tested motor neurone disease. It has been reported that about 50 per cent of sera from patients with motor neurone disease were toxic for cultured nerve cells. We have found in this study that cerebrospinal fluid from patients with chronic and acute myelitis and encephalitis of unknown aetiology were also toxic for the cultured nerve cells.

Dr. Burke: Clinically, there seems strong evidence that lots of cases of the Guillain-Barré polyneuritis type syndrome are immuno-allergic phenomena. I was wondering if you have any among your controls or similar parallel work on the cytoxicity of serum or cerebrospinal fluid from that condition?

Dr. Lamoureux: No, we have not studied patients with this diagnosis.

Dr. L. S. Basser next raised an important issue; namely, whether the myelin-forming cells in the central nervous system were oligodendroglial, as he believed, while Schwann cells were responsible in the peripheral nervous system.

Prof. Kakulas agreed and commented: I think there is a very important issue here apart from just terminology. I think it is perfectly clear that the oligodendrocyte is responsible for myelination within the central nervous system and that the Schwann cell is responsible for myelination without the central nervous system, and that there are points of biological difference, particularly that regeneration will occur outside of the central nervous system. There is a possibility that regeneration does not occur particularly of myelin in the central nervous system, but this is not firmly established, and is by no means to the degree as occurs with the peripheral nervous system. There is a further point in the context of the presentation, and that is that there is probably a tremendous difficulty encountered in the exact identification of cells *in vitro*. It is difficult enough, and I don't think that any neuro-pathologist is satisfied that he is able to identify very accurately the myelination glia that is present in the foetal developing brain which is laying down myelin, and differentiating it from other forms of astrocyte, so that probably there were difficulties encountered here or that the cultures were contaminated by peripheral nervous tissue. I think the point of issue which is very important is the specificity of the reaction you have demon-

strated, and I think that you will agree that other workers in the field have shown very clearly that there is tremendous specificity between the experimental allergic neuritis serum and the experimental allergic encephalitis serum, and that the serum you have tested would (I believe other workers have shown this) not have any effect on central nervous tissue and the reverse is true.

Dr. Lamoureux: That is right. In fact, if you inject an animal with peripheral nervous tissue, you do not produce EAE but peripheral neuritis, and the serum of these animals does not destroy the central nervous tissue. The encephalitogens are different.

Dr. Rail: Could you tell us a little about the effect of ACTH in reducing these globulins that you are referring to?

Dr. Lamoureux: I wish I had an answer to this important question. This study presents the results obtained with only seven cases of multiple sclerosis in the acute phase. The only characteristics of our study were that none of these patients received more than three injections of ACTH; and secondly that we have not been able to demonstrate in these acute multiple sclerosis patients an increase of the gamma globulin in the cerebrospinal fluid. Further work is necessary to clarify this point.

VIRUS-LIKE PARTICLES IN PROXIMITY TO MYELIN IN A CASE OF PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY

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The term "Progressive Multifocal Leukoencephalopathy" (P.M.L.) was suggested by Åström, Mancall and Richardson (1958) for a new clinico-pathological entity which they identified as a demyelinative disorder with unique histological features. Three patients with the disease were described; two were elderly women with chronic lymphatic leukaemia and one was a 42-year-old man with Hodgkin's disease. In addition, attention was directed to five other cases in the new literature where similar neuropathological findings were recorded. Five of these eight occurred on a background of either chronic lymphatic leukaemia or Hodgkin's disease so that it seemed justified to consider that progressive multifocal leukoencephalopathy was chiefly a complication of neoplastic disease of lymphatic tissue. In 1961 Richardson was able to review 22 examples including 10 of his own and 12 others which had appeared in the literature. In this study it became clear that although progressive multifocal leukoencephalopathy was more frequently associated with the lymphoproliferative disorders it also occurred in association with chronic myeloid leukaemia and carcinomatosis.

More recent reports include those of Mancall (1965) and of Richardson (1965). Of the 45 cases now in the literature 21 were associated with lymphoproliferative disease, 7 with myeloproliferative disease, 10 non-neoplastic reticuloendotheliosis (sarcoidosis, tuberculosis, primary hypersplenism, Whipple's disease) and 7 were associated with miscellaneous conditions, including 3 with carcinomatosis, 1 with anthraco-silicosis, 1 with coronary heart disease, with splenomegaly of unknown origin and 1 with hepato-splenomegaly with liver and adrenal necrosis of uncertain cause.

In the same report (Richardson, 1965) described the lesions in the C.N.S. as being widely disseminated and typically demyelinative in type. There was total disappearance of myelin sheaths and relative preservation of axis cylinders. Myelin breakdown into familiar sudanophilic lipids within macrophages occurred. The lesions varied greatly in size, some were barely visible to the naked eye, others occurred as massive foci of myelin destruction involving large parts of the cerebral hemispheres. The appearance of the lesions and the clinically progressive course suggested that the disease advanced by means of enlargement and confluence of small lesions, together with the continuous addition of new ones. The brain stem and cerebellum were affected in many cases but to a lesser degree. However, the typical location of the lesions was in the deeper layers of the cerebral cortex in which necrosis and disappearance of nerve cells regularly occurred as well as the primary demyelinative lesions. A remarkable feature of the histopathology was the presence of gigantic and bizarre astrocytes often showing abnormal mitotic figures. The presence of inclusions in many cells, particularly in oligodendrocytic nuclei, was another unusual finding. Oligodendrocytes enlarged at the periphery of the demyelinative foci and the inclusions were often Feulgen positive. It was suggested that these changes preceded the death of the oligodendrocytes since these cells could not be found at the centre of the lesions where the pathological

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process was more advanced. The most attractive hpothesis was that of Waksman (1961) who suggested to Richardson that the lesions of progressive multifocal leukoencephalopathy could be the result of viral infection of the central nervous system in patients whose immunological responsiveness was impaired by the presence of pre-existing chronic disease.

Considerable support to this theory was provided by the observations of Zu Rhein and Chou (1965) who reported that formalin fixed brain tissue obtained from a patient with progressive multifocal leukoencephalopathy contained virus-like particles when examined with the electron microscope. These particles were invariably found within oligodendrocytic nuclei. They suggested that demyelination resulted from the cytocidal effect of the virus on oligodendrocytes. The diameter of these particles was 33 to 36 μ and showed a para-crystalline arrangement. These observations suggested a resemblance to the Papova group of viruses. Silverman and Rubinstein (1965) confirmed the observations when they examined tissue from another case of progressive multifocal leukoencephalopathy. Howatson, Nagai, Zu Rhein (1965) conducted further observations including the application of negative staining techniques, and brought forward evidence in favour of the virus-like particles belonging to the group of polyoma, vacuolating (SV40) or mouse pneumonitis virus (K). These viruses resemble each other closely and form a subgroup of the Papova viruses.

The present observations were conducted from tissue obtained from a patient with progressive multifocal leukoencephalopathy, complicating lympho-sarcoma and reported by Mercy Sadka in 1963. The patient was a 49-year-old musician who suffered initial symptoms of weight loss, abdominal pain and lymph node swelling. A biopsy of a cervical lymph node disclosed lymphosarcoma. He was treated with radiation with a temporary good result, but relapse occurred six months before his final admission. He was then treated first with nitrogen mustard and later with prednisolone because of the presence of haemolysis. Initially, response to this treatment was satisfactory, but a recurrence of the anaemia followed two months later and was treated with further radiation to the spleen and by blood transfusion. Shortly afterward weakness of the right arm appeared and on neurological examination six weeks later he was found to be vague in giving his history and he veered inexplicably from apprehensive depression to inappropriate jocularity. He was then fully oriented and cooperative. There was flattening of the right nasolabial fold and severe flaccid weakness of the right arm without sensory changes. The tendon reflexes were decreased in the right arm, but brisk elsewhere. The plantar reflexes were normal. The cerebrospinal fluid was normal in all respects. His condition gradually deteriorated and three weeks after the initial neurological examination spasticity developed in the weak right arm, with exaggerated reflexes. Replies during sensory examination were unreliable. Testing showed defects in memory and judgement and calculating ability. An electroencephalogram was diffusely abnormal with somewhat more pronounced changes on the left. Dysarthria and an obvious dysphasia then developed and he died about three months after the onset of the neurological illness and 2½ years from the beginning of the lyphomatous disease. Postmortem examination showed severe right upper lobe pneumonia with little gross evidence of lyphoma which, however, was evident microscopically in liver, spleen, para-aortic lymph nodes and bone-marrow. Examination of the nervous system disclosed multiple bilateral lesions, demyelinative in type and situated within the deep cortical layers and in adjacent white matter. These were generally 1-3 mm in diameter but many were confluent, being most numerous in the frontal and parietal lobes. Smaller lesions were present in the basal nuclei of the hemispheres but none were present in the cerebellum, brain stem or spinal cord. The lesions microscopically revealed myelin loss (Fig. 1) with relative preservation of axons. The oligodendrocytic nuclei were swollen, deep staining and many contained eosinophilic inclusions. Monstrous astrocytes were numerous and these showed bizarre hyperchromatic nuclei often with abnormal mitotic figures (Fig. 2).

Tissue for electron microscopic study was available from the right frontal cortex and subcortical white matter. This tissue had been pre-

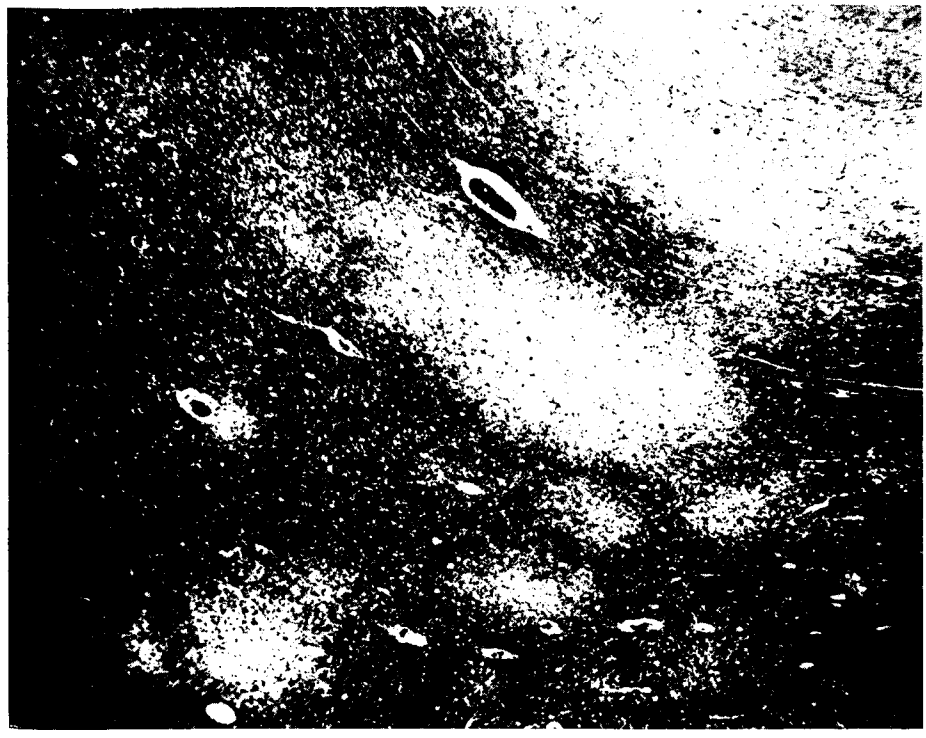

FIG. 1—Subcortical demyelinative foci, frontal lobe. Weigert-Pal. × 29·5.

served in formalin since the time of the necropsy six years before. A number of the lesions were dissected, cut into small pieces, washed in phosphate buffer and fixed in 1 per cent osmium tetroxide. Following dehydration in graded solutions of ethanol, the tissue was embedded in Araldite. Sections were cut on a Huxley microtome using glass knives, stained with lead and examined on a JEM T6 electron microscope at an accelerating voltage of 60 KV. Small pieces of normal cerebral tissue from a brain kept in formalin for five years were treated similarly and used as controls.

In the electron micrographs an appreciable degree of preservation existed. Cell types could be identified from their morphological appearance or from contiguous thick sections which were examined with the light microscope after staining with methylene blue.

The significant finding was the presence of virus-like particles in a number of the electron micrographs obtained. Their diameter varied from 32 μ to 35 μ. They were arranged in a paracrystalline manner and less often in scattered clumps. These virus-like structures were invariably found in the vicinity of the cytoplasm of oligodendrocytes or in intimate association to myelin sheaths (Figs. 3-6). When observed in close relation to myelin, the particles were often arranged in a single row at the periphery of the myelin whorl. Virus-like particles were not seen in nuclei of oligodendrocytes nor were they present in close association to any other cell type. No structure resembling virus aggregates could be found in sections of the control brain, thus the possibility of artefact was excluded.

The virus-like particles demonstrated were similar in size and shape to those described by Zu Rhein *et al.* (1965), which were found to resemble the Polyoma-SV 4-K subgroup of the Papova viruses. However, it may be significant that in the present material the virus-like particles were extranuclear and exhibited a tendency to be closely aligned along the periphery of myelin sheaths, possibly within the

outermost portion of the whorl of oligodendrocytic cytoplasm. This observation has not been made previously and is possibly of great pathogenetic significance since the lesions of progressive multifocal leukoencephalopathy have an important demyelinative component.

Morphologically, progressive multifocal leukoencephalopathy is quite distinct from other demyelinative diseases such as multiple sclerosis, acute disseminated encephalomyelitis or experimental allergic encephalomyelitis. In these, although the lesions are typically demyelinative in character, an inflammatory cellular infiltrate accompanies the changes and inclusions are not observed. Nevertheless a point in common is the disappearance of the oligodendrocytes from the demyelinative lesions in these conditions. The astrocytic changes in progressive multifocal leukoencephalopathy together with the existence of mitotic figures is strongly reminiscent of the bizarre forms encountered in glioblastoma multiforme. However, there is no sign of a truly malignant process, i.e. the development of a mass lesion or of infiltration. The astrocytic changes also resemble those produced by irradiation as first suggested by Åström *et al.* (195), and they bear a close resemblance to altered cells in tissue culture. Features of cells transformed *in vitro* include heteroploidy, numerous mitoses, cellular pleomorphism and the property of continued and unlimited subculture. Transformation *in vitro* may be induced by a great variety of agents including radiation and viruses, especially SV40 (Hayflick, 1964). As may be expected many altered cells do in fact show the characteristics of true malignant neoplasia *in vivo*. The changes in the astrocytic nuclei in progressive multifocal leukoencephalopathy may represent this change in modified form, i.e. the presence of cells *in vivo* which have the morphological characteristics of transformed cells *in vitro* but lacking in malignant biological behaviour, i.e. seemingly anarchic proliferation. Up to the present, growth properties *in vitro* of such cells have not been explored. Whether the

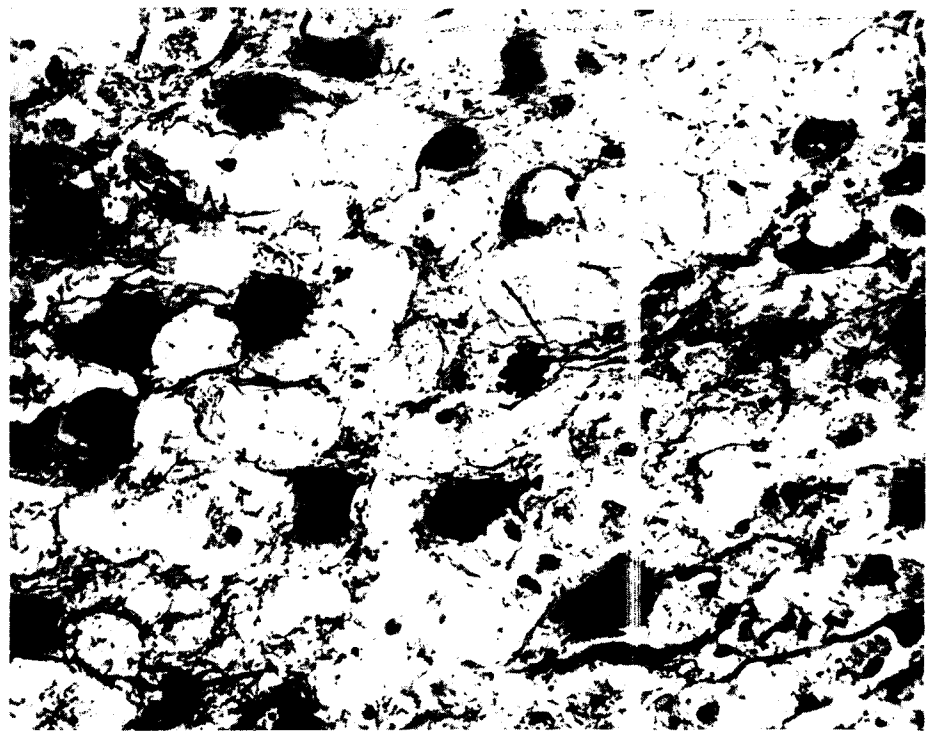

FIG. 2—Large, atypical astrocytes showing an abnormal mitosis (right of centre of the field). Haematoxylin and eosin. × 415.

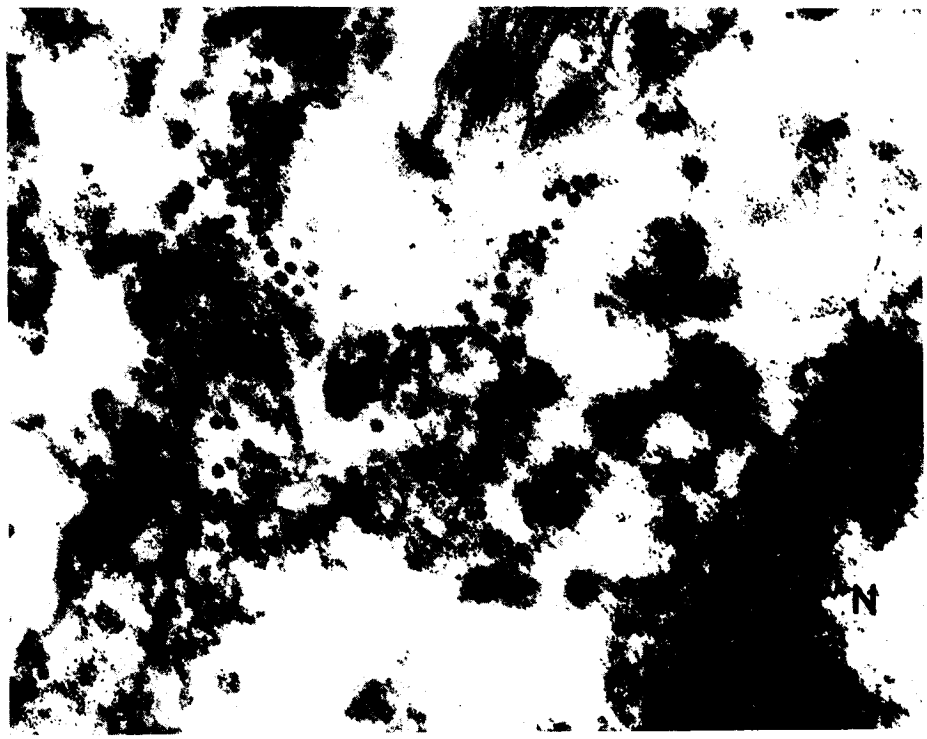

FIG. 3—Scattered virus-like particles (V) near the nucleus of an oligodendrocyte (N). Magnification 34,000 ×.

virus in progressive multifocal leukoencephalopathy is exogenous or whether it be a latent virus which has been stimulated by the underlying disease process or by some form of medical manipulation remains to be defined.

SUMMARY

Virus-like particles measuring 32-35 μ in diameter were found in the brain of a patient with lymphosarcoma and progressive multifocal leukoencephalopathy. Additional to confirming previous observations of similar structures in this disease, the presence of virus-like particles in close proximity to myelin is reported.

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DISCUSSION

Mr. D. A. Simpson: I have one very small technical question to ask arising out of that very fascinating case. We have recently had a child at the Adelaide Children's Hospital who may have been somewhat similar; who also had a long drawn-out illness of reticulo-endothelial type, who had similar histological appearances in the brain, but I am afraid we interpreted it as neoplastic. I should like to ask, Sir, whether these very flagrant glial changes could, in Dr. Kakulas's opinion, be mistaken for a glial tumor. Is this a possible diagnostic error that may arise when small fragments of brain are examined in smears or allied method?

I think that it does become very difficult sometimes if one relies upon cytological criteria in the diagnosis of malignancy. The existence of mitotic figures in a condition which is clearly non-neoplastic is quite remarkable, because up to the discovery and description of this disease, it was considered that astrocytes multiplied by amitotic division and that mitoses were confined to astrocytomas or more malignant varieties of these tumors. I think that for this reason one has to turn to the more basic criteria of malignancy, and to determine whether or not there is in fact a massed lesion, and whether or not the pathological reaction has infiltrating qualities. Now, in progressive multifocal leukoencephalopathy, these criteria do not hold. There is no development of a mass lesion, and there is certainly no sign of infiltration. I think there is

FIG. 4—Paracrystalline array of virus-like particles near a myelin sheath. Magnification 65,000 ×.

FIG. 5—Virus-like particles near the myelin sheath of an axon (A). Magnification 52,300 ×.

FIG. 6—Myelin sheath of an axon with a single row of virus-like particles at its periphery. Magnification 53,000 ×.

a very interesting parallel to *in vitro* transformation, where cells do take on cytological features of malignancy but where, again, they do not necessarily behave as malignant tumors *in vivo*, although usually there is such a relationship.

In the patient that you describe, I think that it would depend upon whether or not a mass lesion did in fact exist, and whether or not the other components of the lesions of progressive multifocal leukoencephalopathy were present. This would depend not only on inclusions, since inclusions will occur with tumors, but whether or not the lesions are strictly demyelinated, and whether the enlargement of the oligodendrocytes occurred, and whether the granule cells of the cerebellum showed the characteristic changes which Richardson now has come to believe as being typical of the disease, where these granule neurones undergo enlargement. It would be quite remarkable and very important to determine whether or not your patient —a two-year-old child?—did in fact have this disease, because I believe that it has not been described in children. I would like to see the material if I may.

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