M. J. Cadie

Proceedings of the Australian Association of Neurologists

VOLUME 8, 1971

Published by

The Australian Association of Neurologists

Editor:
E. GRAEME ROBERTSON
with the collaboration of
JOHN BILLINGS
GEORGE SELBY
JOHN GORDON

Registered at the G.P.O., Melbourne for transmission by post as a book

Business and Subscriptions Manager:
DR. A. C. SCHWIEGER

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Melbourne, Victoria
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MUSCLE DISEASES

Proceedings of an International Congress on Muscle Diseases,
Milan, 19-21 May, 1969

Editors: J. N. WALTON, N. CANAL and G. SCARLATO

This collection of final manuscripts of paper presented at the 1st International Congress on Muscle Diseases, is an up to date source of current research in primary myopathies and neuromuscular conditions.

Although many of the papers are brief as necessitated by the large number of participants, no essential information is omitted. For this reason, and because of the extensive bibliographies, a ready source to the modern literature on the subject, is provided.

The participants include the world's most outstanding authorities with representation from the major schools of America, the United Kingdom, France, Germany, Italy and Japan. The papers are conveniently classified into the following sections: Histology and Histochemistry, Electronmicroscopy, Electrophysiology, Biochemistry, Pathology and Genetics. In addition, the papers presented at a round-table discussion, are included.

The monograph, therefore, serves the purpose of collecting into one volume, all the significant current research, achieving two aims—firstly, the recording of progress to date and, secondly, stimulating and directing further research.

The large number of papers give witness to the much increased interest in this expanding field and the consequent explosive increase in knowledge. Thus, further International Congresses on Muscle Diseases are planned for Australia, 1971, and Newcastle upon Tyne, 1974.

The work is well produced, and the illustrations are of a high standard. It can be recommended to research workers in the field, and to hospital and medical libraries.

MEMORY

A. K. McINTYRE*

It is a great pleasure to have the opportunity of speaking at this combined meeting of our Associations in Dunedin, of which I have so many pleasant memories. "Memory" is the subject of my talk, and it is perhaps appropriate to recall that I began some work here 20 years ago in collaboration with John Eccles on a possible synaptic basis of learning (Eccles and McIntyre, 1953; McIntyre, 1953), the relevance of which to the establishment of memory-traces has recently been revived by some results of research by colleagues in my present department (Watts and Mark, 1970). There would be general agreement among students of brain science that the nature of learning and memory constitutes one of the most baffling puzzles in the whole field of brain function, and there is no need for me to remind this audience of the crucial importance of these processes to function at all but the very simplest levels of nervous organization. However, before looking at the problem of neural memory in the light of recent and current research, I would like to touch on some aspects of the phenomenon of memory in a broader context.

What do we mean by the term "memory"? There is no need to define the personal experience of conscious memory as it presents itself, uniquely, to each one of us. But the word nowadays has much wider usage, including many non-biological examples. For example, artifacts such as books, photographs, tape-recorders and computers make possible the recording of events or ideas directly or via symbolic representations, including written or spoken words, and can thus be regarded as having the attribute of memory. The commonplace reference to "memory-capacity" in the specifications for computers illustrates well this wider usage. Though non-biological, this form of memory through cultural artifacts is nevertheless of the greatest importance to mankind as a species—our species-memory or cultural heritage, to which Medawar (1960) has applied the term *exosomatic,* as opposed to *endosomatic* or genetic heredity. Leaving this aside, however, direct biological memory undoubtedly embraces more than the neural phenomenon which is its most immediate manifestation. A broad definition of biological memory may be given as follows: any mechanism that enables living organisms to store some aspect of experience, which may subsequently alter form or function.

Within this broad framework, three kinds of biological memory can be recognized: genetic, immunological and neural. *Genetic* memory is, of course, the long-term biological memory of a species; its endosomatic inheritance. Over immense stretches of time, a species "learns" from exposure to environmental influences and either changes appropriately—that is, adapts and survives, or perishes. The basic mechanism of this remarkable process is, of course, the handing on of information from parent to offspring over countless generations, the information being chemically coded in the incredibly compact DNA filing system.

Immunological memory is the other non-neural example, whereby an individual organism "learns" to make appropriate antibodies to foreign substances or antigens, so that on subsequent exposure, the invading agent can be dealt with by rapid mobilization of the specific antibody. The spectacular recent advances in elucidating the nature of immune responses and the basic question of how the immunological defences are able to distinguish between foreign cells or substances and those which are part of the organism itself emphasize the complexity of these mechanisms, and are well summarized

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in recent publications such as Burnet's *"Self and Not-Self"* (1969).

Neural memory includes much more than its personal manifestation or "conscious recall" aspect. The phenomenon also embraces, for example, the development of motor skills, largely subconscious in nature, and the building up of the perceptual framework of the body itself in relation to its surroundings: the body-image so essential to perception and consciousness. The mechanisms underlying neural memory—that is the nature of the processes conferring the ability to store and retrieve neural information—have so far eluded clearcut identification, and their elucidation must surely be one of the highest-priority aims of those studying brain function. Other equally puzzling and important, and by no means unrelated questions, are the nature of the processes determining growth and development of the nervous system, the "blueprint" as it were which permits the vast assemblage of cells, processes and specific interconnections to take place in the proper manner; and the nature and significance of consciousness.

The baffling complexity of central nervous structure has, of course, been known since the light microscope first revealed its cellular nature. Estimates of nerve-cell numbers in the human brain are of the order of ten to twelve thousand million, and the surface of each cell is encrusted with many hundreds of thousands of synaptic endings of other cell processes. Consider, for example, the great number of thorny projections from dendrites of Golgi-stained cortical pyramidal cells, as in Cajal's classical pictures: each is now known to receive an excitatory presynaptic ending, and there are many more synapses, inhibitory as well as excitatory, elsewhere on the dendrites and cell body. Sholl (1956) has estimated that a single incoming fibre to the cortex may branch to connect with up to 5,000 cortical cells spread through about one tenth of a cubic millimetre; and in addition to these structures are the myriads of glial cells with their processes closely investing almost every part of the nerve cells and fibres themselves, except where they make synaptic contact. Imagination boggles in attempting to visualize the fantastically complex packing of these astronomical numbers of overlapping nerve cell bodies with intermeshing dendritic, axonal and glial-cell processes in the cerebral cortex and other parts of the brain, and the vast numbers of potential pathways within this unique three-dimensional network which must be available for signal-transfer.

It is, of course, generally accepted that the neuronal basis of all aspects of behaviour from simple reflex activities to the most complex cerebral transactions must be specific patterns of interactions between populations of nerve cells, patterns involving impulse-propagation and synaptic interactions in nerve cell circuits often of fantastic complexity; processes in addition to the well-known propagated impulses, after potentials, and synaptic inhibitory and excitatory intercellular transactions (for example, in glia cells), may also participate in creating and sustaining such patterned activity. For the tremendous range and variety of learned behavioural responses a correspondingly vast array of possible alternative pathways must be expected. Recent electron microscope studies have shown at the ultrastructural level (for example, Cragg, 1967) a far greater richness of detail and complexity of synaptic interconnection than could have been conceived as a result of light microscopic examination alone. His careful counts of numbers of nerve cells and synaptic profiles in a given volume of tissue, and of size and density of synaptic vesicles, now suggest that there might be a great redundancy of connections and pathways for even the most extravagant notions of range and variety of circuit-paths in the brain; in other words that there could be a vastly greater number of synaptic contacts between the nerve cells of a given brain than are necessary for mediating its full range of activities.

This notion of redundancy of synaptic connections in the genetically determined structure of the brain is the basis of a new approach to the fundamental basis of memory towards which we are groping.

Hypotheses of Neural Learning and Memory

Almost all theories acknowledge that in the process of learning, some lasting change in the functional state of nerve cells, and especially their synaptic linkage-points, must be brought about so that particular spatio-temporal patterns of impulse-traffic, etc., become established

by refinement and repetition during the learning procedures, and that recall depends upon virtually the same patterns becoming reactivated, or, as it were, re-played like a record. What constitutes the ultimate structural and functional basis of this record or "engram", to use Lashley's term, still eludes us, despite currently popular notions of specific molecular patterns (nucleic acid or protein) in which the vast potential information-content of such molecules is postulated as the basis of long-term neural memory, as is now established for genetic information-storage and transfer. There is indeed much evidence pointing to the involvement of protein synthesis in learning, but so far no theory has been able to show how nerve-cell activity-patterns could be re-coded chemically and later "read out" to re-create the original pattern of nerve impulses, etc., associated with recall. Two aspects of learning mechanisms are now, however, gaining general recognition. Firstly, despite the known special involvement of particular brain regions such as the hippocampus in the establishment of memories, it is likely that most memory-patterns involve activity in circuits which engage vast numbers of neurones not only spread widely in the cerebral cortex itself, but probably throughout many sub-cortical structures as well. Furthermore, individual nerve cells are likely to be involved in very many different memory patterns, though doubtless playing rather different roles in each. The permutations and combinations in a population of ten to twelve thousand million units would permit a richness of possible different patterns probably far exceeding the total number of electrons in the universe.

Secondly, it is firmly established that learning takes place in two stages, a short-lasting initial phase of temporary storage, which does not survive episodes of deep anaesthesia or other depressing procedures such as concussion, and a later long-term phase of consolidated memory which persists after severe temporary disruption of central nervous activity. It is this second phase of long-term memory which is believed to depend ultimately upon chemical, molecular changes, themselves somehow wrought by the processes operating during the short-term phase.

The Basis of Short-term Memory

The vulnerability of this phase of memory to procedures which disrupt the normal ceaseless shuttling of impulse-patterns in the brain, such as anaesthesia, injury, convulsive episodes or hypothermia, strongly suggests that a continued reverberatory circulation of specific spatio-temporal impulse-patterns constitutes its essential basis. It is not unreasonable to postulate that a pattern of this kind set up during learning might after a few repetitions become self-sustaining as a result of the reiterated action, excitatory or inhibitory, at the particular synapses involved in the pattern, if the transmitting efficacy of impulses at each synaptic junction in the nerve-cell chain were altered by repetitive activation.

Lloyd provided the first experimental demonstration that enhancement of transmitter action at synapses in the central nervous system can occur as a result of repetitive presynaptic activity (Lloyd, 1949), and this phenomenon (termed post-tetanic potentiation) was shown to last for several minutes after a brief presynaptic tetanus. We showed in further experiments (Eccles and McIntyre, 1953; McIntyre, 1953) that abolition for periods of days or weeks of presynaptic impuse-input in simple spinal reflex arcs, by operative section of dorsal roots distal to their ganglia, resulted in depression of synaptic transmissive efficacy which could be partly restored for long periods (hours in some instances) by a few episodes of tetanic stimulation of the silenced afferent fibres. These and other related studies provided a possible basis for the short-term phase of memory, for it is reasonable to postulate a basic similarity of mechanism at spinal synaptic junctions and those of higher levels. Recently, very striking and enduring post-tetanic potentiation has been demonstrated in a synaptic system in the hippocampus (Bliss and Lømo, 1970); even brief bursts of presynaptic stimulation at 15/sec led to striking potentiation of synaptic action enduring for hours.

Current experiments by Dr. Mark and Mrs. Watts in my department also provide support for a role in short-term memory of a mechanism akin to post-tetanic potentiation, which is known to be related to the cell-membrane ionic mechanisms responsible for impulse-generation

and recovery processes. They have made use of a simple learning situation in groups of day-old chicks, each of which is taught to peck at a bright object (a banana plug dipped in water) and then presented with a similar plug dipped in methylanthranilate, at which the chicks peck once, and react with obvious distaste and aversion. The retention of this learned task—not to peck at a banana plug after this single unpleasant exposure—is measured by simple methods and plotted, and the effects on this retention of injecting into the chicks' forebrain small volumes (0·025 ml) of various substances or saline controls have been studied. The experiments have shown that substances such as ouabain, with actions on membrane ionic mechanisms, interfere with short-term retention, but have no effect on the conversion of short-term to established memory. On the other hand, a substance such as cycloheximide, which inhibits protein synthesis but has no action on membrane ionic processes, does not interfere with the early phase of memory-retention, but prevents its consolidation as long-term memory (Watts and Mark, 1970).

These simple but elegant experiments not only demonstrate a basic difference between the two stages in establishment of a memory, but strongly support the notion that the short-term phase involves repeated circulation of impulses in a pattern, the reverberation perhaps persisting because of synaptic changes like those responsible for post-tetanic potentiation (McIntyre, 1953; Kandel and Spencer, 1968). During this time, the more permanent changes which lead to the long-term memory engram are gradually brought about, by unknown mechanisms involving protein synthesis.

Mechanism of Long-term Memory

I return briefly to consideration of the two other examples of long-term biological memory —genetic and immunological—and their basic mechanisms. Until the general acceptance of Darwin's hypothesis of natural selection to explain the evolution of species, it seemed obvious that in the course of evolution a species "learned", by an *instructive* process, to adapt. This was the Lamarckian notion that, for example, constant stretching of a pre-giraffe's neck to reach higher and tastier leaves would somehow modify its germ cells in the direction of neck-lengthening. Today scarcely anyone questions the view that evolutionary mechanisms are not instructive but *elective* (Lederberg, 1958). Vast pools of possible DNA coded hereditary characteristics, arising through mutation and successive recombination, are present in the germ cell pool of a species, from which those with fitness for survival are selected.

Until about 10 years ago, immunological memory was also generally thought to be an instructive process, the immunocytes being pictured as learning to make a variety of antibody molecules of appropriate configuration to fit a variety of different foreign substances or antigens. Since the appearance of Burnet's clonal selection hypothesis (Burnet, 1959), it has become apparent that immunological memory is also based, at least in large measure, upon selection and stimulation, by contact with an antigen, of particular cells with the in-built capacity to make the appropriate antibody, from a vast array of immunocytes each with a different inbuilt antibody-making programme.

To return to neural memory, it occurred to us in looking at the staggering numbers of synaptic contacts shown by electron micrographs of the brain, that perhaps this vast number of possible routes of communication between the nerve cells could be far in excess even of those required to mediate all the reflex and learned responses of a lifetime. On this view, the genetically determined brain structure has all possible potential pathways for any conceivable neural patterned activity, just as an individual's population of immunocytes has pre-formed, as it were, information for making all conceivable patterns of antibody molecules. Could the mechanism of memory in the brain depend similarly upon selection of pre-formed patterns, by depression of the transmitting efficacy of presynaptic endings with effects inappropriate to the goal of each episode of learning? This would imply a basically similar mechanism for the different kinds of biological memory, all three being essentially *elective* in nature (McIntyre, 1969; Mark, 1970). A somewhat similar view has been propounded by J. Z. Young (1966), except that his hypothesis envisages the "turning off" of whole nerve cells by inhibition, rather than depressing or abolishing the functional effectiveness of individual

presynaptic endings with inappropriate function. Our suggestion has the merit of providing a far wider range of possible memory-patterns.

One difficulty in accepting this notion is that all the vast numbers of presynaptic endings seen in electron micrographs look the same, in the sense of containing synaptic vesicles, membrane modifications, and so on. If our view of memory is correct, there seems to be no ultrastructural correlation with degree of synaptic effectiveness of endings. Quite recently, Dr. Mark and his colleagues have demonstrated, by means of successive re-innervation experiments in the eye muscles of fish, that synaptic endings from inappropriate motor axons (for example, on denervated superior oblique muscle fibres by inferior oblique motoneurones) can be made ineffective within hours of the arrival of growing axon terminals from their own (trochlear) motoneurones. Such "turning off" of previously effective nerve terminals has no effect on their ultrastructural appearance (Mark, 1970; Marotte and Mark, 1970). Fish extraocular muscle fibres are normally innervated by a number of nerve terminals, unlike mammalian muscle fibres. In this respect, therefore, they resemble central neurones, and can be regarded as models of such cells. Mark and Marotte's experiments at least suggest that normal-looking synaptic terminals in the brain might be functionally depressed or even totally lacking in transmitter action.

This hypothesis of a basically elective mechanism for long-term memory is obviously incomplete and still largely speculative. For example, there is as yet no clue as to the nature of a feedback system which might signal goal-achievement in the learning task, nor any indication of how the postulated early reverberatory patterned impulse-flow could lead to establishment of the long-term memory engram. Perhaps the enduring change may depend upon synthesis and release of specific proteins from the active terminals, with antigen-antibody-like reactions with the postsynaptic cell and other terminals ending upon it, and the degree of changed synaptic effectiveness might be a finely graded rather than an all or nothing process. An elective hypothesis of this kind for the mechanism of neural memory has the attractiveness of bringing into line, despite their superficially quite different nature, all three varieties of biological memory.

ACKNOWLEDGMENTS

I am indebted to my colleagues Drs. Cragg, Mark and their co-workers in my department for permission to cite their results, and to the National Health and Medical Research Council, the Australian Research Grants Committee and the Van Cleef Foundation, who have generously supported our work.

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KURU AND CREUTZFELDT-JAKOB DISEASE: CLINICAL AND AETIOLOGICAL ASPECTS

MICHAEL ALPERS and LEONARD RAIL

INTRODUCTION

Kuru is the model disease for slow virus infections in man, and for the new class of transmissible degenerative neurological diseases. This class now includes Creutzfeldt-Jakob disease.

Both diseases have been transmitted to the chimpanzee (Gajdusek, Gibbs and Alpers, 1966; Gibbs *et al.*, 1968). This paper will present our own findings on the clinical aspects of kuru and of the two diseases in the chimpanzee, and will then examine some of the aetiological implications of these slow virus diseases.

KURU IN MAN

The clinical picture of kuru may be divided into four arbitrary stages—

(1) prodromal
(2) stage 1
(3) stage 2
(4) stage 3

Prodromal stage

Before the first sign of neurological defect a history of vague pains in the limbs, headaches, gastrointestinal symptoms or malaise is often obtained; occasionally patients complain of diplopia.

Stage 1

The earliest neurological signs are instability of posture and ataxia of gait. On standing there is a wavering of the body and head with dancing of the dorsal tendons of the feet. Gait may appear normal on level ground but there is a loss of the sure-footedness so characteristic of the normal New Guinean in less favourable terrain. Gradual deterioration occurs—the gait becomes wide-based and staggering. Falling occurs with increasing frequency and after a few months a stick is used to help maintain balance.

There is no tremor of the hands or legs in the early stages. Finger-nose and heel-knee tests are performed readily. The shivering tremor referred to in several descriptions is difficult to elicit clinically but may be observed occasionally, especially in the colder weather.

As the disease advances increasing disturbance of the anti-gravity muscles is noted. A tremor at the rate of 5-6 per second occurs whenever unsupported movements are made.

When the patient stands, tremor of the agonist and antagonist muscles of the thigh and leg is noted. At this stage efforts to place the fingers on the nose or finger-finger tests result in a coarse rhythmic tremor which increases as the finger reaches its target.

There is little to find on examination of the cranial nerves. Nystagmus is not present early. Later, coarse jerks and irregular movements of the eyes on gaze are noted but no sustained nystagmus. The extra-ocular muscles do not develop individual palsies but strabismus is not uncommon in the advancing stages of the disease; occasionally it is present from the very first.

Vision and hearing are unaffected throughout the course of kuru. Convulsions do not occur. No sensory deficits are found. Electroencephalographic and electromyographic changes throughout the disease are minimal.

Stage 2

The second stage of the disease is reached when walking becomes impossible without support by one or more helpers. In other words the upright posture is impossible to maintain. In spite of the loss of effective voluntary move-

ment due to ataxia and tremor, muscle strength is preserved although poorly sustained.

The patient seeks to steady herself by sitting with legs extended and the arms gripping the body or head to minimize tremor. The muscular tone is decreased or normal in the early stages but as the tremor increases a rise in tone is commonly found. The type of increased tone varies but is usually plastic rather than spastic in type. Tendon reflexes which are at first normal or pendular commonly become increased towards the end of the second stage, and clonus at the ankle and knee may be elicited.

Stage 3

The third stage is accompanied by increasingly impaired voluntary movement. The patient is still capable of pulling herself to a sitting posture with the aid of a stick driven into the ground or a bar over the bed, but is unable to maintain a sitting posture without these aids. Marked tremor of the head, body and limbs occurs. A child or other relative will spend hours supporting the patient in a more comfortable position and in feeding her. When she is bed-ridden, contractures of the legs and arms commonly occur.

At this stage rigidity of a cogwheel or plastic type may be noted. Tendon reflexes are raised with clonus in many instances. Speech is so dysarthric as to be understood with greatest difficulty. However, even in these advanced stages, answers to questions are usually accurate and obtained with little delay. Dysphagia is severe and progressive. Dementia is difficult to demonstrate. Incontinence occurs in these later stages but it is not easy to establish whether this is due to sphincter problems or to difficulty in obtaining help for being carried outside. There may be extensive decubitus ulceration.

Death finally occurs from starvation, intercurrent infection or bulbar paralysis. The average duration of the disease from first symptoms to death is 12 months, with a range of 3 months to 2 years.

Conclusion

It is not an easy matter, it seems, to describe the clinical features of a new disease, for while all observers are agreed that kuru is remarkable in the uniformity of its clinical features, there is, at first glance, a notable lack of uniformity in the published clinical descriptions. However, if the descriptions of Gajdusek and Zigas (1959); Simpson, Lander and Robson (1959); Rail (1962); Alpers (1964); Hornabrook (1968); and Hornabrook and Field (1969) were mixed together and distilled, the result, we believe, would not differ very much from the brief description just presented. Kuru is a disease confined to the central nervous system, and involves the cerebellum primarily. As the disease progresses—and this is assessed by the severity of the cerebellar signs—clinical evidence of other motor system involvement becomes apparent.

This makes sense, too, pathologically. The work of Klatzo, Gajdusek and Zigas (1959), Fowler and Robertson (1959), Kakulas, Lecours and Gajdusek (1967), and Beck *et al.* (1968) indicates prime involvement of the cerebellum, especially the palaeo-cerebellum. But in addition there are widespread neuronal changes throughout the brain, especially in the striatum, thalamus, limbic system, hypothalamus and brain stem; and although the cerebrum and spinal cord are involved to a much lesser extent, no part of the central nervous system can be considered free of the disease.

This means, too, that in the later stages of the disease there is a richness and rapid progression of clinical signs not brought out in our brief description. These, however, are short-lived and variable. The standard clinical pattern of the disease is remarkably uniform, extending across all age groups, though the course tends to be more rapid and florid in younger patients.

Has this clinical pattern changed at all in the period in which kuru has been observed? Considering the striking changes that have taken place in the epidemiology of the disease since 1957, we were particularly interested in this question. As a result of our visit to New Guinea in 1969, and examination of patients and our previous records, we have found no evidence of change in the basic clinical pattern of kuru over the previous decade.

Kuru in the Chimpanzee

Kuru in the chimpanzee, as in man, is first manifested by postural instability and ataxia. Titubation is an early sign. The animals walk in a clumsy fashion; their dexterous grasping

movements when climbing around the cage begin to deteriorate. There is a tendency to miss when gripping an overhead bar, necessitating a second attempt. Increase in disability occurs inexorably—tremor of the arms and legs becomes apparent during feeding and standing, and the animal will remain huddled in a corner of the cage.

The facial appearance changes—the lips become pendulous and the face lacks its usual animated expression. Piloerection readily occurs. Some cases show exaggerated grimacing and an associated high-pitched fearful scream. A characteristic sign is the picking up of food directly into the mouth rather than taking it first with the hands.

In a few months, increasing difficulty results in inability to progress—the animal sprawls with its limbs at various angles and will feed only when food is held to its lips. As in the human subject there is no lateralization of defect but a uniform bilateral disturbance. Tone changes—hypotonia and hypertonia—are seen during the evolution, and clonus is not uncommonly noted. Occasionally there is strabismus. Decubitus ulcers may occur.

The course of the disease, from the first signs to a moribund state, varies from 3 to 9 months. Most animals have been killed for experimental purposes long before the full clinical evolution of their disease.

Creutzfeldt-Jakob Disease in Man

Since the first descriptions by Creutzfeldt in 1920 and Jakob in 1921, Creutzfeldt-Jakob disease (or, if you like, Jakob-Creutzfeldt disease) has grown to include many clinical and pathological variants. May (1968) lists 20 synonyms and 4 basic clinical forms. The essential clinical features are presenile dementia, with motor signs, electroencephalographic changes and a subacute progressive course. Primary pathological changes are confined to the grey matter and consist of neuronal loss and degeneration, proliferation of hypertrophic astrocytes, and spongy change; the characteristic distribution involves the neo-cortex, striatum, thalamus and, in some cases, neocerebellum.

No special pathognomonic features occur, as in Alzheimer's disease or Pick's disease, although chromatolytic swollen neurones, first described by Jakob (1921), are sometimes found. In view of the fact that this distinctive neuronal change was frequently seen by Beck *et al.* (1969) in the brains of experimental animals, it may in time come to assume more specific significance (Fig. 1), though similar cells have also been seen in chimpanzees with experimental kuru (Beck, personal communication).

Unlike kuru, Creutzfeldt-Jakob disease is rarely familial, although at least 3 family aggregations have been described. Its onset is usually in middle age, but if, as Crompton (1968) suggests, we include Alpers' disease as a clinical variant of it, then the age range is as wide as in kuru. Unlike kuru, where adult males are relatively free of the disease, in Creutzfeldt-Jakob disease males and females are equally affected at all ages. The duration of disease is comparable to kuru, the mean varying between 1 and 2 years in different series, and the median duration less than 12 months, though the range is a little wider.

Two extremes of the syndrome are described. One has a rapid progression over some months with dementia, myoclonus and epileptic phenomena, with marked EEG changes, and often including ataxia. It is characterized pathologically by widespread status spongiosus, remarkable hyperplasia of astrocytes and neuronal degeneration and loss. This form is frequently called spongiform encephalopathy. The other extreme is more slowly progressive, with dementia and motor system disease which may be cortical, striatal, nigral or spinal or some combination of these four. This form is characterized pathologically by neuronal loss, astrocytosis and gliosis and, in some cases, minor status spongiosus. The pathological differences are attributable to the differences in time course of the two extremes; and with regard to status spongiosus, Beck *et al.* (1969) have discovered that the spongy change clearly evident in a biopsy may disappear by the time of autopsy. Since cases are described which fit into the middle of this spectrum there seems good reason to include them all under a single syndrome. Following the *Lancet* of 1970 and Beck *et al.* (1969) we shall call this wide syndrome Creutzfeldt-Jakob disease. We should point out, however, that some British neurologists insist

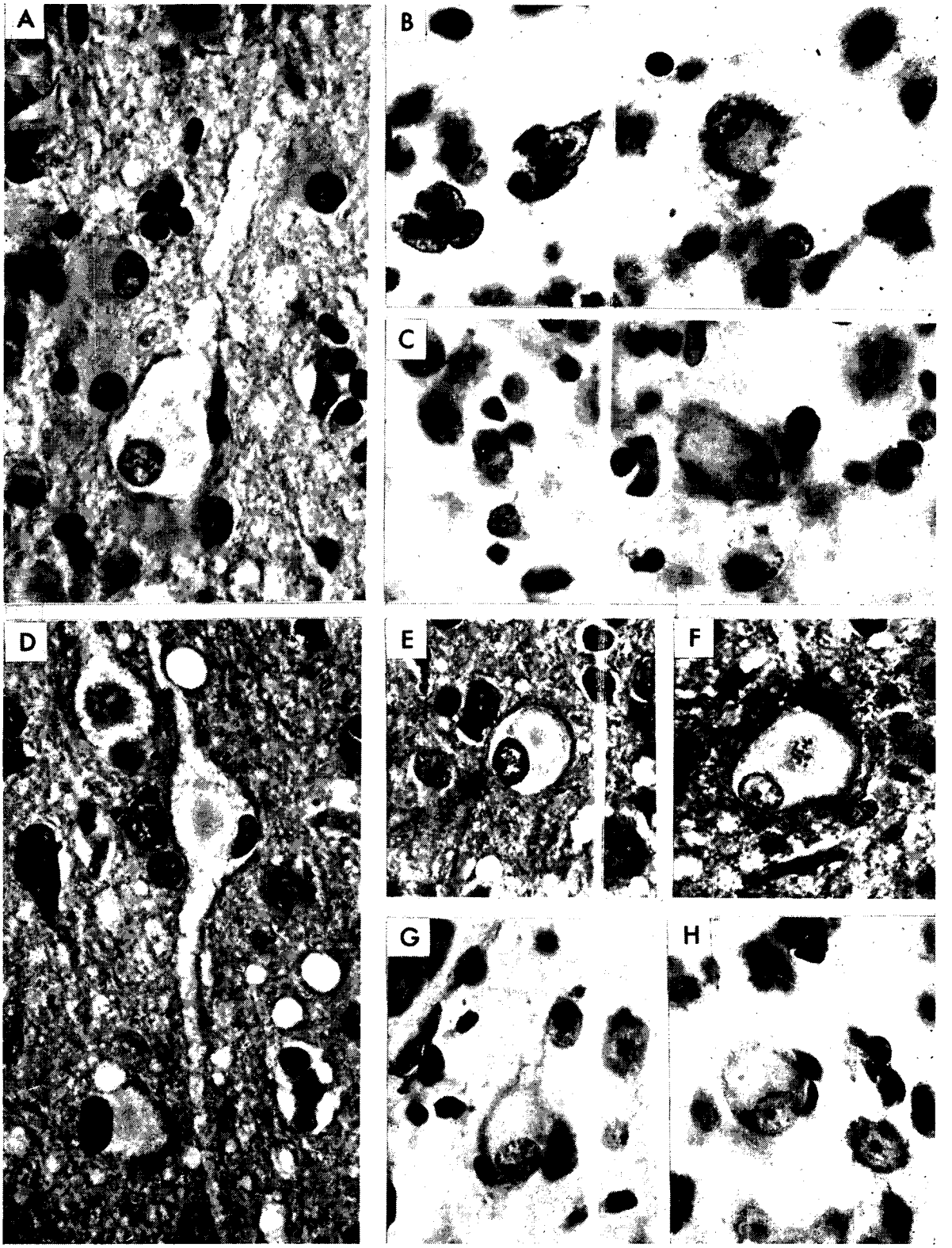

FIG. 1 (see opposite).

that the eponym should apply only to the slowly progressive cortico-striato-nigral disease (see Nevin, 1969); and that common American usage (see Richardson, 1967) and the *British Medical Journal* of 1969 would limit the eponym to the rapid spongiform encephalopathy.

Brownell and Oppenheimer (1965) use the term subacute presenile polioencephalopathy, which is suitably descriptive of the complex syndrome, and could be adopted for it, but Creutzfeldt-Jakob disease, despite the confusion in its use and its somewhat tenuous connection to the cases first described by Creutzfeldt and Jakob, seems to be here to stay. The name encompasses an important and not altogether rare group of cases. Such a concept, covering the whole syndrome, is at present a more convenient and useful one than any restricted to a single clinical or pathological variant. Whether it ultimately proves more meaningful will depend not on further clinical and pathological considerations but on the outcome of current experimental studies.

Creutzfeldt-Jakob Disease in the Chimpanzee

The syndrome which occurs in chimpanzees injected with suspensions of Creutzfeldt-Jakob-diseased brain can be distinguished from kuru clinically. Although an ataxia of gait is usually one of the first signs, other signs soon predominate: for example, in several animals a hemiparesis followed shortly after the onset. There is evidence of hemianopia, and signs suggesting dementia appear early in the course of disease. Myoclonus or other involuntary movements are found. Titubation and trunkal instability are not found. This, together with the evidence of lateralized defect, myoclonus

TABLE 1

Comparison of Kuru and Creutzfeldt-Jakob Disease in Man and Chimpanzee

Clinical	*Ataxia*	*Dementia*	*Extrapyramidal Signs/ Myoclonus*	*Pyramidal Signs*	*Focal Signs*	*Epilepsy*	*EEG Changes*
Kuru man	++	−	−+	−+	−	−−	−+
ape	+	−+	−+	−+	−	−−	−+
CJD man	+−	++	++	+	+	+−	+
*ape	+	++	++	+	+	−	+
Pathological	*Cerebellar Damage*	*Cortical Damage*	*Neuronal Degeneration*	*Astrocytosis*	*Status Spongiosus*	*Jakob Cells*	*Kuru Plaques*
Kuru man	++	+	++	++	+	−	+
ape	+	++	++	++	++	−+	−
CJD man	+−	++	++	++	+	+−	−+
*ape	+	++	++	++	++	+	−−

−− Never
− Rarely, or to a very minor degree
−+ Occasionally, or to a minor degree
+− Frequently
+ Characteristically
++ Always
* Generalizations based on the small number of animals so far examined have only limited validity.

FIG. 1—Chromatolytic nerve cells (Jakob cells) from human and experimental Creutzfeldt-Jakob disease, A, B, C. *Human.* A: Swollen neurone with eccentric nucleus from occiptal cortex; note also the swollen, degenerating apical dendrite. B, C: Chromatolytic neurones from the fifth layer of the insular cortex; note rim of dustlike Nissl substance and extreme peripheral displacement of nucleus which forms a protuberance on the surface of the cell. D, E, F, G, H. *Chimpanzee.* D, E: Swollen neurones with peripherally displaced nucleus from the fifth layer of the frontal cortex; note dense area in centre and distended processes still attached to the cell body. F: Many such cells have a PAS-positive membrane-like edge and contain a small cluster of PAS-positive granules. G, H: Nissl preparations showing the characteristics of chromatolysis; note their close resemblance to similar cells in the patient's brain (C). H. and E. (A, D, E); Nissl stain (B, C, G, H); Periodic acid Schiff reaction (F). All × 770.

Reprinted with the permission of Mrs. Elisabeth Beck and *Brain* from Beck, Daniel, Matthews, Stevens, Alpers, Asher, Gajdusek, and Gibbs (1969), *Brain,* **92**, 699-716, figure 6.

and dementia, clearly distinguishes this syndrome from kuru in the chimpanzee. Further clinical details are given by Gibbs and Gajdusek (1969). So far, insufficient animals have succumbed to the disease to allow for much useful generalization. The clinical range from which possible specimens for inoculation have been gathered has been a wide one, and it will be interesting to see whether this is reflected in any way in the clinical expression of the experimental host.

In order to draw these observations on kuru and Creutzfeldt-Jakob disease together, we have summarized the major similarities and differences between the diseases in the form of a table (Table 1).

Aetiological Aspects

Viruses are becoming more and more difficult to define and classify. We seem to have separated them satisfactorily at the top end from rickettsial, chlamydial and mycoplasmal organisms, but the bottom end continues to give trouble. In essence, a virus contains one of the nucleic acids, DNA or RNA, but not both, and is a means of getting this genetic information from one cell to another. In the process of moving its own genetic material a virus may carry with it information from the host cell. On the other hand, a virus need not move out of its host cell at all, but may stay there for the life of the cell, with its nucleic acid more or less integrated with that of its host. We are beginning to realize that this may be the most common mode of the virus-cell relationship: all the unpleasant, acute diseases which enabled us first to recognize the presence of viruses may merely represent the end-result in the small number of cases where this relationship has broken down. The first to realize the subtle possibilities in the interaction between virus and host were those working with bacteriophages. It now looks more and more that these same possibilities hold for mammalian viruses.

There is little point at this stage in discussing further the definition of virus. Work with scrapie, bacterial episomes and a steadily increasing number of transfer factors has suggested that genetic or copying information may be carried between cells by means quite different from the traditional virus; and any insistence on a particular defining line would, in this state of the art, be quite arbitrary.

From the point of view of the human or mammalian organism we must distinguish different modes of interaction between virus and host (Fig. 2). Note that this figure classifies *modes* of virus-host interaction. A given virus may exhibit all modes in different circumstances and at different times, and in a given host may change from one mode to another. For this reason "tumor virus" or "slow virus" does not say anything absolute about the virus. Measles virus provides an excellent example of a virus known for causing a characteristic acute disease which may on occasion act in a slow mode—to cause subacute progressive panencephalitis. The term "slow virus" is put in quotes, because this usage, relating only to degenerative—usually neurological—diseases, though common, is a restricted and unsatisfactory one. Many of the original slow virus diseases were in fact proliferative in nature, so the term in the restricted sense does not even have historical accuracy.

We have already said that the latent mode may be the most common way in which viruses and cells interact. This is also likely to be true for the virus-mammalian host relationship. Gajdusek *et al.* (1969), for example, were able to isolate 56 strains of viruses which were latent in chimpanzee tissues, that is, without causing apparent disease, by explanting pieces of tissue obtained at autopsy and growing them *in vitro*. Many of these isolations were made from brain. And they still continue to get new viruses out.

We start then with the premise that human beings normally have viruses latent within their tissues, including their brains. On occasion, for one reason or another, these viruses may

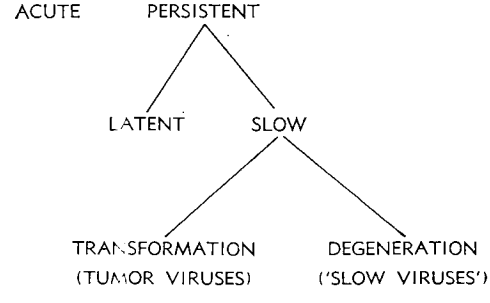

Fig. 2— Modes of virus-host interaction.

change their mode and cause disease. The viruses may be either of the public or private kind. As an example of a public virus we have reovirus, widely found in most mammalian species, including man. Occasionally it causes hepatoencephalitis or other syndromes in children (Stanley, 1967). Each primate species seems to have its own characteristic herpes viruses and vacuolating viruses, which are normally latent. Herpes simplex of man is undoubtedly the best-known of all latent viruses: on occasion it too may cause a specific acute encephalitis in its normal host. Of the vacuolating viruses of man we know little or nothing —but one cannot help wondering whether such a virus may not cause the spongiform change so striking in kuru and certain forms of Creutzfeldt-Jakob disease. In this context it is interesting to note that progressive multifocal leucoencephalopathy, another of the acknowledged slow virus diseases of man, is associated with a papova virus morphologically similar to SV40, the latent vacuolating virus of the rhesus monkey.

In addition, we postulate private viruses which have a close link-up with the genetic properties of the host. A mutation occurring in such a latent virus in one individual, or an environmental change in the host, may result in the virus causing slowly progressive damage to certain cells and the production of degenerative disease in the host; in other words, a change from a latent to a slow mode. This would explain the sporadic occurrence in the community of diseases like Creutzfeldt-Jakob and possibly motor neurone disease. Very rarely such occurrences occur in families: either because of spread of the mutant virus, to other genetically susceptible individuals, or through the long intimacies of family life; or because of vertical transmission of the virus followed by an incubation period of many decades; or because of the sharing of the same latent virus and the same triggering environmental change. Kuru-like disease occurring in an Austrian (Seitelberger, 1962) and a German family (Schaltenbrand *et al.*, 1968), the spinocerebellar degenerations, familial forms of Creutzfeldt-Jakob disease, amyotrophic lateral sclerosis, and the like, may arise in this way. Because of the stringent requirements of the virus these small familial outbreaks soon die out. The genetic requirements of the virus for its host are, at least initially, specific; also specific are the cells which the virus can damage. Thus, in the few instances in which the virus has grown after experimental transmission to another host—and the only one in which this has so far been achieved from man is the chimpanzee—the clinical and pathological syndrome is reproduced almost exactly.

We believe that kuru began in a similar way, as a single sporadic mutation—about 50 years ago, if one can judge from local accounts of its origin. The "family" in which the disease arose was an extended one, the Fore linguistic group of the Eastern Highlands of New Guinea. Such a phenomenon is not unknown in neurology—for example, some of the diseases of the Hebrew linguistic group—but the remarkable geographical localization of cases is unique to kuru. The spread of the virus through this population we have postulated as being the result of cannibalism (Alpers, 1968): these people used cannibalism as a means of disposal of their dead, including those who had died of kuru. Cannibalism will explain the manner in which the disease spread, slowly at first but reaching epidemic proportions by the time it was first investigated in 1957. It will explain the unusual sex and age incidence of the disease, for the practice was confined essentially to the women and the young children of both sexes who associated with them. Once a boy reached the age of about 6 he joined the male group and was prohibited from eating human flesh. The disease, when first investigated, was common in children of both sexes; beyond the age of 18 very few males were affected, whereas in adult females the bulk of cases, about 70%, occurred. The practice of cannibalism ceased abruptly in 1957, except in a few outlying hamlets. This readily explains the fact that no children born since this period, except for one or two in outlying hamlets, have suffered from the disease. The disease then has died out amongst children, and is declining amongst adults. Vertical transmission of the virus clearly has not occurred. The persistence of adult cases we can attribute to a long incubation period following exposure to the virus before 1957. If this hypothesis is correct we would predict a continual rise in the age of the youngest case, and, as the mean of the incubation period since the

last exposure is passed, a subsequent rather sudden decline in the incidence across all remaining susceptible age groups. We are therefore following the yearly changes in the epidemiological pattern of kuru with great interest.

Finally, we may draw our discussion of kuru and Creutzfeldt-Jakob disease together by asking whether one virus, acting slightly differently in different populations or individuals, might not cause both syndromes. The remarkable differentiation of the two diseases in the same experimental host, the chimpanzee, and their obvious similarity to the diseases of origin, argues unequivocally for different viruses being involved. One of the interesting things we can expect to learn from further work with Creutzfeldt-Jakob disease is how much of the clinical and pathological variation of the syndrome is specific to the causative virus or viruses.

SUMMARY

Kuru is the model for degenerative slow virus disease in man. Creutzfeldt-Jakob disease has recently been shown to belong to this category. The clinical aspects of the two diseases in man and in the experimental host, the chimpanzee, are briefly described and compared.

The relationship between persistent virus and its host is discussed. We suggest that diseases like Creutzfeldt-Jakob disease and kuru may be initiated in an individual by the change of a persistent virus from the latent to the slow mode. In the case of kuru the population affected by this change has been augmented by the practice of cannibalism.

ACKNOWLEDGMENTS

We both acknowledge the assistance and collaboration of Dr. Carleton Gajdusek and Dr. Joseph Gibbs in relation to the experimental diseases in chimpanzees. M.A. is a Research Fellow of the National Health and Medical Research Council.

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LYMPHOCYTE STUDIES IN KURU PATIENTS

M. J. SIMONS, MARGARET G. FITZGERALD and M. P. ALPERS

INTRODUCTION

It is now established that part of the lymphocyte population of mammals is long-lived and belongs to a recirculating pool (Gowans, 1966). The functional competence of lymphocytes can be studied *in vitro* by quantitation of the stimulatory effect of agents such as phytohaemagglutinin (PHA) (Ling, 1968; Simons, Fowler and Fitzgerald, 1969). The reaction to PHA is evidently given by the long-lived subpopulation of recirculating lymphocytes (Simons, 1970), and it is this thymic-derived cell population which has been shown to underlie cell-mediated immunity (Miller, 1967). Thus the reaction to PHA can be regarded as a measure of general cellular immune competence. Similarly, the presence or absence of specific cellular immune competence can be determined by quantitating the response of lymphocytes to specific antigens.

Although kuru has been investigated since 1957, no studies of lymphocyte function have been reported. Immunological mechanisms may be involved both in the host's response against the kuru agent, and in the pathogenesis of the disease itself. Consistent with this possibility is the transmission of kuru by lymphoid tissues as well as by brain (Gajdusek *et al.*, 1969). Apart from Mantoux testing of some kuru patients, previous immunological studies have been limited to a search for humoral antibodies. Cell-mediated immunity has not been directly studied, either to assess cellular immune competence or to examine the possible role of such immune mechanisms in the pathogenesis of kuru.

Experimental kuru has been induced in chimpanzees by peripheral as well as by intracerebral inoculation (Gajdusek *et al.*, 1969). Thus haematogenous spread must occur in the experimental disease. Viraemia in general can be of two types, plasma or cellular. Lymphocyte viraemia has been demonstrated in the course of disease due to viruses such as measles (Gresser and Chany, 1963), cytomegalovirus (Foster and Jack, 1968) and rubella (Simons and Jack, 1968). The presence of intralymphocytic virus can be detected by *in vitro* studies of lymphocytes (Simons and Fitzgerald, 1968): in short-term culture the response to phytohaemagglutinin (PHA) may be impaired; in long-term culture survival and transformation of lymphocytes is associated with, and perhaps dependent upon, the presence of intracellular virus (Glade *et al.*, 1968).

Our aim, therefore, was to study the *in vitro* characteristics of blood lymphocytes from kuru patients from the points of view of both cellular immunity and cellular viraemia. For this reason we undertook short-term and long-term culture of leucocytes from kuru patients and, as controls, an equal number of normal Fore people from the kuru region.

MATERIAL AND METHODS

Nineteen patients with kuru have thus far been studied, 9 in stage I and 10 in stage II of the disease. These comprised 15 females (aged from 21 to 54 years) and 4 males (aged from 17 to 22 years). Eighteen controls (9 females, 9 males, age range 16-61 years) from the same villages as the patients were tested at the same time. The blood samples were transported to Goroka Hospital, Eastern Highlands, in heparinized bottles or heparinized plastic tubes at ambient temperature. In 1969 cultures were set up there within 24 hours of collection; in 1970 the heparinized blood was sent on to Melbourne, where cultures were set up within 36 hours of collection.

Lymphocytes from 11 patients and 10 controls were put up in short-term culture. Buffy coat suspensions were obtained by centrifugation, the cells washed once in Dulbecco's phos-

phate buffered saline, and resuspended at a concentration of 1×10^6 lymphocytes in 1 ml medium (TC 199/20% calf serum). Phytohaemagglutinin (Burroughs Wellcome, batch K 7035) and two preparations of brain were used in a range of concentrations in order to establish dose-response curves. A whole human myelin suspension (obtained through the courtesy of Mrs. B. Bencina) was prepared according to the method of Eng *et al.* (1968). A purified basic protein (obtained from Dr. P. Carnegie) was prepared from this myelin by the procedure described by Kies, Thompson and Alvord (1965). Lymphocytes were cultured in round-bottomed glass Wasserman tubes at 37°C, maintained stationary and vertical. The tubes were stoppered in New Guinea, while in Melbourne they were covered with loose-fitting metal caps in an atmosphere of 95% air/5% CO_2. At the end of 3 days (PHA cultures) and 5 days (brain antigen) 2 microcuries of tritiated thymidine was added to each culture for a two-hour pulse. The radioactivity incorporated into lymphocytes was determined in a Packard Tricarb liquid scintillation counter, following washing of the cells with saline (2 times), precipitating with 5% trichloracetic acid, and dissolving the precipitate in Hyamine (Packard).

For long-term cultures buffy-coat leucocytes from 11 patients and 10 controls were used. In most cases these individuals were different from those whose blood was tested in the short-term cultures. The cells were washed once in Dulbecco's phosphate buffered saline, and resuspended in medium TC 199 with 20% bovine serum at a concentration of approximately 3×10^6 lymphocytes per 1 ml. To each culture 20 μg PHA was added as a sub-stimulatory dose. The medium was changed, without PHA, at 3 days, then at 7-day intervals.

Smears were prepared by placing a drop of cell suspension on a microscopic slide and allowing it to spread by tilting the slide. The

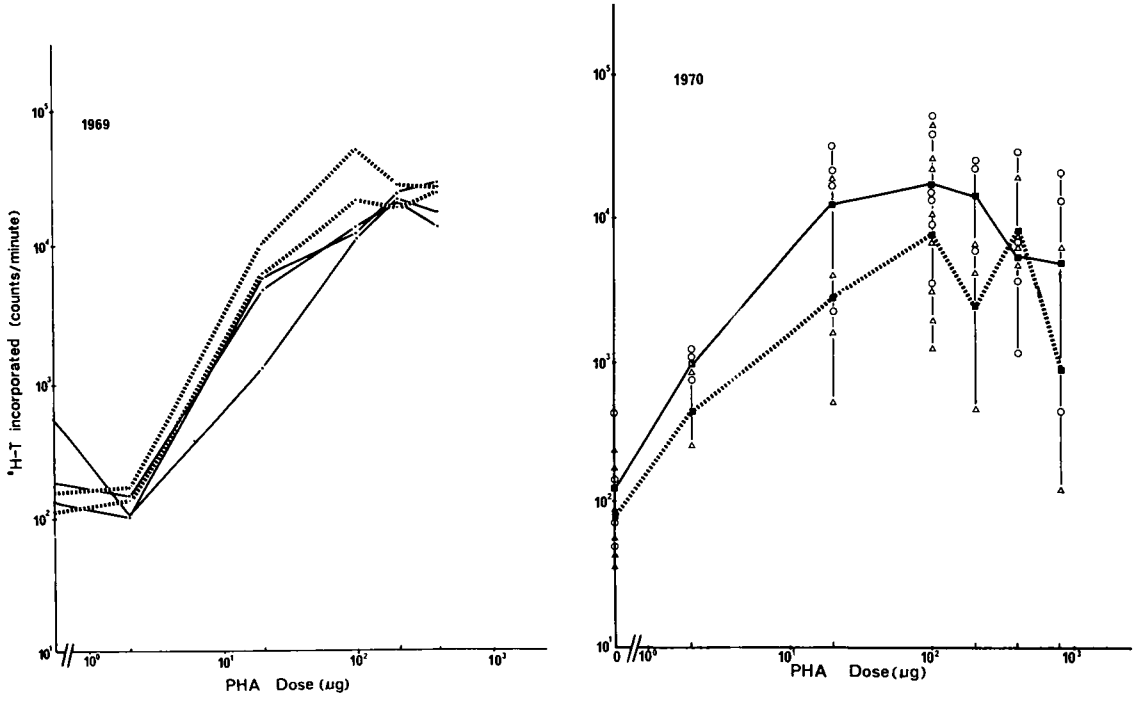

FIG. 1—Dose-response curves for PHA transformation of lymphocytes from kuru patients and controls. Transformation is quantitated by incorporation of radioactive thymidine.
(a) 1969 patients and controls. Curves plotted individually, —— Patients - - - controls.
(b) 1970 patients and controls. Curves connect the mean of results at each PHA dose ○ single value from patients △ single value from controls ■ mean value, —— joins patient's means - - - joins control means.

smears were fixed in methanol (3 min.) and stained with Leishman's stain (30 sec.; buffer 4-4½ min.).

RESULTS

Short-Term Cultures

With PHA as stimulant

In Figures 1-2 the radioactivity of the thymidine incorporated in counts per minute has been plotted against PHA dosage in micrograms. Figure 1 shows the results obtained from kuru patients and normal controls from the Fore region of the Eastern Highlands of New Guinea. There is no difference between the results of kuru patients and controls. Both show a normal shape for the dose-response curve with PHA. The radioactivity counts as plotted have not been corrected for quenching. When such corrections were made no significant change resulted in the relative positions of the dose-response curves.

If the results for kuru patients and controls are combined separately and their means and standard deviations determined we get the values plotted in Figure 2. Also plotted are results from other normal New Guinea Highlanders. Analysis shows no significant difference between kuru patients, Fore controls and other Highland controls. This indicates that the lymphocytes from Fore people, both kuru patients and unaffected individuals, respond to PHA in a way similar to that found in other New Guinea Highlanders.

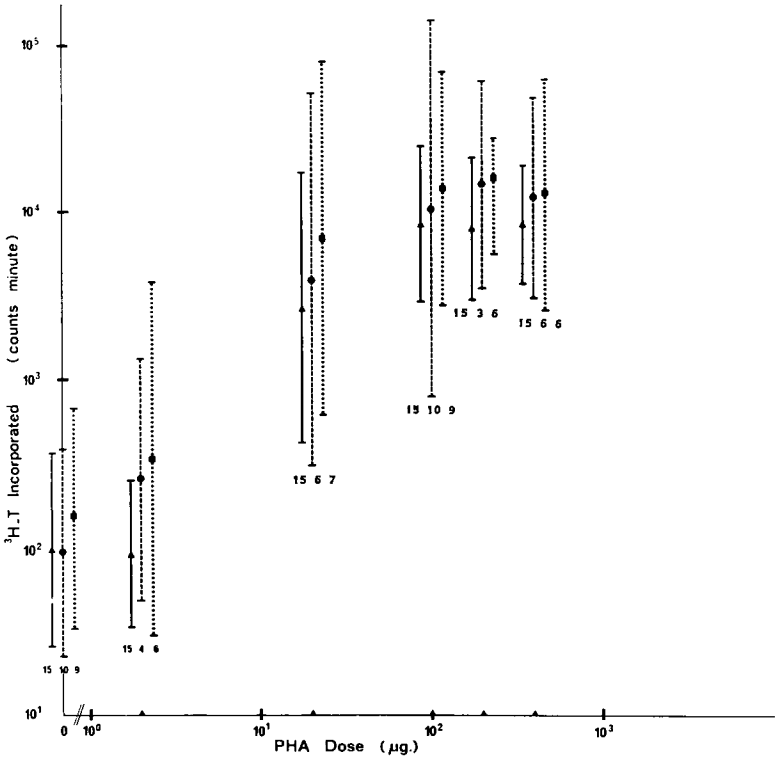

FIG. 2—PHA dose plotted against radioactive thymidine incorporation, for PHA transformation of lymphocytes from Fore patients with kuru, normal Fore and normal New Guinea Highlanders. For each PHA dose the geometric mean ±2 standard deviations of the radioactivity incorporated is plotted. ■ Fore with kuru ● normal Fore ▲ New Guinea Highlanders. The numbers refer to the number tested in each group.

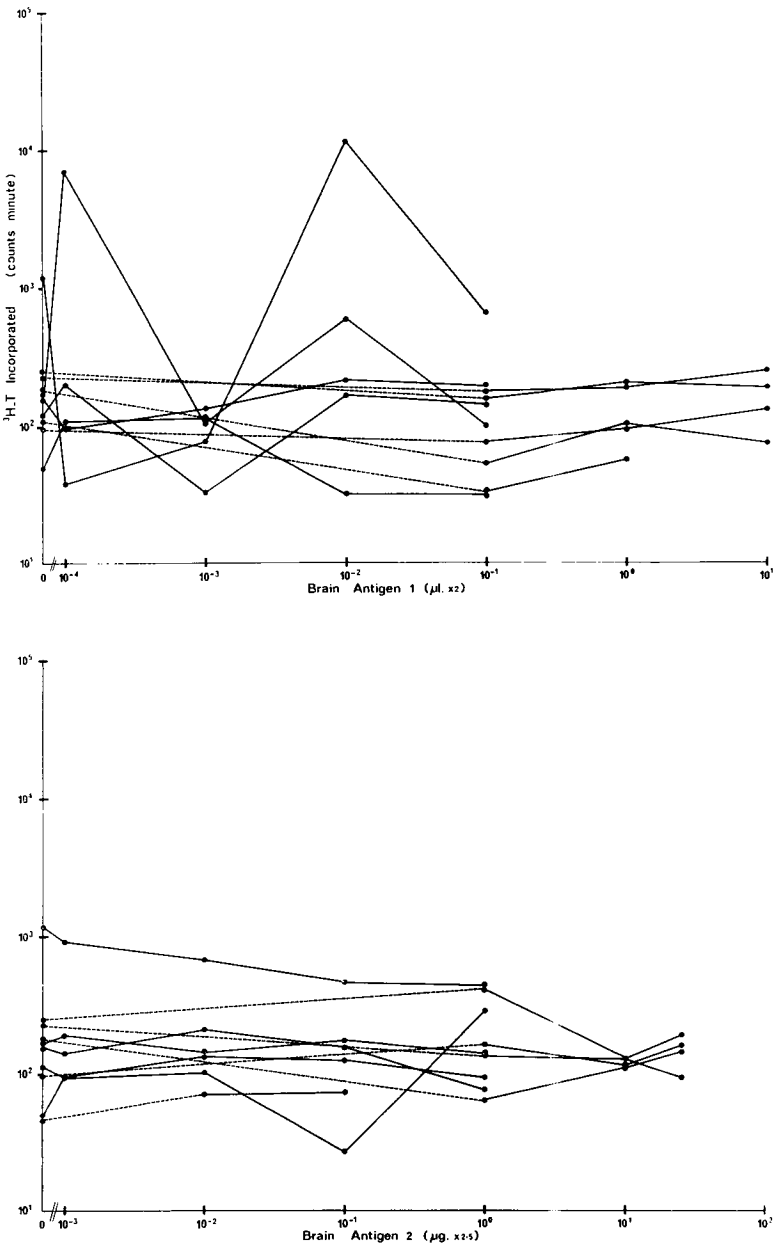

FIG. 3—Dose-response curves of leucocytes in culture to brain antigen. Stimulation is quantitated by incorporation of radioactive thymidine. Brain antigen 1 = whole human myelin suspension in the dose range 0·0002 to 20 μl per culture. Brain antigen 2 = purified basic protein of myelin in the dose range of 0·0025 to 50 μg per culture.

With brain antigen as stimulant

Using the two different brain antigens with 3 or 4 dilutions of each (depending on the number of cells available), we found the lymphocyte response to be essentially negative (Fig. 3). The cells were capable of responding to PHA, but there was no consistent response to either of the normal brain extracts. Of the two specimens which gave a response to whole myelin (brain antigen 1) in a single dilution, one was from a kuru patient and the other from a control.

Long-Term Cultures

No cultures from kuru patients became established into permanent cell lines, all dying out by 60 days and many by 30 days. One culture from a kuru patient and one from a control became lymphoblastoid but did not proliferate rapidly enough to become established as permanent lines.

We found an unusually high number of multinucleate giant cells in some of our cultures. We saw them first and most prominently in cultures from kuru patients, but subsequently found them in a number of control cultures as well. Therefore, whatever their significance, we cannot attribute them specifically to kuru. Similar cells have been reported in passing by other workers in connection with leucocyte cultures (Danes and Bearn, 1969). By first passing leucocytes through a glass-bead column to trap granulocytes and macrophages, we have been able to prevent the formation of multinucleate giant cells in culture. It is therefore unlikely that they are derived from lymphocytes.

Discussion

It has frequently been suggested that the cellular immune system may be involved in the pathogenesis of certain neurological degenerative diseases. This involvement may take place in two fundamentally different ways.

In the first, it has been suggested that the cellular immune system is deficient, thereby enabling a virus to become established and continue to proliferate despite the presence of humoral antibody against it. This has been suggested as an explanation for the way in which measles virus causes subacute sclerosing panencephalitis (Burnet, 1968).

The second possibility is that the cellular immune system mounts a reaction against brain antigen and by an autoimmune process contributes to the pathogenesis of the disease. On the basis of an analogy with experimental allergic encephalomyelitis it has been proposed that multiple sclerosis may be caused in this way (see Paterson, 1969). Furthermore, there is good evidence that in lymphocytic choriomeningitis infection of mice the disease in the central nervous system is the result of a cellular immune reaction and not of the virus infection itself (Gledhill, 1967).

We feel that in general terms both these pathogenetic mechanisms are likely to be important in neurological disease and that it was essential to examine them in kuru. Therefore, our studies of lymphocyte function have been directed towards testing both these possibilities.

1. The hypothesis that the pathogenesis of kuru involves cellular immune depression.

Our results indicate that cellular immune responsiveness is comparable in both kuru patients and controls. Our early results suggested that the response of lymphocytes from both groups was depressed relative to that from Caucasians resident in New Guinea and Melbourne; however, later results indicate that this difference is probably not significant. The results of Mantoux testing after BCG inoculation are also relevant to this hypothesis. In the Eastern Highlands of New Guinea, it often takes two BCG inoculations before definitive conversion takes place (J. Huon, personal communication), and decline in response is rapid. Though kuru patients show the same dampening of delayed hypersensitivity response, at least one patient tested produced an unequivocally positive response.

2. The hypothesis that the pathogenesis of kuru requires the presence of lymphocytes with anti-brain activity.

Our results provide no support for this hypothesis, in that there was no consistent *in vitro* response to dilutions of normal brain antigen. The fact that aggregations of lymphoid cells in the brain of kuru patients are very rare is in keeping with these experimental findings.

Taken together, the results of cultures using PHA and brain antigen indicate that simplistic versions of these hypotheses do not provide an explanation of the pathogenesis of kuru. However, more subtle approaches will be necessary

to investigate the possible role of a cellular immune depression or tolerance specific for kuru virus, or an immune response directed not against normal brain antigens but against virus-induced antigens present in kuru brain.

Long-term cultures did not disclose any circumstantial evidence for a kuru leucocyte viraemia. To test this directly, cells in culture have been sent to Dr. D. C. Gajdusek for inoculation into chimpanzees, at present the only known host available for primary transmission of kuru. The reason for the high proportion of multinucleate giant cells in some cultures from kuru patients is not known, but it is unlikely to have any specific relationship to the disease.

SUMMARY

Leucocytes from 19 patients with kuru and an equivalent number of controls from the same area of New Guinea were studied in short-term and long-term culture. In short-term culture no difference was found between the responses of lymphocytes from kuru patients and controls either to PHA or normal brain antigens. In long-term culture no cell lines became established; multinucleate giant cells were seen in both kuru and control cultures.

ACKNOWLEDGMENTS

We thank Dr. Patrick Carnegie and Mrs. B. Bencina for preparing the brain antigens, Dr. F. Rhodes for his help during fieldwork in New Guinea, and Mr. F. W. Mears and Dr. C. S. Hosking for their help away from the field. M.J.S. and M.P.A. were supported by the National Health and Medical Research Council. Field expenses were supplemented for M.P.A. by a grant from the University of Western Australia and for M.G.F. by a special grant from the Department of Pathology, Royal Melbourne Children's Hospital.

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MYASTHENIA GRAVIS AND HORROR AUTOTOXICUS

A review of the current literature with two case presentations

ERIC DAVIS

INTRODUCTION

Consideration of auto-immunity may be said to have begun when Erlich said it could not occur and the phrase Horror Autotoxicus was coined. A few years later Donath and Landsteiner (1905) demonstrated the presence of an auto-antibody in paroxysmal cold haemoglobinuria.

Auto-immunity may be defined as a condition in which there is a reaction of immunity involving immunocytes versus self-antigens. As a result of which auto-immune antibody globulins are produced which in turn bring about a self-destructive process.

The system of immunocytes (reticulo-endothelial cells, primitive precursor cells—immunoblasts—lymphocytes and plasmocytes) is set up to react against foreign antigens either directly or through the intermediary of specific globulin fractions. The immunocytes will regard as "foreign" those materials to which it has not been previously exposed and will react with the production of globulin. Damashek (1959) speculates that an abnormal proliferation of immunocytes reacts against normal tissue or cellular antigens to produce auto-antibodies as compared with the usual immunological reaction, i.e. normal immunocytes versus abnormal antigens.

The hypothesis otherwise stated is "normal antigens coming in contact with abnormal or genetically dissimilar immunocytes result in a self-destructive immunological response, i.e. an auto-immune response".

One may further speculate that in many non-leukaemic auto-immune disorders clones of abnormal but non-leukaemic immunocytes—the "forbidden clones" of Burnet may be present. The term forbidden clones as used by Burnet refers to the hypothetical group of immunocytes of a specific type which, for one or another reason, are tolerated by the body and simultaneously tolerate the body sometimes for very lengthy periods.

Burnet has speculated that the site of the forbidden clones is the Thymus and that, in turn in Myasthenia Gravis, the germinal follicles are the forbidden clones. It is postulated therefore that Myasthenia Gravis is an auto-immune disease in which presumably abnormal lymphoid clones may attack directly or through abnormal antibodies, muscle, motor end-plates or even the Thymus.

The concept of auto-immunity in relation to Myasthenia Gravis has received much attention since 1960. The evidence for this rests primarily on two findings—

(a) the presence of serological abnormalities (Nastuk *et al.* and Strauss and his co-workers) and

(b) evidence that the Thymus plays a role in the development of immunological capacity.

The association of Myasthenia Gravis with thymic hyperplasia and tumour has long been recognized and the beneficial effects of thymectomy on the course of the disease has been known for 35 to 40 years. The exact relationship between the Thymus and Myasthenia Gravis is still incompletely known. The clues to the nature of the relationship have stemmed from the recent elucidation of the role of the Thymus as an immunological organ and from the numerous studies suggesting an auto-immune aetiology for Myasthenia Gravis. The pathology of the Thymus gland in Myasthenia Gravis has one of three microscopic pictures—

1. Approximately 10% of patients have true neoplasms (Thymomas).
2. 18% have completely normal glands

(microscopically and macroscopically).

3. 72% have abnormal Thymus glands microscopically although the gross appearance and weights of the glands are usually within normal limits.

Microscopically the striking feature in the last group is the presence of increased numbers of germinal centres in the medulla that are exactly like those observed in any lymph node. The germinal centres with their cuff of mature lymphocytes may be so numerous as to cause compression of the cortex which appears like a cap over the expanding medulla. Hassal's corpuscles are present in their usual numbers.

Most if not all of the increase in the numbers of the cells in thymic hyperplasia is in the medulla and affects only the lymphocytes. The epithelial cells are not increased and therefore the thymic hyperplasia in Myasthenia Gravis is nothing more than lymphoid hyperplasia of the medulla in the form of germinal centre proliferation. There is no generalized hyperplasia in Myasthenia Gravis.

In the classic form of Thymomas associated with Myasthenia Gravis there is an admixture of lymphocytes and large pale ovoid epithelial cells. In the second type there is a preponderance of epithelial cells with only a scattering of lymphocytes. The Thymomas associated with Myasthenia Gravis are usually well encapsulated, very slowly growing and only rarely do they lead to the death of the patient as a tumour *per se*. The prognosis appears to be better in those patients with lymphoid hyperplasia than in those with lymphomas.

Thymic hyperplasia has been demonstrated in such diseases as Agammaglobulinaemia, Haemolytic anaemia, Sjögren's syndrome, Systemic Lupus Erythematosus, Myasthenia Gravis, Thyreotoxicosis and Rheumatoid Arthritis.

These findings, M. H. Lessof (1959) considers as supportive of the function of the Thymus as a central organizer of immunological function.

Nastuk *et al.* (1959) demonstrated a complement fixing reaction with Myasthenic serum lysing frog muscle cells. In 1960 Strauss (1959) and his co-workers demonstrated anti-muscle antibodies in Myasthenic serum. Coers and Woolf (1959) stated that structural changes may occur at the neuro-muscular junction interfering with transmission. These changes are presumably due to a substance elaborated by the Thymus and capable of passing the placental barrier—probably a globulin of the antibody type. The best method to demonstrate the presence of muscle antibodies is the indirect immuno-fluorescent technique of Marshall which Joshua *et al.* found more sensitive than the complement fixation fluorescein technique.

Joshua and his co-workers (1967) found that the intensity of the antibody reaction in the immunologically positive patients correlated directly, i.e. the highest titres were found exclusively among the severely ill patients whilst the mild cases had the lowest titres. Conversely in the immuno-negative group the mild cases predominated. Anti-cholinesterase treatment *per se* did not seem to influence the antibody titres. Using the indirect fluorescent method, staining of striated muscle was obtained with the sera of 11 out of 17 Myasthenic patients.

In positive tests striations of normal human muscle appear clearly under the microscope ($\times$ 400). In negative controls fluorescence of the sarcolemma only occurs. The incidence of fluorescent muscle antibody has been found to fluctuate within a range of 30-52% by other authors. Joshua and his co-workers found 64% positivity by the indirect staining method.

Thus all investigators have demonstrated by means of immuno-fluorescence, haemagglutination and complement fixation techniques that the sera from a considerable number of cases of Myasthenia Gravis contain immune gammaglobulins directed against normal striated muscle.

Summarizing the situation it may be stated that it has been generally postulated that an auto-immune mechanism plays a role in Myasthenia Gravis based on the finding of specific antibodies in the sera of patients with this syndrome.

The aetiological significance of the findings of auto-immune phenomena remain to be elucidated. A relationship with other idiopathic auto-immune diseases has been suggested by many investigators. Although the production of multiple antibodies points primarily to a disturbance of the antibody producing system there appears to be a certain specificity in the incidence of antibodies reacting with skeletal

muscle and Thymus tissue.

Although it is very unlikely that the pathophysiology of Myasthenia Gravis, which is primarily a disturbance of acetylcholine mechanism at the neuromuscular junction, is fully explained by antibodies reacting with acetylcholine receptors at the endplate one is tempted to suppose that the functionally disturbed neuromuscular transmission in Myasthenia Gravis is influenced by a circulating substance which has something to do with antigen-antibody reactions (Oosterhuis, Van der Geld and Feltkamp, 1967).

For the clinical Neurologist the results of the newest immunological investigations have a limited but definite value. Antibodies reacting with muscle and Thymus tissue strongly support the diagnosis of Myasthenia Gravis. If no such antibodies can be demonstrated in the patient with proven Myasthenia Gravis a Thymoma is very unlikely. Although multiple antibody formation and clinical traits place Myasthenia Gravis in the group of idiopathic auto-immune diseases this provides no causal relationship with the observed defect of acetylcholine mechanism at the neuromuscular junction.

Case Reports

Case 1—J. W., female, aet 26 years.

This patient was first seen by the author in October 1961 at the age of 19 years with Group II (a) (Osserman and Genkins) Myasthenia Gravis. The principal complaints were diplopia, dysarthria and dysphagia. The bulbar symptoms had been present intermittently for 12 months prior to the first consultation. Examination revealed the classic features of Myasthenia Gravis involving the face and proximal limb musculature.

She was admitted to hospital where a Tensilon test was positive and x-rays of her anterior mediastinum revealed no evidence of thymic enlargement. Electromyographic studies were confirmatory. She was discharged on Cholinergic medication.

Her disorder ran a typical remitting but slowly progressive course and she was submitted to thymectomy in February 1963. The histology of the excised gland revealed typical lymphoid hyperplasia. Her anticholinesterase requirements dropped dramatically post-operatively and an almost complete remission was maintained through 1964 and 1965. During this latter year she miscarried at 18 weeks and her Mestinon requirements were one tablet (60 mg) t.d.s.

In 1966 the patient was admitted to a peripheral hospital with the diagnosis of "Acute Nephritis". However at this stage the following findings were pertinent. A cardiac friction rub was present, blood pressure was 180/110, albumen was found in the urine. The blood pressure steadily rose, retinal exudates were seen and erythematous patches were observed on both lower limbs. Lupus erythematosus cells were demonstrated on at least two occasions. A diagnosis of Systemic Lupus Erythematosus was entertained.

The patient was reviewed in 1966. The Myasthenia Gravis was still in remission and her blood pressure was 210/120. She was on maintenance steroid hormone therapy.

In 1968 she was again admitted to a peripheral hospital wih a severe degree of anaemia. Her haemoglobin on admission was 3·1 gm per cent with a white blood cell count of 4,400 and a platelet count of 70,000. The attending physician considered that the pancytopaenia was due to either drugs or Systemic Lupus Erythematosus. Numerous investigations were carried out including repeated blood studies, bone marrow biopsy and a search for L.E. cells (latter positive again). Serum B12 and folate levels were considered to be low. The haemogram showed marked anisocytosis of the red cells with moderate microcytosis and macrocytosis. Serum proteins were abnormal with a hypergammaglobulinaemia. The patient was treated symptomatically and made a good recovery.

In late 1969 the patient was seen by the author following three Grand Mal seizures. At that time her blood pressure was 240/120, her skin was mottled, there was a well marked L.E. rash and the patient looked ill.

She was admitted to The Royal North Shore Hospital where further seizures occurred. During her stay she was submitted to a renal biopsy which showed a mixed membrano-proliferative picture, with a lot of glomerulosclerosis and also some hypertensive change —a complex pattern and one certainly suggesting that she will have progressive renal impairment. It was considered that the findings were consistent with Systemic Lupus Erythematosus. Anti-nuclear factor and immuno-fluorescent studies were performed and these were positive. Further evidence of her multisystem disease was offered by continued seizures and E.E.G. studies which showed a diffuse encephalopathy.

The patient was reviewed in April 1970 and her condition remained surprisingly stationary. She is not on steroids as it was considered unwise to prescribe these. Her Myasthenia Gravis remains in complete remission and she is no longer taking Cholinergic drugs.

Case 2—V.I., female, single, aet 21 years.

First admission, 9/10/65. At the age of 20 years she presented with a history of diplopia and was first seen by an ophthalmologist. Shortly afterwards she developed ptosis and early signs of bulbar weakness—palatal paresis, dysphagia and dysarthria. When examined the findings were typical of bulbar Myasthenia Gravis without involvement of axial or limb musculature. A Tensilon test was positive and E.M.G. studies were also confirmatory. Full blood studies were normal and an x-ray of her chest and mediastinum revealed no masses. She was discharged on anti-cholinergic therapy.

Second admission, 19/8/66. The patient was re-

admitted to hospital with progressive bulbar symptomatology. Recent severe exacerbations had occurred during intercurrent infections and drug requirements had increased. She was admitted during a Myasthenic crisis. Examination at this time revealed bulbar involvement only, limbs were normal. She was given increasing doses of Prostigmin and Mestinon. On 2/9/66 thymectomy was performed. The convalescence was uncomplicated. Histopathology report—A thinly encapsulated thymus (10 gm). The appearance was that of a normal Thymus gland. Microscopy revealed normal thymic tissue with lymphoid hyperplasia of the germinal centres. No tumour was disclosed. She was discharged on Mestinon and Prostigmin in lesser dosage.

Third admission, 13/7/67. Again in a severe relapse ? Myasthenic crisis, ? Cholinergic crisis. On admission her medication included Mestinon to a total dosage of 480 mg and Prostigmin 120 mg per day. A gentle drug reduction was carried out under close supervision and her schedules were readjusted. The patient was found to be suffering from an atypical viral pneumonia.

Fourth admission, 9/6/69. In the intervening two years there had been a steady decline in the patient's wellbeing. Her bulbar weakness was constant and for the last 12 months she had been unable to swallow solid foods. There was a gradual reduction in all activities to the stage of a virtual invalidism being confined to chair and bed rest at home. This period was punctuated by remissions and relapses. During February to May 1969 Spironolactone was tried without any good results. Her weight loss was marked and now there was proximal limb girdle muscle wasting. She wore a constant patch over one eye to avoid her diplopia. A course of end-organ steroids was commenced. This produced a further acute deterioration and was withdrawn over 10 days. She did not need assisted breathing. A slight remission followed withdrawal of the steroids. She was discharged on three Mestinon every 4 hours plus two Prostigmin every 2 hours.

Fifth admission, 6/8/69. Again a further acute deterioration had occurred. The patient was thin, in constant respiratory distress and was now subject to choking attacks at night. With the help of her local medical practitioner the family were instructed in the giving of parenteral Prostigmin. A further acute crisis developed and she was admitted to the Intensive Care Unit of The Royal North Shore Hospital. It was obvious that the patient had become refractory to all drugs. It was then decided to withdraw all anticholinesterases. This was done under controlled measures and tracheotomy and gastrotomy were carried out. With superb management in the Intensive Care Unit with the Director of Anaesthesia in close consultation she was maintained in a state of virtual complete paralysis for 33 days and then small doses of Prostigmin only were introduced—15 mg two hourly. A remission was established with the return of a normal airway and oral feeding.

Sixth admission, December 1969-January 1970. The patient had remained home for 8 weeks with very little spontaneous activity. A further acute relapse had developed with urgent nocturnal symptoms. It was now thought that the patient would die of respiratory failure (both central and peripheral). She was again re-admitted in a critical state and it was decided to try the effects of massive A.C.T.H. therapy—100 international units were given daily for 10 days with Prostigmin only. A rapid worsening of her condition developed within 72 hours of the commencement of therapy. She was again tracheotomized and fed by gastrotomy. Bulbar and limb paralysis was virtually total. On the 7th day improvement was noted and this was quite extraordinary and sudden. After 1,000 units on the 10th day the patient was moving her limbs and could open and close her eyes. Ocular movements were almost back to normal (she had been permanently diplopic). Again normal feeding and breathing were instituted and the patient returned to a general ward on oral Prostigmin only. This has produced her best remission and on discharge from hospital was walking, talking and eating in complete comfort—solid foods for the first time in 12 months. She no longer wore a patch over her eye.

At the time of this report the patient is being maintained on 100 units of A.C.T.H. weekly and her present remission is the best in the last four years.

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Discussion

Question: Dr. J. W. Lance: Was Spironolactone used in the second patient and, if so, in what dosage.

Answer: No, it was not used in the acute phase of her illness but had been tried early without any good results accruing.

Question: Dr. R. Rischbieth asked whether it was found necessary to continue the cholinergic drugs during the massive A.C.T.H. therapy.

Answer: Yes.

Question: Dr. S. Gubbay brought up the question of immuno-suppressive drugs in the treatment of Myasthenia Gravis.

Answer: Dr. Davis stated, in his experience, and in his reading of the literature, these particular drugs were of no value.

NON-PROGRESSIVE SENSORY NEUROPATHY

RONALD E. VLIETSTRA* and MARTIN POLLOCK†

INTRODUCTION

A number of congenital syndromes in which diminished response to painful stimuli is a common feature have been reported in recent years. Dearborn's (1932) description of his renowned vaudeville patient, Edward H. Gibson, "The Human Pin-cushion", appears to have contributed to the early enthusiasm of case finding. Although pathological studies are few it is apparent that there is great aetiological diversity, some being identified as peripheral neuropathies (Ogden, 1959), and others as ganglioradiculopathies (Swanson, 1965). A small group justify inclusion in the Riley-Day syndrome (Brown, 1964). In a number of cases, careful autopsy study has revealed no significant abnormality (Baxter, 1960; Magee, 1963). In the group which show neuropathological changes, the most frequently described clinical pattern is one of a peripheral sensory loss, predominantly of a dissociated type, of a progressive nature, and commonly associated with trophic changes of the hands and feet (Spillane, 1969). A number of these cases are probably linked with the progressive disorder "hereditary sensory radicular neuropathy", described by Denny-Brown (1951), and have the same autosomal dominant gene transmission.

One case, however, has been described (Ogden, 1959) where although pain perception was markedly impaired, both centrally and peripherally, in the presence of myelinated fibre loss, there were no signs of progression or familial occurrence. This paper describes a further case of this non-progressive non-familial variety of congenital sensory neuropathy, together with data obtained on light, phase, and electron microscopy of biopsied peripheral nerve tissue.

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CASE REPORT

The patient is a 22-year-old European office worker. She presented to the Dunedin Hospital Neurology Unit in August 1969 with a life-long history of clumsiness of the fingers, and with difficulty in appreciating pain and temperature. She was born at full term, a breech presentation, following a normal pregnancy. At birth she was found to have bilateral hip dislocations, but no other congenital abnormality was noted. Although her motor development in the legs was slow she showed no retardation in her other milestones. She said her first word at fifteen months of age, and spoke short sentences at 2 years. At 3 years she sustained a severe burn to her legs. She showed no sign of distress. Throughout her childhood she suffered many such incidents when the sight of blood would attract her attention to a cut. Her teeth are in poor condition but she has never suffered toothache. Her fine movements are clumsy. She has had great difficulty in fastening buttons and tying shoe-laces. Her handwriting is poor. She has difficulty in appreciating textures. Since the age of 18 years she has noted a total inability to sense smell. She has diminished awareness of bladder sensation and is, at times, incontinent. At other times she is quite embarrassed because she is not aware that her "period" has started. She is adamant that her symptoms have not been progressive.

Examination of her parents and siblings revealed no neurological abnormality. Her parents and siblings are right-handed.

She is a right-handed girl of normal intelligence. Psychiatric history does not elicit anything suggestive cf an hysterical disorder.

There is an inability to sense a large variety of odours, including coffee, cloves, almond and tar. The optic fundi show no abnormality. Corrected visual acuity is 6/6. There is a complete loss of pain, light touch and temperature sensation bilaterally in all divisions of the 5th nerve. The corneal reflexes are absent. There is no weakness of facial musculature. Taste perception is absent to galvanic stimulation at 400 micro-amps, and to solutions of salt, sucrose, urea and hydrochloric at concentrations of 1 mole per litre. Lacrimation is normal, as is the audiogram. There are poor palatal movements, absence of the gag reflex and impaired oro-pharyngeal sensation. There is great difficulty in depressing or protruding the tongue. Numerous small scars are present on her arms, hands and legs. There is no chronic infection or ulceration of her hands and feet. X-rays of her ex-

tremities are normal. Peripheral nerves are not enlarged and there is no calf tenderness. Muscle power and tone are normal. Co-ordination is unimpaired. All deep tendon reflexes including the jaw jerk are absent. There is a cervical scoliosis to the right. Hip movement is markedly reduced but other joints are normal.

There is a marked sensory loss. Superficial pain and light touch are lost over the entire body except about the anus and in the thoracic dermatomes T10 and T11. Light pressure sensation is preserved over the trunk but lost in the limbs. The ability to detect temperature is entirely lost in the limbs. Normal temperature discrimination exists only in the peri-anal and in T10 and T11 dermatomes. Immersion of the hands in ice cold water occasions no distress. Position sense is lost in the fingers and toes. There is no loss of vibration sense. There is no appropriate response to compression of the tendo Achilles nor to heavy pressure on the anterior surface of the tibia. No pain is induced after one hundred contractions of the fist in one hundred seconds with the arterial circulation occluded. A sural nerve biopsy was performed without anaesthesia and without discomfort.

The cerebrospinal fluid contained 16 mg of protein per 100 ml. Serum cholesterol estimations, 150-200 mg per 100 ml. Serum lipoproteins and phytanic acid were normal. The rectal biopsy was negative for amyloid. Chromosome study showed a normal female karyotype. A sweat test showed no impairment. There was no postural hypotension. An intradermal injection of 0·05 mg histamine acid phosphate produced a normal flare response on the feet, forearms, and abdomen, while an intravenous injection of 0·25 mg histamine produced anxiety, tachycardia, and a typical histamine headache. The E.E.G. was normal.

Cystometry revealed a bladder capacity of 1,100 ml. No denervation contractions were present. Bladder sensation was markedly reduced but power was preserved.

Peripheral Nerve Examination

Motor nerve conductions in the left ulnar, right median and right ulnar nerves were normal. No sensory action potentials could be recorded percutaneously from the left and right ulnar and median nerves. However, with computer averaging of one hundred stimuli, an action potential of greatly diminished amplitude (0·5 μv), and of increased distal latency 3·75 msecs), was obtained from the left median nerve.

Fascicular nerve biopsies were performed on the right sural nerve and the left lateral cutaneous nerve of the thigh (Dyck, 1966).

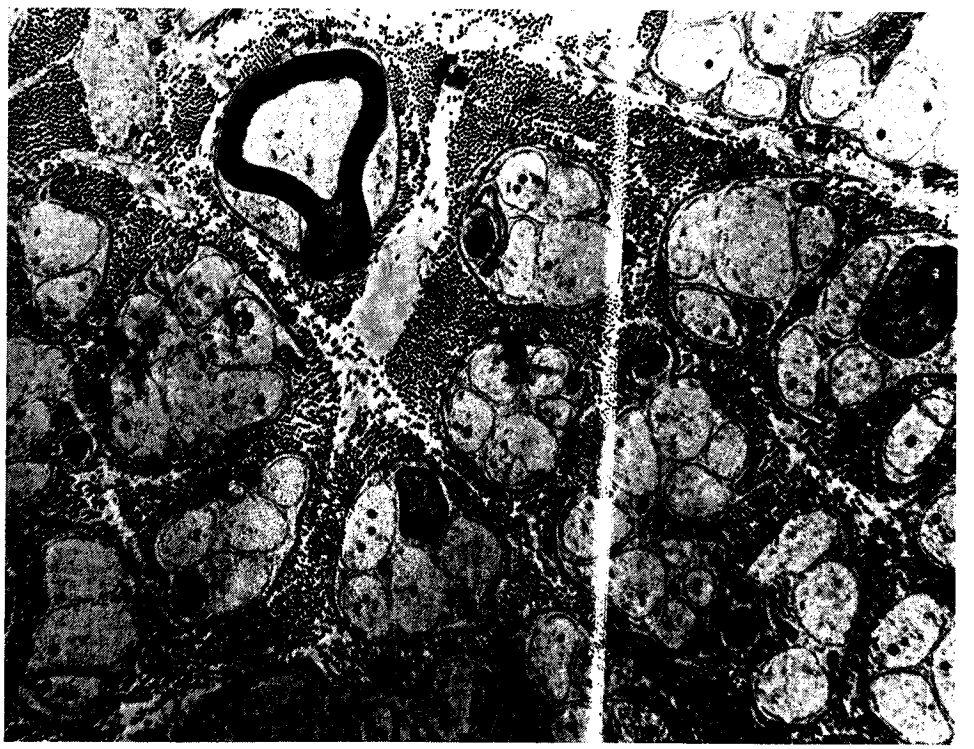

FIG. 1—Electron-micrograph of lateral cutaneous nerve of the thigh (7,000 ×). A single small myelinated nerve fibre is seen. Unmyelinated fibres and associated Schwann cells are normal.

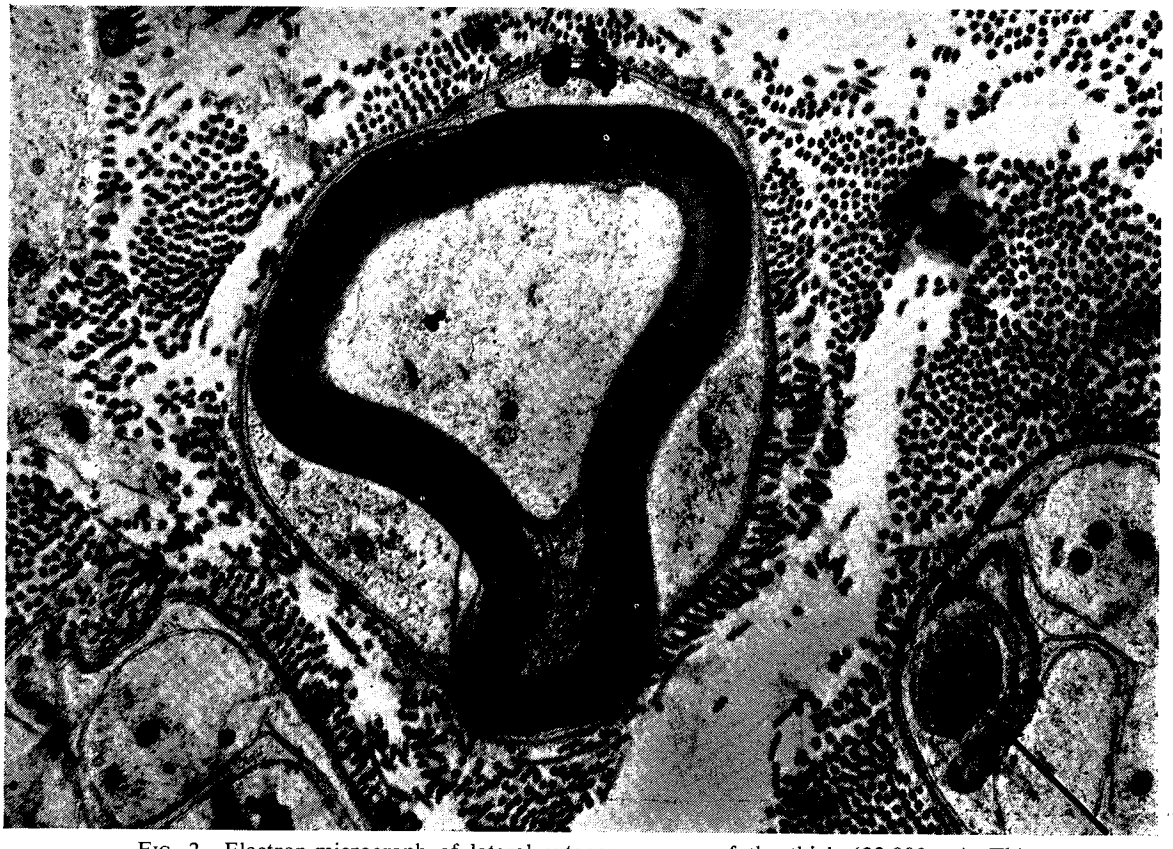

FIG. 2—Electron-micrograph of lateral cutaneous nerve of the thigh (23,000 ×). This small myelinated fibre shows normal lamellar arrangement. A Schwann cell enfolds a collagen bundle at lower right.

Specimens were studied with light, phase, and electron-microscopy. The striking finding was a great reduction in the number of large myelinated fibres, with a moderate loss of small myelinated fibres, but no decrease in the number of unmyelinated fibres (Fig. 1). Teased myelinated fibres showed no evidence of segmental degeneration. Two hundred serial sections stained with methyl violet were examined without evidence of amyloid. The few myelinated fibres appeared normal on electron-microscopic study but there was an excessive amount of collagen in the interstices of the fasiculus (Fig. 2).

DISCUSSION

The striking finding in this patient was of a virtual absence, both by light and electron-microscopy, of large myelinated fibres in peripheral nerve. Many workers have attempted to correlate peripheral nerve fibre size and function (Zotterman, 1959). It has been postulated that the modalities of pain and temperature are mediated by small myelinated or unmyelinated fibres, and vibration and proprioception by large myelinated fibres (Bishop, 1959). The observations in this patient contradict such a view. Vibratory and proprioceptive senses were retained despite great reduction in the number of large peripheral myelinated fibres. By contrast, pain perception was markedly diminished although small myelinated and unmyelinated peripheral nerve fibres were present.

The afferent arc of the flare response has similarly been attributed to specific fibres; those in the dorsal root afferents subserving pain (Celander and Folkow, 1953). Flare responses were demonstrable however in the present case in multiple analgesic areas.

These findings emphasize the limitations of relating both a specific modality theory to cer-

tain fibre diameter groups, and the flare response to a specific fibre type.

The long non-progressive clinical history, and the absence of macrophages and segmental demyelination in peripheral nerve biopsies, suggests that this rare syndrome results from a congenital absence of large peripheral myelinated fibres.

Summary

A case of non-familial, non-progressive congenital sensory neuropathy is described. Light, phase and electron-microscopy examination of peripheral nerve revealed a marked loss of large myelinated fibres. This finding is discussed.

Acknowledgments

We are grateful to both Dr. P. Dyck, Department of Neurology, Mayo Clinic, and Dr. E. G. Jones, Department of Anatomy, Otago Medical School, for examining specimens of biopsied peripheral nerve.

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HISTOCHEMICAL AND BIOCHEMICAL STUDIES IN PSEUDOHYPOPARATHYROIDISM

MARTIN POLLOCK* and JOHN GRAHAM SNEYD†

INTRODUCTION

Phosphorylase exists in skeletal muscle in two forms: "active" phosphorylase "a" and "inactive" phosphorylase "b". Cyclic adenosine 3′, 5′-phosphate (c AMP), adenosine 5′-phosphate (AMP) and adenyl cyclase are concerned with the activation of phosphorylase "b".

Chase *et al.* (1968) have drawn attention to a defective excretion of c AMP in pseudohypoparathyroidism and to the possibility that this results from an insensitive renal adenyl cyclase system.

An absence of phosphorylase in skeletal muscle in pseudohypoparathyroidism has also recently been reported (Cape, 1969). The interesting possibility is thus presented of a generalized deficiency of adenyl cyclase as the underlying defect in pseudohypoparathyroidism.

This report further evaluates the excretion of c AMP and skeletal muscle phosphorylase in the following case of pseudohypoparathyroidism.

CASE REPORT

The patient, aged 31 years, was admitted to the Dunedin Hospital on 29th April, 1969, because of epilepsy and involuntary movements. His first generalized seizure had occurred at the age of 19 years. Despite Phenytoin he continued to have infrequent seizures. In May 1968, jerking movements began in his right leg. Five months later he first noted a coarse tremor in his hands.

He presented the classical features of pseudohypoparathyroidism. He had a rounded facies and a high forehead (Fig. 1). Early subcapsular cataracts were present. Small hard subcutaneous nodules were palpable in his scalp, hands and feet. The fourth and fifth metacarpal bones were shortened bilaterally (Fig. 2).

His mentation was impaired. He was disorientated in time, had poor calculation ability and was unable to repeat the Babcock sentence. His gait was halting because of proximal irregular jerking movements. A moderate "plastic" increase in tone was present in the right limbs. The general physical and neurological examinations were otherwise normal.

Serum calcium ranged between 4·4 and 6·5 mg per cent and serum phosphorus between 5·5 and 8·1 mg per cent. A radiographic survey showed numerous areas of ectopic calcification in the scalp, basal ganglia, trunk and limbs. An electrocardiogram had a prolonged Q-T interval. On two occasions parathyroid

FIG. 1—The patient with pseudohypoparathyroidism.

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FIG. 2—Characteristic shortening of fourth and fifth metacarpal bones.

hormone injections failed to produce a phosphaturic response or an increased urinary excretion of c AMP.

The patient was treated with 100,000 units of vitamin D daily. His mentation dramatically improved as his calcium and phosphorus imbalance was corrected. Nine months later he reported no involuntary movement and no further seizures.

METHODS

Biopsies were taken from the right sternomastoid and quadriceps muscles. For histochemical studies part of each biopsy was apposed to control muscle to form a "sandwich" block. The blocks were frozen by being placed on solid carbon dioxide, and serial sections, 12μ thick, were cut in a cryostat. Sections were placed directly into incubating solutions for the demonstration of phosphorylase "a" and total phosphorylase activity by the method of Takeuchi and Kuraiki (1955).

Part of the patient's quadriceps biopsy and four quadriceps biopsies from control patients were assayed for phosphorylase (Cornblath *et al.*, 1963).

RESULTS

Phosphorylase "a" activity was absent in the axial muscle fibres of resting skeletal muscle (Fig. 3). However, phosphorylase "a" activity was present in peripheral muscle fibres of both sternomastoid and quadriceps muscles (Fig. 4). c AMP added to the incubation media resulted in activation of phosphorylase "b" (Fig. 5). Enzymatic activity was unaltered by the addition of calcium or magnesium to the incubation media.

The normal content of phosphorylase "a" was confirmed by muscle assay (Table 1).

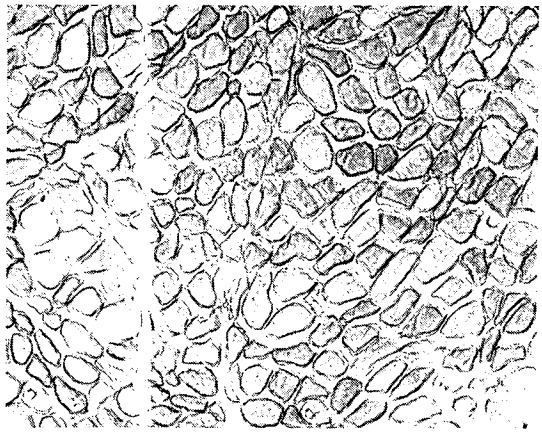

FIG. 3—Absence of phosphorylase "a" activity in deep fibres of quadriceps muscle.

FIG. 4—Presence of phosphorylase "a" activity in peripheral fibres of quadriceps muscle.

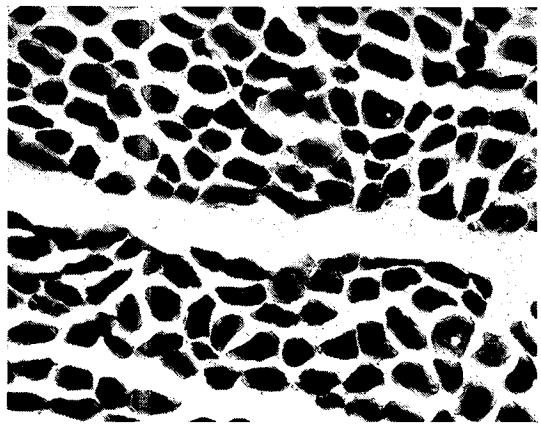

FIG. 5—Normal total phosphorylase activity (phosphorylase "a" and "b") in quadriceps muscle.

TABLE 1

Phosphorylase Content of Quadriceps Muscle in Pseudohypoparathyroid Patient and Controls

	Total Phosphorylase (units/g wet wt)	*% of Phosphorylase in "a" form*
Patient	29·2	20·7
Control 1	30·2	5·6
2	24·1	16·7
3	20·4	4·8
4	32·6	3·9

DISCUSSION

The enzyme adenyl cyclase catalyses the formation of c AMP and is stimulated by parathyroid hormone. Thus, giving parathyroid hormone to normal patients results in an increased renal excretion of c AMP. This report confirms the defective renal excretion of c AMP in response to parathyroid hormone in pseudohypoparathyroidism and supports the view of an underlying deficiency of adenyl cyclase in renal cortex. However, this investigation failed to confirm an absence of phosphorylase "a" in pseudohypoparathyroidism. Both histochemical and quantitative biochemical investigations showed a normal content of total phosphorylase and its active component in skeletal muscle. Thus, it is likely that skeletal muscle adenyl cyclase has normal activity in pseudohypoparathyroidism.

A deficiency of adenyl cyclase in pseudohypoparathyroidism is therefore unlikely to be generalized and may be localized to renal cortex and bone.

SUMMARY

Histochemical and biochemical findings in a patient with pseudohypoparathyroidism are reported. Renal excretion of c AMP was defective, but a normal content of phosphorylase "a" was present in skeletal muscle. This investigation does not support the concept of a generalized deficiency of adenyl cyclase in pseudohypoparathyroidism.

ACKNOWLEDGMENT

This study was supported by Golden Kiwi grant I.A. 200/10240.

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FURTHER OBSERVATIONS ON AN OUTBREAK OF DIPHENYLHYDANTOIN INTOXICATION

J. H. TYRER, M. J. EADIE and W. D. HOOPER*

INTRODUCTION

During 1968 an outbreak of anticonvulsant intoxication occurred in several cities in Australia. Evidence has accumulated that this outbreak was related to a change in the excipient in one maker's brand of 100 mg diphenylhydantoin (phenytoin) capsules. Although a preliminary report of investigations into the Brisbane outbreak has been given (Tyrer, Eadie and Sutherland, 1969) it is here proposed to review the evidence that the change in excipient was responsible for the outbreak, and to describe further studies in which an attempt has been made to investigate how the change in excipient produced its effects.

THE OUTBREAK

In Brisbane a total of 51 persons affected by anticonvulsant intoxication was seen personally in 1968 and early 1969. All these patients were taking one manufacturer's brand of 100 mg phenytoin sodium capsule and, while many of these patients were receiving multiple anticonvulsants, 6 of the 51 affected persons took no therapy except phenytoin. All the patients had taken phenytoin for months or years previously without side effects, and anticonvulsant dosages had been altered in only 6 of the 51 patients in the 4 weeks preceding the onset of their symptoms.

The clinical picture in the affected patients (ataxia of gait, diplopia, drowsiness and nystagmus) was compatible with phenytoin intoxication. Measurements of blood phenytoin concentration were carried out in 39 of the 51 patients, and were above the upper limit of the therapeutic range (20 μg/ml) in 34 (87%) of the 39 patients. Therefore it was highly probable that the epidemic was one of phenytoin intoxication, and related to one manufacturer's 100 mg phenytoin sodium capsules.

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THE CAUSE OF THE OUTBREAK

Measurement of the phenytoin content of 100 mg phenytoin sodium capsules being taken by three patients when they became intoxicated showed that these capsules did not contain excess drug (contents assayed at 92-98 mg of phenytoin), and phenytoin sodium capsules (labelled 100 mgm) produced by the same manufacturer some years earlier assayed at 99-100 mg of drug. Therefore it was unlikely that changes in the phenytoin content of the capsules had caused the outbreak. However, it appeared that in November 1967 a change had been made in the excipient of the phenytoin sodium capsules responsible for the outbreak. In the excipient, calcium sulphate dihydrate had been replaced by lactose, and the amounts of magnesium stearate and magnesium silicate in the capsules had been increased. It would usually take several months from manufacture for a particular batch of capsules to be dispensed to patients; the outbreak of phenytoin intoxication had begun in April 1968, the majority of patients who were affected being involved by June 1968. These time relations suggested that the change in excipient in the capsules might be responsible for the outbreak. Further studies, to be described below, showed that in all patients so far tested, blood phenytoin levels rose when patients were changed from the phenytoin capsules (calcium sulphate excipient) to the same phenytoin dose in capsules with lactose as excipient. The problem remained as to whether one or more of the following were operative:

(i) calcium sulphate accelerated phenytoin metabolism or decreased its absorption;
(ii) the increased magnesium stearate or magnesium silicate slowed phenytoin metabolism or increased its absorption;
(iii) lactose decreased phenytoin metabolism or increased its absorption.

The Effects of Three Different Phenytoin Excipients

Blood phenytoin levels were compared in a "cross-over trial" on patients taking three different brands of phenytoin capsule (A, B and C), containing different excipients, to see whether there was any correlation between these excipients and blood phenytoin concentrations. Ten patients took the same daily total phenytoin dosage over a five-month period, patients' therapies being changed at monthly intervals from one commercially available phenytoin capsule to another, according to the following schemes: A → B → A → C → A. (5 patients), A → C → A → B → A (5 patients). The details of the excipients in the 3 brands of capsules are set out in Table 1. Blood phenytoin levels were measured in samples taken one hour apart after each patient had completed one month's therapy with each brand of phenytoin. Without entering into a detailed analysis of the results, there was no statistically significant difference in blood phenytoin concentrations produced by the different brands of phenytoin capsule with their different excipients (Table 2). Unlike phenytoin capsules brands B and C, phenytoin with calcium sulphate as excipient differed from phenytoin capsules brand A as regards the blood phenytoin levels produced. For this reason the following investigations were carried out to evaluate possible "excipient" activity further.

Table 1

Composition of Capsules (in mg)

	Brand		
	A	*B*	*C*
Phenytoin	100	100	100
Lactose	124	0	33·6
Magnesium stearate	9·6	10	0·6
Talc (Mg silicate)	6·5	0	6·0
Calcium stearate	0	0	1·4
Miscellaneous components	0	0	4·4

Table 2

Mean Blood Phenytoin Concentration (μG/ml)

	Sample 1	*Sample 2*
Brand A	6·2	6·2
„ B	6·8	7·0
„ A	7·1	7·4
„ C	7·7	8·0
„ A	5·3	5·1

After analysis of variance the effects of changing treatment prove to be not statistically significant.

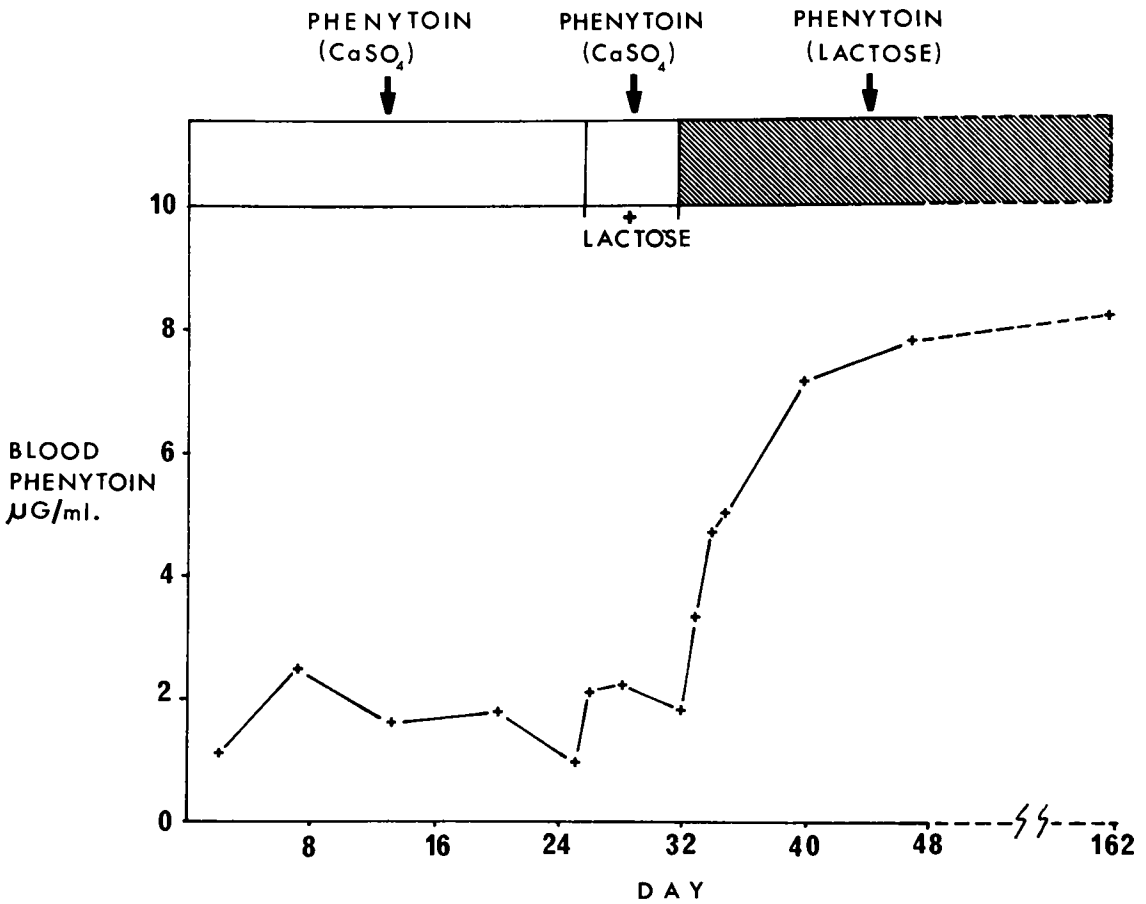
Studies on Phenytoin Metabolism

(i) A patient took a constant daily oral dose of phenytoin and other anticonvulsants throughout the study. When therapy was changed from phenytoin (lactose excipient) to phenytoin (calcium sulphate excipient) there was a rapid and profound fall in blood phenytoin concentration, but no increase in faecal phenytoin loss. Blood phenytoin levels rose when phenytoin (lactose) was used again. When blood phenytoin concentrations had been stable for some time with the subject taking phenytoin (lactose) additional calcium sulphate was given separately, in a dose nearly 3 times that contained in the phenytoin (calcium sulphate) capsules. Blood phenytoin levels rose further and clinical phenytoin intoxication appeared; blood phenytoin levels fell when this calcium sulphate was withdrawn.

These observations suggest that the administration of calcium sulphate alters blood phenytoin concentrations, that this effect cannot be accounted for by altered phenytoin absorption from the gut, and that the effect of calcium sulphate may be bidirectional, depending on dosage. It is not yet shown that magnesium stearate and magnesium silicate have no action.

(ii) Another patient began therapy with phenytoin (calcium sulphate). When his blood phenytoin concentration was stable he was given lactose by mouth in addition, his phenytoin dosage being unchanged. His blood phenytoin level did not alter. Replacement of phenytoin (calcium sulphate) by the same phenytoin dose as phenytoin (lactose) caused a considerable increase in blood phenytoin concentration (Fig. 1).

This is further evidence that lactose itself does not alter phenytoin metabolism, but that withdrawal of calcium sulphate does.



(iii) A third patient commenced phenytoin (calcium sulphate) therapy. When his blood phenytoin concentration had become steady lactose was added to his therapy; blood phenytoin concentration did not change. An amount of magnesium sulphate calculated sufficient to make up the difference between the magnesium contents of the phenytoin (calcium sulphate) and phenytoin (lactose) capsules was added to his therapy; blood phenytoin concentration still did not change. However, when the patient was given the same phenytoin dose as phenytoin (lactose) there was a prompt and marked increase in blood phenytoin concentration.

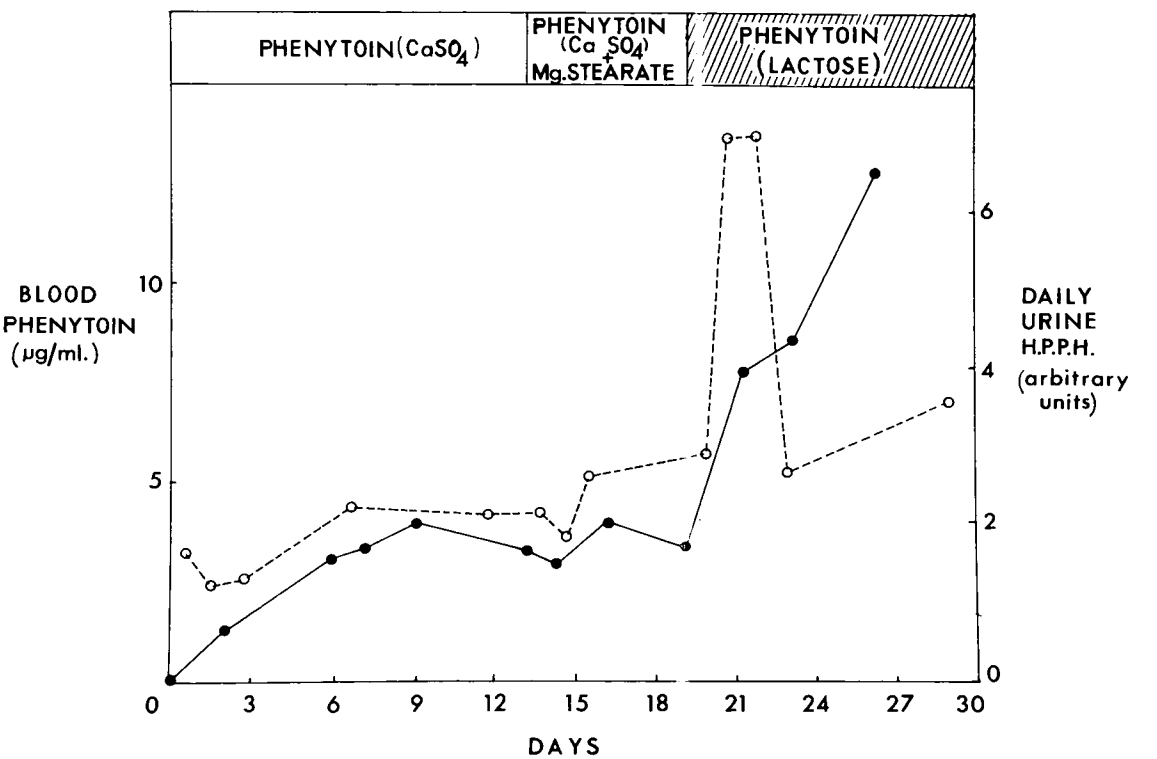
It thus appeared that both lactose and magnesium were unlikely to be responsible for the effects of the changed excipients.

(iv) A further patient took phenytoin (calcium sulphate) till his blood concentration of drug was stable. The addition of magnesium stearate then had no effect on blood phenytoin level, but this rose once phenytoin (lactose) replaced the phenytoin (calcium sulphate), there being no change in the dose of phenytoin itself (Fig. 2). In this patient, 24-hour urinary excretions of the main phenytoin metabolite (p-hydroxyphenyl-phenylhydantoin, commonly abbreviated to HPPH) were monitored at intervals, using the gas-chromatographic assay developed by Chang and Glazko (1970). HPPH excretion was relatively steady until the two phenytoin preparations were exchanged. There was then a rapid and marked increase in urine HPPH excretion once phenytoin (lactose) was introduced, but after 48 hours HPPH excretion fell briefly, and then rose again to a level higher than that while the patient was taking phenytoin (calcium sulphate). Unfortunately the study could not be continued beyond this stage.

This study shows that magnesium stearate has no effect on phenytoin metabolism. All the data so far considered suggest that calcium sulphate is the only active ingredient in the "excipients" in the two forms of phenytoin capsule. The HPPH excretion findings are not consistent with the possibility that calcium sulphate acts by accelerating the metabolism of phenytoin to HPPH. If this were so, removal of calcium sulphate should have caused HPPH production, and therefore excretion, to fall, whereas HPPH excretion rose. A simple explanation for the rise in HPPH excretion would be that calcium sulphate has been preventing the absorption of phenytoin from the gut. However, this hypothesis does not easily explain the secondary fall in HPPH excretion (if indeed this fall is valid: it occurred on one occasion only) and it is not compatible with the faecal phenytoin excretion data obtained in study (i) above. This leads one to consider an alternative hypothesis, that calcium sulphate may alter HPPH metabolism, with secondary "mass action" effects on phenytoin.

Phenytoin is hydroxylated to HPPH in the liver, and the HPPH is conjugated with glucuronic acid and in part excreted in the bile as a glucuronide. The conjugate splits in the gut and the HPPH is reabsorbed. If we postulate that calcium sulphate traps the HPPH in the gut, possibly as a sulphate, then less HPPH returns to the blood stream to be excreted by the kidney, and the increased faecal HPPH loss would not be detected by the Wallace (1966) method used for measuring phenytoin in the present study. Because blood and tissue HPPH levels are reduced the conversion of phenytoin to HPPH might be accelerated (following the law of mass action) thus causing tissue and blood phenytoin levels to be lowered; once calcium sulphate was withdrawn from the gut more HPPH (perhaps even a "back-lag") might return to the blood, urine HPPH excretion rise, and higher blood HPPH levels might slow phenytoin metabolism and thus produce a rise in blood phenytoin.

The hypothesis that calcium sulphate interferes with HPPH in the gut seems to fit the facts that we have so far established better than do the other hypotheses considered, but clearly



further studies are required. All that has been established with reasonable certainty is that calcium sulphate is capable of altering the body's handling of phenytoin, and that this capacity of an "excipient" to be active biologically led to an outbreak of anticonvulsant intoxication.

Discussion

Dr. Davis: What is the usual oral phenytoin dose to produce a therapeutic blood level? I do not like to use more than 200 mg per day.

Dr. Eadie: We find 400 mg per day is required by most patients though it is too high a dose for some.

Professor Lance: Do you consider it desirable to monitor blood levels in all patients with epilepsy?

Dr. Eadie: It is very helpful to use blood phenytoin measurement to find why therapy is going wrong, or to see if the patient is taking enough drug to give optimal possible protection against epilepsy, but one must be careful not to assume that blood level is directly proportional to dose when one intends to increase the dose. As dose is increased a stage is reached where metabolism tends to become saturated and beyond this stage drug levels in blood rise rapidly.

Dr. Selby: I have noticed that patients who had phenytoin intoxication, once the intoxication was relieved by suspending the drug, often could tolerate again their original phenytoin dose.

Dr. Eadie: This was not our experience in Brisbane and I find it difficult to explain your observation, other than to suspect that patients may not have taken their drug regularly. We are becoming increasingly aware of how often patients fail to take regularly the anticonvulsant therapy prescribed for them.

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WHOLE BLOOD HISTAMINE AND PLASMA SEROTONIN IN CLUSTER HEADACHE

M. ANTHONY and J. W. LANCE*

INTRODUCTION

Cluster headache (Kunkle *et al.*, 1952; Friedman and Mikropoulos, 1958), or periodic migrainous neuralgia (Harris, 1926) or histaminic cephalgia (Horton, 1941), in addition to a host of other names and eponyms, has until hitherto been classified as a variant of migraine (*Ad Hoc* Committee on Classification of Headache, 1962). The episodic nature of the headache, the occurrence of typical migraine both in the patient as well as in his family (Bickerstaff, 1959), the dilatation of the extracranial vessels during the attack, together with the relief of pain by compression of the superficial temporal artery (Horton *et al.*, 1939) or the administration of ergotamine tartrate (Horton, 1952; Symonds, 1956), all bespeak the migrainous nature of the headache. On the other hand the predominance of male sufferers, the brevity of the attacks (usually less than two hours and frequently less than one), the occurrence of the attacks in bouts or clusters, the long intervals of freedom between bouts, the absence of prodromal symptoms, the rarity of gastrointestinal symptoms, the presence of autonomic phenomena (e.g., conjunctival congestion, increased lacrimination, nasal obstruction, rhinorrhoea, Horner's syndrome, and rise in skin temperature on the side of the headache), together with the tendency to strike consistently on the same side of the head, tend to suggest a separate and distinct entity for the disorder.

Notwithstanding the difficulty regarding the classification of cluster headache, it is generally agreed that the headache is due mainly to dilatation of the extracranial vessels (Horton *et al.*, 1939; Kunkle *et al.*, 1952), with some contribution from the internal carotid artery, if some of the eye and nasal symptoms are to be accounted for (Friedman and Mikropoulos, 1958). The mechanism of the vasodilatation, however, is far from clear. Many authors have implicated abnormal efferent nerve impulses originating at the nerve, or ganglia supplying the area of the headache. The neural structures implicated so far have been the sphenopalatine ganglion (Sluder, 1908), the vidian nerve (Vail, 1932), the ciliary nerve (Harris, 1936) and the greater superficial petrosal nerve (Gardner *et al.*, 1947). None of the treatments suggested, e.g., cocainization of the sphenopalatine ganglion, alcoholic injection of the Gasserian ganglion or petrosal neurectomy, produced any consistent relief and all have failed to stand the test of time.

The theory that the headache is due to the local release of a vasodilating agent is not without its proponents. Kunkle (1959) implicated acetylcholine in the mechanism of vascular headache, but made no distinction between cluster headache and migraine. He found acetylcholine-like activity in the cerebrospinal fluid of 5 of his 22 patients during attacks of such headache. Increase in the excretion of both lysine and 17-ketosteroids during cluster headache have been reported suggesting some biochemical derangement during the attack (Friedman and Mikropoulos, 1958), but no further detailed studies have been published. Horton (1941) himself suggested that histamine could be the locally released factor, as he was able to induce headache in 35 out of 72 of his cases with subcutaneous injection of histamine and claimed relief of the condition following histamine desensitization.

Serotonin (5-hydroxytryptamine, 5HT) is known to be involved in the mechanism of

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migraine headache, where the attack is accompanied by a fall in plasma levels of the amine (Curran *et al.*, 1965; Anthony, *et al.*, 1967, 1969). However, no comparable study has been undertaken in relation to cluster headache.

In an attempt to define the role of both serotonin and histamine in the mechanism of this particular variety of headache, we here report the findings of a study involving estimations of plasma levels of both amines before, during and after an attack.

Materials and Methods

All 20 patients admitted to this study suffered from typical cluster headache and all were male. Their ages varied from 20 to 57 years and they had been subject to attacks of headache for 1 to 24 years. In 5 patients the headache was induced with 1 mg of sublingual nitroglycerin (Ekbom, 1968) and in a further 4 by the ingestion of alcoholic drinks, which from past experiences were known to precipitate attacks in these patients. In the remainder the headaches studied developed spontaneously.

The 20 patients experienced 22 headaches, and estimations of plasma serotonin were performed in all, whilst whole blood histamine was estimated during 13 of these. Blood specimens were collected at 15 to 30-minute intervals so as to establish a baseline until the headache developed, and then at 10 to 15-minute intervals until the conclusion of the attack. One or more specimens were collected after the headache had ended.

Plasma serotonin was estimated fluorometrically by the method of Crawford and Rudd (1962), and whole blood histamine by condensation with o-phthalaldehyde (Shore *et al.*, 1959).

Results

Mean levels of whole blood histamine for the pre-headache, headache and post-headache periods were 0·045, 0·053 and 0·049 μg/ml respectively. Values for plasma serotonin for the corresponding three periods were 0·48, 0·57 and 0·59 $\mu g/10^9$ platelets (Table 1). Whilst the rise of whole blood histamine levels during headache is followed by a fall once the attack is concluded, those for serotonin continued to rise (Fig. 1).

Nonparametric analysis of histamine values shows a statistical difference between the three periods which is significant at the 1-2% probability range. Similar analysis of serotonin values shows the difference between the three periods to be outside statistical significance (probability range 20-30%).

Discussion

The results of this study suggest that an attack of cluster headache is associated with a release reaction involving histamine. As plasma serotonin levels are higher during the attack, although this rise does not reach statistical significance, it is possible that serotonin is similarly involved in the headache process.

As far as biochemical changes are concerned an attack of cluster headache appears to differ from that of migraine in two respects—

(1) Blood histamine levels rise during the attack, whereas no such change was observed during attacks of migraine in seven cases studied (unpublished observations).

(2) Plasma serotonin levels possibly rise during cluster headache, whereas the migrainous attack is accompanied by a marked fall in the majority of cases (Curran *et al.*, 1965; Anthony *et al.*, 1967).

On the other hand it bears some resemblance to the biochemical changes during the initial phase of the migrainous attack, when plasma levels of serotonin are higher when compared with

Table 1

Mean Levels of Plasma Serotonin and Whole Blood Histamine, Before, During and After an Attack of Cluster Headache

	Serotonin $\mu g/10^9$ *platelets*	*Histamine* $\mu g/ml$
Preheadache	0·48	0·045
Headache	0·57	0·053
Postheadache	0·59	0·049
Number of Headaches Studied	22	13

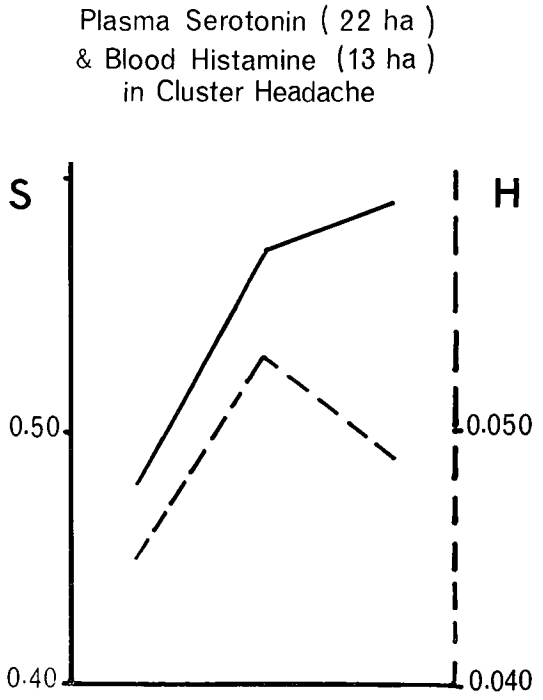

FIG. 1—Mean levels of whole blood histamine and plasma serotonin before, during and after an attack of cluster headache.
(Histamine - - - - - - -
Serotonin ————)

baseline values (Anthony *et al.,* 1969), just before the precipitous fall.

Release of preformed histamine occurs explosively, within a few seconds, as it has been demonstrated in several species, including man (Kahlson, 1966), whereas that of other vasoactive substances, e.g., serotonin, catecholamines, kinins, prostaglandins, etc., is a slower process. The rise in blood histamine during cluster headache, reported in this study, could reflect such a release and could explain at least some of the clinical features of the attack, e.g., the rapid onset of headache, the quality of the pain or the rise in skin temperature on the affected side of the head. If the rise in plasma serotonin, which outlasts that of blood histamine, could be proven to be significant, it is possible that it represents a compensatory response to the released histamine, as its vasotonic effect would tend to neutralize the histamine-induced vasodilatation and bring the clinical attack to an end.

The observation that BC105, a cyclohepta-thiophene derivative, possessing antihistaminic and antiserotonin properties, is useful in preventing spontaneous attacks of cluster headache (Sicuteri *et al.,* 1967; Ekbom, 1968), as well as blocking the ability of nitroglycerin to precipitate attacks during a bout (Ekbom, 1968), serves to emphasize the role of histamine in cluster headache.

It appears that the above facts and observations would tend to give credence to Horton's unproven hypothesis that an attack of cluster headache is due to the release of histamine, as suggested by the relief afforded to some of his patients following histamine desensitization.

SUMMARY

Whole blood histamine was estimated during 13 and plasma serotonin during 22 attacks of cluster headache. Blood histamine levels rose during and fell at the conclusion of the attack, whereas levels of plasma serotonin rose during the attack, but showed no fall at its conclusion. Analysis of the figures showed that the rise in histamine levels reached statistical significance, whereas those for serotonin did not do so.

It is suggested that an attack of cluster headache is associated with a release reaction, involving mainly histamine and possibly serotonin, and that this release could explain, at least, some of the clinical features of the attack. It is further suggested that serotonin release could be a compensatory response, its vasotonic effect neutralizing the histamine-induced vasodilatation, thus bringing the clinical attack to an end.

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THE INFLUENCE OF HORMONAL CHANGES UPON MIGRAINE IN WOMEN

B. W. SOMERVILLE*

INTRODUCTION

Clinical observation suggests that changes in levels of sex hormones influence migraine attacks in some women. For example, migraine may recur with predictable regularity just before or during menstruation. Migraine is improved or remits completely in the majority of women during pregnancy (Friedman and Merritt, 1959), although exacerbation during pregnancy has been reported (Callaghan, 1968). Migraine is frequently aggravated by taking oestrogen-progestogen combinations for contraception (Whitty, Hockaday and Whitty, 1966; Grant, 1968).

The tendency for migraine to recur regularly in some women around the time of menstruation suggests that migraine may be precipitated by withdrawal of progesterone, or oestrogens, or both. Alternatively, alteration of the oestrogen/progesterone ratio could be responsible.

Plasma levels of the two major female sex hormones, progesterone and oestradiol, can now be measured accurately by competitive protein-binding techniques which require only small quantities of plasma (Murphy, 1964). Thus, it is now possible to follow daily changes in plasma levels of these hormones in women subject to menstrual migraine.

PLASMA PROGESTERONE LEVELS IN MENSTRUAL MIGRAINE

The aim of this study was to follow plasma progesterone levels daily throughout the entire menstrual cycle, in normal, migraine-free women serving as controls, and in women who suffered from regular menstrual or premenstrual migraine, to determine whether any significant difference could be detected between these two groups. In the migrainous women, the exact timing of the onset of menstruation and of migraine were recorded, and related to the level of plasma progesterone.

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METHODS

Migrainous women: Five women, all suffering from recurrent severe throbbing headaches, lasting in each case for several hours, and accompanied by nausea with or without vomiting, were studied. In three of these, the headache was unilateral, and in one there were prodromal visual symptoms. In each case there had been one severe attack each month, clearly related to menstruation in timing, and recurring monthly for at least twelve successive menstrual cycles immediately prior to the investigation.

Non-migrainous controls: Five normal women volunteers from the State Reformatory for Women, Malabar, were studied. Two of these subjects were multiparous. All had regular menstrual cycles, and were aged from 19 to 40 years; none was taking any hormonal preparation.

All women were asked to record accurately the onset and duration of menstruation. The migrainous women were asked to record the onset and duration of migraine. Venepuncture was performed daily at about 9 a.m., beginning in the middle of the cycle, and continuing until the corresponding point in the following menstrual cycle had been reached. About 15 ml of plasma was obtained each day from each subject, and this was divided into aliquots of suitable volume for the measurement of plasma progesterone, oestradiol, and serotonin (5-hydroxytryptamine).

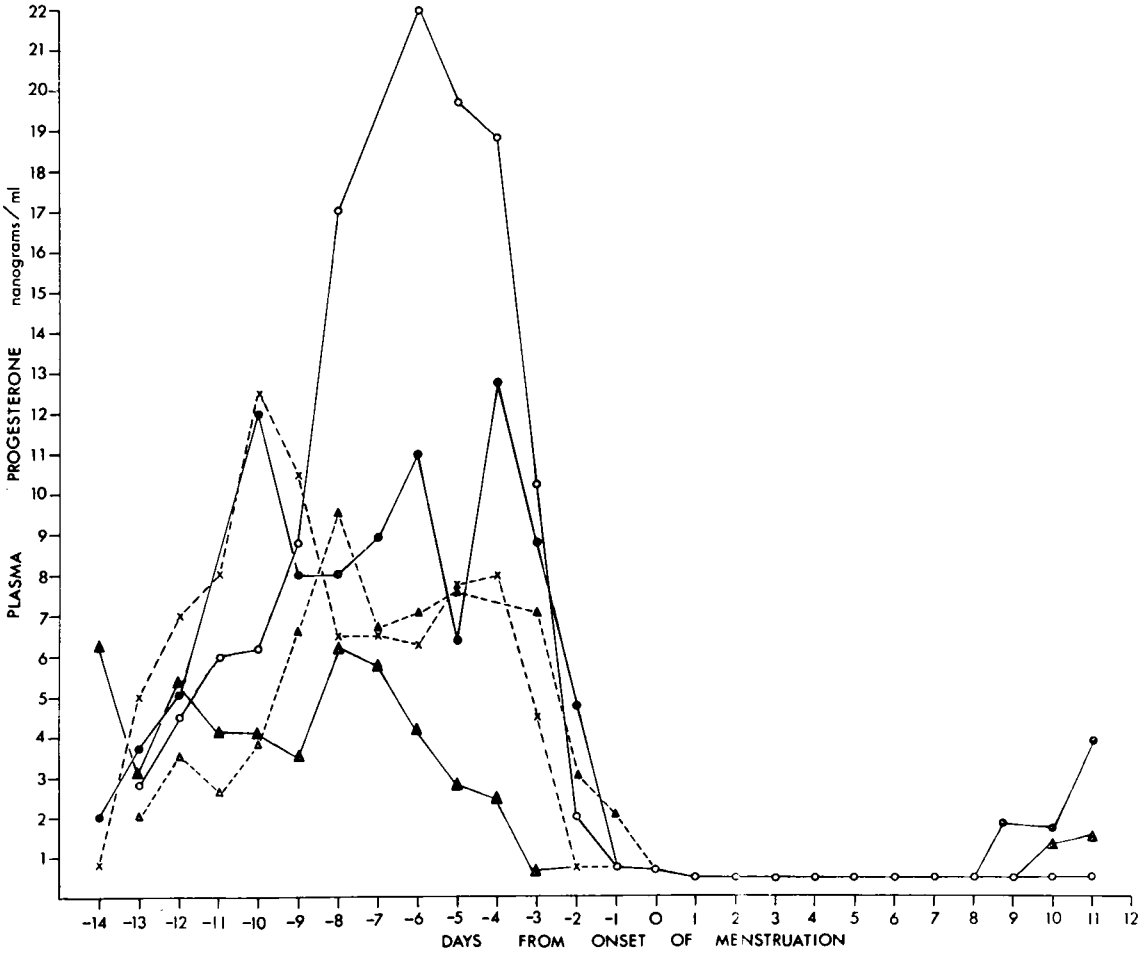


FIG. 1—Plasma progesterone levels in five normal, non-migrainous women.

Plasma progesterone levels were determined by the rapid method of Johansson (Ref. 7, 1969). The only departures from his technique were the use of canine cortisol-binding globulin as the binding protein, and the use of multiple small columns of Sephadex to separate protein-bound from displaced, free corticosterone. Both of these modifications have been described by Bassett and Hinks (1969). Plasma progesterones were determined in duplicate using 0·5 ml aliquots of plasma.

RESULTS

As individual cycles varied in length, the progesterone values have been plotted around the first day of menstruation ("Day 0"). In both groups, the plasma progesterone rose sharply after presumed ovulation in mid-cycle (approximately 14 days before the onset of the next menstruation). In the non-migrainous group, the mean of peak plasma progesterone levels was $12{\cdot}7 \pm (5{\cdot}95)$ nanograms/ml, and in the migrainous women, the mean of peak progesterone levels was $15{\cdot}3 \pm (2{\cdot}21)$ nanograms per ml. The difference between the means of these two groups was not statistically significant ($p > 0{\cdot}10$).

The majority of subjects in each group showed a period of sustained, though widely fluctuating, high levels of progesterone for some days following the peak; then, from about 6 days before menstruation, there was a rapid reduction in progesterone levels, until at the onset of menstruation, the progesterone level

in every case was less than 2·0 ng/ml. In both groups, progesterone levels remained low throughout menstruation and during the early follicular phase, reflecting the absence of corpus luteum activity.

The five migrainous subjects each developed their typical headaches, as shown in Fig. 2. In three patients, the onset of migraine occurred during the phase of rapid reduction of progesterone levels. In one patient, migraine occurred on the second day of menstruation, at the "tail end" of the falling phase; and in the remaining patient, the onset of headache occurred just before the phase of rapid progesterone withdrawal.

The Effect of Progesterone Upon Menstrual Migraine

As the previous study had shown that menstrual migraine occurred during the phase of progesterone withdrawal in the subjects studied, it was decided to investigate the effect of artificially maintaining high levels of progesterone on menstrual migraine. Two subjects have been studied to date. Daily progesterone levels were estimated for several days before and during menstruation for one control (untreated) cycle. During the subsequent cycle, progesterone 75 mg in oil was injected intramuscularly daily for several days before menstruation, and plasma progesterone levels were estimated daily as before. The injections were continued for at least two days after the expected onset of menstruation in each of the regularly-cycling subjects studied.

Results

Figs. 3 and 4 illustrate that the daily injection of progesterone 75 mg produced a high, though fluctuating, level of progesterone in the plasma. These levels were somewhat higher than those encountered during the luteal phase of the control (untreated) cycle. One patient menstruated on the expected date, but de-

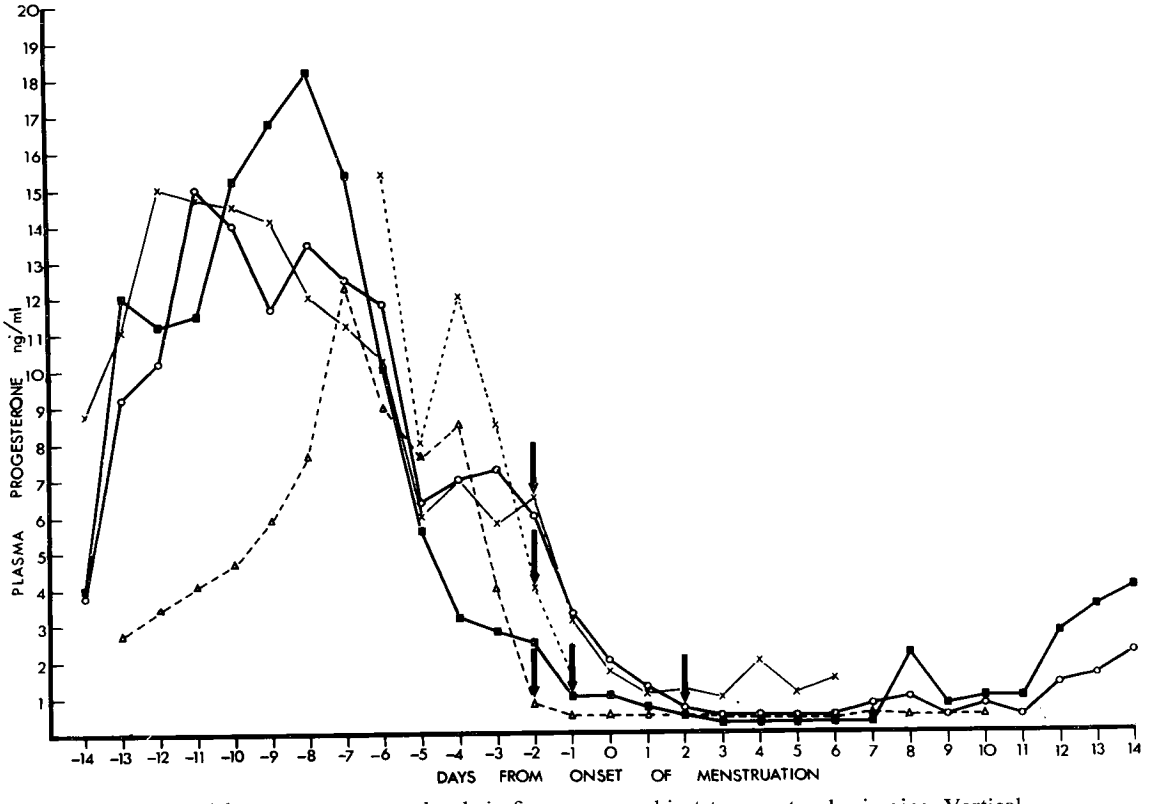
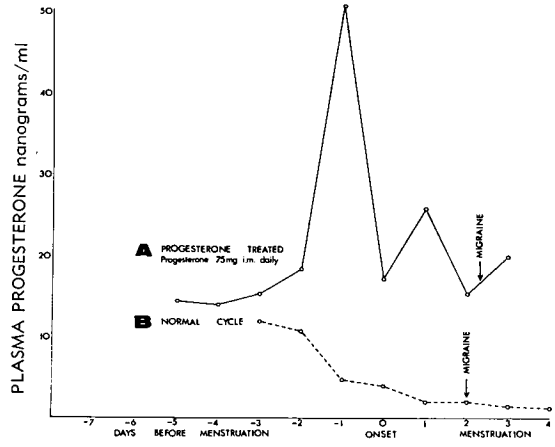
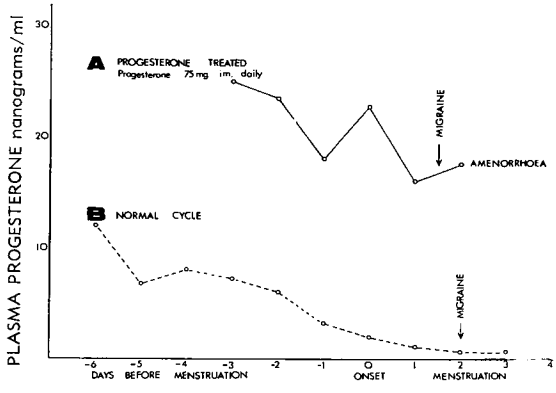


Fig. 2—Plasma progesterone levels in five women subject to menstrual migraine. Vertical arrows indicate the onset of headache.





FIGS. 3 and 4—The effect of progesterone on menstrual migraine.

veloped her usual migraine at the customary time. The other patient developed amenorrhoea, but also experienced her usual headache at the expected time, although menstruation occurred only several days later, i.e. when the injections of progesterone had ceased.

PLASMA PROGESTERONE AND MIGRAINE DURING PREGNANCY

The relief of migraine in the majority of women during pregnancy prompted this study, which is still in progress. Women attending the antenatal clinic at the Hospital for Women, Crown Street, Sydney, are questioned during the four weeks before the estimated date of confinement regarding headaches. Patients who are subject to recurrent paroxysmal headaches, lasting for hours at a time, and accompanied by nausea with or without vomiting are classified as migrainous, and are further classified according to whether or not they have experienced substantial relief of migraine during the current pregnancy. A single blood sample is taken during the last four weeks of pregnancy from women in three categories, viz.:

(1) Normal, non-migrainous pregnant women.
(2) Migraine sufferers who have obtained relief during the current pregnancy.
(3) Migraine sufferers who have not experienced relief during the current pregnancy.

Plasma levels of serotonin, progesterone, and oestradiol are being estimated in women of each of these groups, to see whether relief from migraine can be correlated with hormonal levels.

Preliminary data (Fig. 5) suggest that there is no significant difference in plasma progesterone levels between each of the three groups. Progesterone levels during the last four weeks of pregnancy are about tenfold higher than during the peak of the luteal phase. Plasma oestradiol levels during the last four weeks of

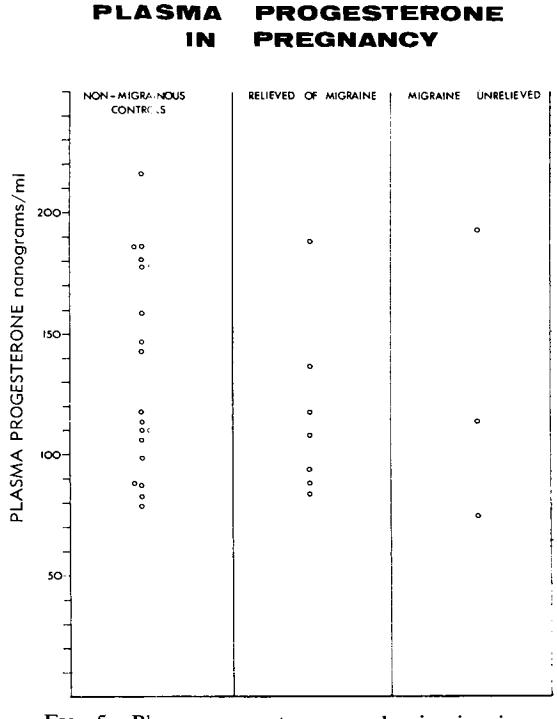


FIG. 5—Plasma progesterone and migraine in pregnancy.

pregnancy are of the order of one hundred times higher than in the non-pregnant state.

It is remarkable that pregnancy is usually associated with improvement in migraine, in the presence of greatly increased plasma levels of both oestradiol and progesterone. This suggests that fluctuations in hormone levels, rather than absolute levels, are more likely to be involved in the precipitation of migraine. During pregnancy, these fluctuations are suppressed, and are replaced by gradual changes in levels of progesterone and oestrogens. This hypothesis is supported by the observation that migraine may occur in particularly severe form within a few days of delivery.

Discussion

In each of the five migrainous women studied throughout the entire menstrual cycle, the migraine occurred during the phrase of progesterone withdrawal. This finding might at first seem to support the hypothesis of Whitty (1966) that menstrual migraine in women on synthetic oestrogen-progestogen combinations might be precipitated by withdrawal of synthetic progestogen.

In the two women studied to date, however, artificially maintaining the plasma progesterone at the high levels found during the luteal phase had no influence on the migraine, and migraine still occurred at a time when the progesterone levels were demonstrably high in each case. It is noteworthy that in one woman, the headache occurred at its usual time, despite the absence of menstruation, demonstrating that the processes of menstrual bleeding and menstrual migraine are separable, and that the appearance of menstrual migraine does not necessarily depend upon menstrual bleeding. Further quantitative hormonal studies, especially of plasma oestrogen levels, are clearly indicated.

It should be emphasized that other sex hormones are also falling concurrently with progesterone in the days preceding menstruation. This applies particularly to the oestrogens, of which oestradiol-17β is probably the most physiologically important. Although no definite conclusions can be drawn at this stage, it would appear that fluctuations in plasma oestrogen levels are a more likely cause of menstrual migraine than fluctuations in plasma progesterone levels.

Relief of migraine during pregnancy does not appear to be related to any significant difference between plasma progesterone levels in women who experience relief of headache during pregnancy, those who do not experience relief during pregnancy, and normal, headache-free pregnant women, when single determinations of plasma progesterone are made during the last four weeks of pregnancy.

Daily Plasma Serotonin Levels in Normal Women and in Women Subject to Menstrual Migraine

The demonstration of a fall in plasma serotonin and the rise in urinary metabolites of serotonin which occur in the migraine attack underlines the importance of serotonin in the migraine syndrome (Anthony, Hinterberger and Lance, 1967). In the present study, the plasma serotonin level was determined daily throughout the menstrual cycle in the two groups of women (normal controls and women subject to menstrual migraine). The normal control group was expanded by the addition of four more volunteers. The aim of this study was to determine whether the clustering of migraine around the time of menstruation could be attributed to changes in daily plasma serotonin values.

Plasma serotonin was measured by the spectrofluorimetric method (Crawford and Rudd, 1962), and expressed as micrograms/10^9 platelets. From Figs. 6 and 7, it can be seen that in neither group was there any significant variation in serotonin levels around the time of menstruation, compared to the intermenstrual period. It should be pointed out, however, that this method of obtaining only one sample of plasma each day would be inadequate to demonstrate the fall in serotonin described by Anthony *et al.* (1967), who took plasma samples at intervals of a few hours before, during, and after the migraine attack.

Summary

Plasma progesterone levels have been measured daily throughout the menstrual cycle in five women subject to menstrual migraine, and in five normal controls. In each of the migrainous subjects, the migraine began during the phase of progesterone withdrawal. There was no significant difference between means of peak

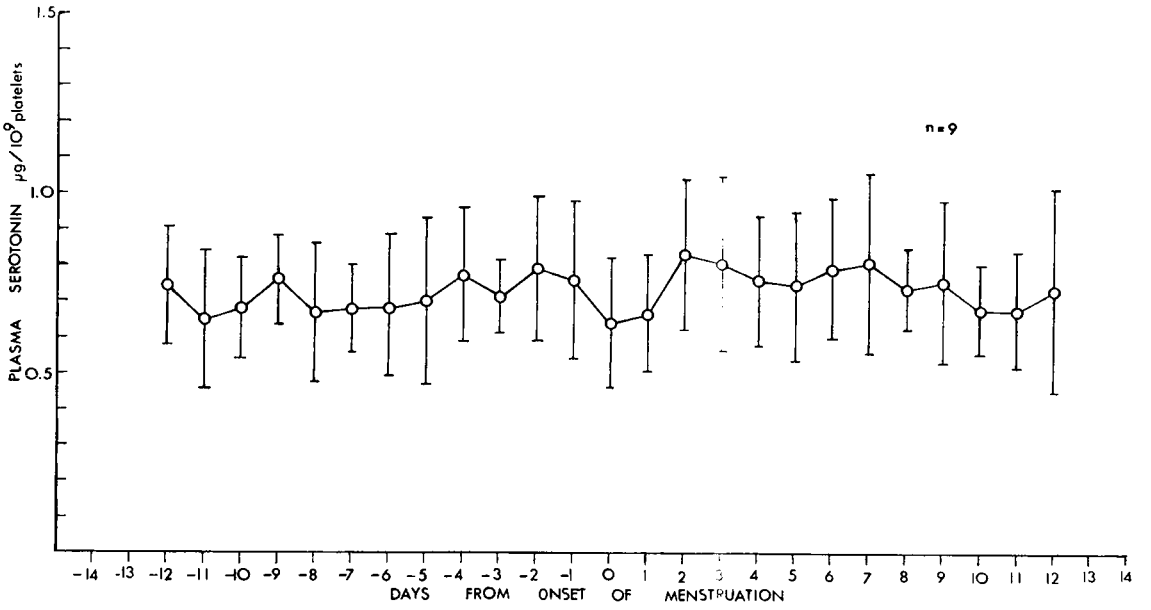


FIG. 6.
Plasma Serotonin: Controls.

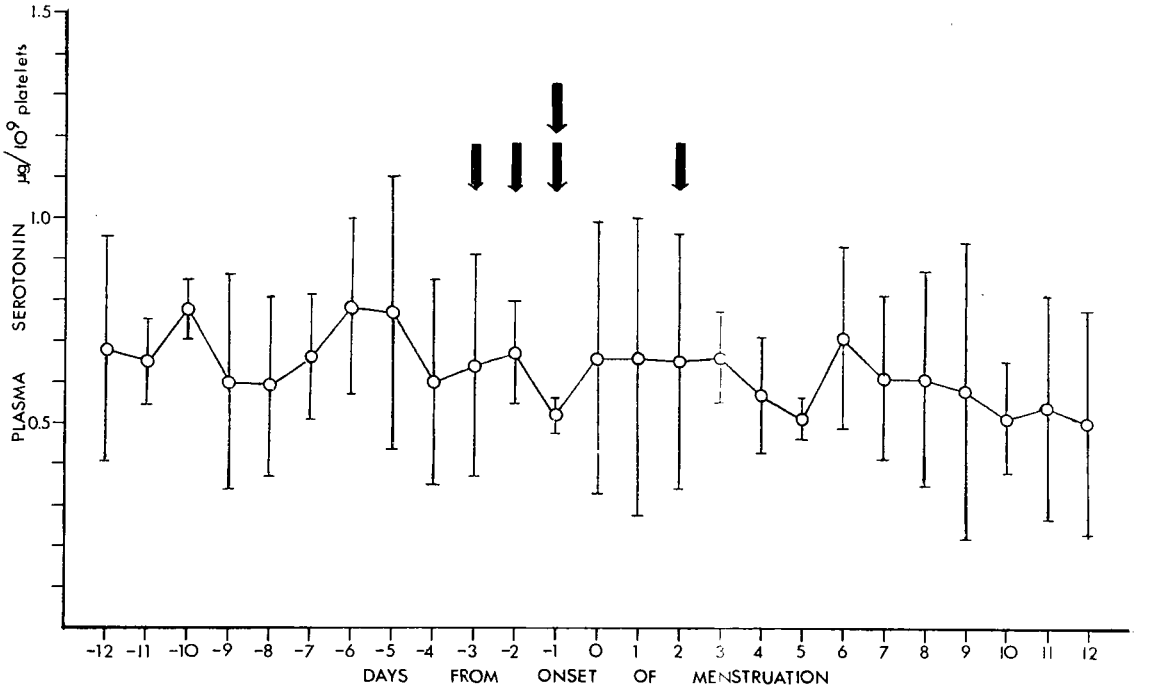


FIG. 7.
Plasma Serotonin: Menstrual Migraine.

FIGS. 6 and 7—Plasma serotonin levels in normal controls and in women subject to menstrual migraine. Vertical arrows indicate the onset of headache.

progesterone levels of the two groups (migrainous group mean 15·3 ± 2·21 ng/ml, non-migrainous group mean 12·7 ± 5·95 ng/ml, $p > 0·10$).

Two women subject to menstrual migraine have been treated with daily injections of progesterone. In both cases, the usual headache developed in the presence of high plasma progesterone levels. Withdrawal of progesterone did not initiate a migraine in either of these subjects.

A current study of migraine during pregnancy is described. There appears to be no significant difference between plasma progesterone levels in non-migrainous pregnant women, in women who have experienced relief from migraine during pregnancy, and in women who have continued to suffer from migraine during pregnancy.

Daily estimation of plasma serotonin throughout the menstrual cycle in women subject to menstrual migraine and in normal controls has not demonstrated any significant fluctuation around the time of menstruation to account for the more frequent occurrence of migraine at this time of the menstrual cycle.

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DISCUSSION

Dr. E. Davis: What about migraine occurring in men?

Reply: It is clear that hormonal changes are only one of several factors precipitating migraine. Given the constitutional predisposition, many factors, for example tyramine in foods, or emotional stress, may also precipitate migraine.

Dr. R. Burns: I notice that oestradiol reaches maximum levels in the middle of the cycle, then falls sharply. If oestradiol withdrawal were responsible for menstrual migraine, would one not expect migraine to occur at this time?

Reply: Mid-cycle migraine due to this mechanism may well occur, although women may fail to notice the connection with the menstrual cycle. Menstrual migraine is obvious to women because it occurs closely related to visible uterine bleeding. It is well recognized that mid-cycle migraine may occur regularly in some women.

Assoc. Prof. J. McLeod: Is there any rational basis for the use of diuterics in menstrual migraine?

Reply: Fluid retention was at one time thought to be the factor responsible for menstrual migraine. However, Schottstaedt and Wolff treated women with diuretics, and prevented fluid retention premenstrually. Unfortunately, their subjects still developed migraine. It would appear that there is no rational basis for diuretics in the prevention of migraine.

ABNORMAL PROTHROMBIN TIMES IN CEREBROVASCULAR DISEASE

J. BARRIE MORLEY, M. KORMAN and W. G. PARKIN

INTRODUCTION

This study arose from the chance observations in two middle-aged males, both with severe cerebral haemorrhage attributed to systemic hypertension. Although there was no evidence of any other medical disease, and particularly none of any bleeding diathesis or liver disease, the intriguing clinical observation was that both exhibited a marked bruising tendency. Both patients had several examinations of platelet counts and prothrombin times, the latter consistently being abnormal. At autopsy, there was confirmation of the absence of liver disease, or disturbance of the reticulo-endothetial system, and of the presence of severe cerebral haemorrhage.

These observations immediately prompted questions regarding any possible relationship between the cerebral insult and this apparent bleeding diathesis, particularly since, if any such relationship existed, it could weigh adversely against the prognosis by increasing the risk of further cerebral haemorrhage. Certainly, it is occasional autopsy experience to find a "secondary" cerebral haemorrhage having complicated an earlier acute cerebrovascular insult.

To attempt to satisfy curiosity, over the last three years, the prothrombin times and other blood coagulation studies have been performed on patients admitted with strokes to Prince Henry's Hospital.

METHOD

Any patient was considered for the study who was admitted with any form of stroke. From the outset, no attempt was made to distinguish between cerebral haemorrhage and cerebral infarction, essentially because of the frequent difficulties encountered in making such distinctions. In fact, as the study proceeded, it became clear that, for the purposes of this study, such distinction would be redundant. Indeed, eventually the study was extended to include patients with subarachnoid haemorrhage, provided there was such clinical evidence of cerebral damage as confusion or impaired consciousness, or clear focal neurological signs.

Grounds for exclusion from the study consisted of known chronic alcoholism or known liver disease, or any generalized medical condition that conceivably could affect the blood coagulative mechanism. In addition, in the earlier part of the study, within the first three days of admission, estimation of serum salicylates and barbiturates, and full liver function tests were carried out on each patient; these were not continued in the final stages of the project, as it was found that these were infrequently elevated, and then to minor proportions.

The study to date has been conducted in three chronological phases, each corresponding to the minor differences in the laboratory technique of the Quick's prothrombin time examination, and in the venepuncture technique. In phase 1, the venepuncture was performed by the Ward Nursing Staff; the blood was sent to the Laboratory with all other hospital specimens in the routine way, to be examined unidentified with all other hospital prothrombin samples for the day; the Commonwealth Serum Laboratory prothrombin reagent was used.

After some time, it became clear that abnormal readings were occurring occasionally among the normal controls. Accordingly, phase 2 of the project was organized. The three medical officers involved in this study performed all these venepunctures, at the same time of the day, and under the same stringent precautions; notably, care was taken to avoid

bubbling of the blood, and the tourniquet was applied for no longer than a minute. The blood immediately was placed in a standard citrated tube, and packed in ice. Within the next twenty minutes, this blood was centrifuged, and the plasma so obtained was frozen. By arrangement with Dr. J. Hirsh, and Dr. A. Gallus, of the Department of Medicine, St. Vincent's Hospital, these plasma samples were delivered every few days to that Department, for examination of prothrombin time, also for examinations for thrombin clotting times and fibrin breakdown product; furthermore, Dr. Gallus provided fibrin plates, on which 0·03 ml of each serum specimen was placed, to be incubated and read after twenty-four hours. After following this procedure with twenty patients, the results were reviewed; as will be indicated, significantly meaningful figures were obtained, and with this reassurance, and because of the inconvenience of continuing with this procedure, phase 3 of the study was organized.

In this third phrase, the procedure has reverted largely to that of the first phase of the study. The only modifications are that the venepunctures are performed by the trained Haematology Nursing Staff, and that the Quick's prothrombin reagent is that produced by Warner. Again, the prothrombin time is estimated as part of the routine Prince Henry's Hospital haematology procedure.

In each of these three phases, the procedure involved the taking of blood on as many days as possible, from as early as possible in the illness, until the tenth day of the illness. Initially, the blood sampling was performed over the first three weeks, but it was found that the additional ten days did not provide further information. In a few instances, patients died or were discharged from hospital before this ten-day period elapsed; if the prothrombin time had not fallen to abnormal levels, these patients were excluded from the study on the basis that the result was inconclusive.

The essential intention of the study was to determine the number of stroke victims in whom the prothrombin times fell to abnormal levels during the first ten days of the illness. A result above 50% was accepted as normal, which, according to Sawers (1964), is the level below which anti-coagulant therapy should be maintained. In the first instance, a control was required against the possibility that abnormal prothrombin readings in the test group were occurring merely by random chance, or by errors of venepuncture technique, or an inherently faulty laboratory technique. During phase 1 of the study, full blood samples were taken from sixteen normal persons (medical and laboratory staff), and were compared with the fifty-nine samples taken from the twenty-three stroke patients in this first phase. Individual samples were compared, rather than the individual subjects, as the errors, and the random chance, under examination would be operating for each blood sample taken. Table 1 demonstrates this comparison, and since, by applying χ^2 test there exists a truly significant difference between the control and test groups, this comparison with normal control subjects was discontinued. Despite this significant difference, the fact that six samples from normal persons were abnormal prompted the setting up of a procedure in which greater confidence could be placed, both in venepuncture technique, and in laboratory procedure. Accordingly, phase 2 was devised, as outlined above.

At this point, the second control was initiated, using the stroke patients as their own controls. This was planned by dividing the patients into two groups according to the severity of the illness. Patients were judged severely ill (group II), if there was mental confusion or clouded consciousness, or if the hemiplegia was absolute. All other patients were classified as group I, which thus comprised the less ill patients. This comparison was based on the premise that any factor operating in stroke patients to cause an abnormal prothrombin time might be more evident in the more severely ill patients. This control was continued into phase 3 of the study after phase 2 concluded.

Results

As already mentioned, the possibility was examined that the abnormal prothrombin times resulted merely by chance, or by errors of technique. Table 1 demonstrates that only six of the sixty-four blood samples from normal subjects were normal, as compared to twenty-one abnormal samples from the fifty-nine samples taken from stroke patients. This difference is statistically significant.

The next question is whether this abnormal

TABLE 1

Comparison of prothrombin times in samples from normal subjects and stroke patients

NUMBER OF BLOOD SAMPLES

	Abnormal Prothrombin Time	*Normal Prothrombin Time*	*Total Examined*
Normal subjects (16)	6	58	64
Stroke patients (23)	21	38	59

prothrombin time, which occurs in the presence of cerebrovascular damage, is merely a non-specific effect. To date, a total of fifty-five patients with strokes have been examined in the study, and Table 2 itemizes the results, comparing the patients in group I and group II respectively as outlined above. These figures represent those numbers of patients in each group, in whom the prothrombin time was abnormal on at least one occasion during the ten-day test period. This manner of representation avoids undue loading of the results by patients in whom there have been several normal or abnormal prothrombin times within the test period. In Table 2, there would appear to be a trend of greater occurrence of abnormal prothrombin times in the severe group (73% compared to 52%); however, statistically these total numbers are not yet sufficient for full confidence that there is a true difference between these groups. However, by applying the χ^2 test, if this trend continues over the next thirty patients examined, statistical confidence can be obtained. Meanwhile, particular attention is drawn to the figures for phase 2 (Table 2): nine of the ten severely ill patients had an abnormal prothrombin time at least once, whereas only three of the ten mildly ill patients did so. By applying Fisher's Exact Test, applicable for these small numbers, the difference between these two groups is clearly significant. It is emphasized that it was at this stage of the study that the techniques were under closest examination, and it is in this phase that there has been the greatest confidence of any.

Also, during phase 2, each blood sample that was taken for estimation of prothrombin times also was examined for thrombin clotting time, fibrin breakdown products, and evidence of fibrinolysins. All specimens tested were negative for these substances. In addition, during phase 2, each patient had formal liver function tests performed within the first few days of the onset of the illness: eight patients had an elevation of the alkaline phosphatase (range of elevation 17 to 21 K.A. units), and five patients had raised S.G.P.T. (range of elevation 32-43 units). Other elements of the liver function test were not significantly abnormal. These findings of the raised alkaline phosphatase or raised S.G.P.T. occurred in patients both with and without abnormal prothrombin times. Platelets were counted at least once in the first four days of the illness, and on no occasion were these abnormally low.

DISCUSSION

Throughout this study, the relative imprecision of the prothrombin time test has been kept much in mind, and has prompted continuous examination of the study. Nevertheless, despite three minor modifications in the technique, there remains no doubt of the significant occurrence of the abnormal prothrombin times in patients suffering from any form of stroke

TABLE 2

Tabulation of numbers of patients in whom abnormal prothrombin times occurred

PHASE

	1		*2*		*3*		*Totals*	
	Abnormal	*Total Patients*	*Abnormal*	*Total Patients*	*Abnormal*	*Total Patients*	*Abnormal*	*Total Patients*
Mild (Group I)	10	16	3	10	2	3	15	29
Severe (Group II)	5	7	9	10	5	9	19	26
Total	15	23	12	20	7	12	34	55

which causes parenchymal damage. Thus, 34 of the 55 patients examined had an abnormal prothrombin time at least once in the first ten days of the illness. That this is a truly significant occurrence is demonstrated by Table 1 as discussed above.

Recently, there have been reports of blood coagulation disturbances in various neurological disorders. Initially these concerned the platelet properties in disseminated sclerosis (Nathanson and Savitsky, 1952; Caspary, Prineas, Miller and Field, 1965; Wright, Thompson, and Zilkha, 1965; Millar, Merritt and Dalby, 1966), and recently fibrinogen abnormalities have been noted in cerebrovascular disease (Bang and McDowell, 1966; Ettinger, Kusonoki, and Fujishama, 1967); Ettinger *et al.* did not observe disturbed prothrombin times.

Perhaps, the abnormal prothrombin time reflects disturbed platelet function, similar to that described in disseminated sclerosis, although the cause-effect relationship may not be necessarily the same. It is likely that the abnormal prothrombin times could be indicating part of a dynamic sequence of disturbed coagulation reactions, for Bang and McDowell observed an initial hypercoagulability in the first hours after the infarction, followed by an excessive bleeding tendency, and clearly a complex series of events could be involved.

The question remains as to whether this prothrombin disturbance is specifically aligned to the cerebral damage in cerebrovascular disease. Coagulative changes are known to occur in a variety of acute illnesses (Hampton and Mitchell, 1966), and any condition which affects liver function even briefly, such as cardiac failure, can disturb the prothrombin time (Sawers, 1964). However, it seems unlikely that hepatic dysfunction is the explanation for the abnormal prothrombin times in this study; liver function tests in patients of this study showed only mild disturbance in a minority of patients and such changes occurred irrespective of whether or not such patient had an abnormal prothrombin time.

In fact, the findings of this study throw no light on the possible mechanisms of any prothrombin time disturbance; such would require more sophisticated examination. One speculative possibility stems from the fact that the cerebral tissues' supply of tissue thromboplastin is used routinely in the Quick's prothrombin test itself; perhaps in cerebral haemorrhage or infarction there is sufficient *in vivo* prothrombin consumption to cause a prothrombin deficiency. In recent times, there has arisen the concept of "consumption coagulopathy" as the basis for a number of widespread general medical disturbances, and, although Bang and McDowell do not use this term, they postulate this mechanism as the basis for their observations. However, against this being operative in this study's patients, is the finding in phase 2 of normal thrombin clotting times. Nevertheless, cerebral tissue is known to be rich in many thromboplastic factors, and although no "anti-prothrombin factor" has yet been identified (Bidwell, 1969), an additional suggestion is that this could be released by cerebral damage.

Whatever may be the mechanism, it is clear that a substantial number of patients, with any form of cerebral damage from a stroke, can be found to have abnormal prothrombin times, if this is sought sufficiently frequently in the first ten days of the illness. In all probability, this arises from multiple disturbances of coagulation factors, some of which may be operating in a dynamic sequence. Whatever the basis, these observations provoke additional thoughts of theoretical consequence. Any possible "auto-coagulative" disorder could be expected to influence adversely the prognosis of patients with acute cerebrosvascular insults; perhaps this could be relevant to the autopsy observations of "secondary" cerebral haemorrhage following earlier acute cerebrovascular insult. In addition, similar propositions should be pursued in other types of cerebral disease, notably head injuries and tumours, and it is of interest that these associations appear to have been the subject of three recent reports (Delli and Arcidiacono, 1967; Morozov, 1967, and 1968).

SUMMARY

A prospective study is being conducted, which indicates that the prothrombin time can be found to be prolonged in the early stages after cerebral haemorrhage or infarction, especially if severe. It remains uncertain whether this is a specific result of the cerebral insults, or part of a general medical disturbance, although the results to date tend to support the former possibility.

ACKNOWLEDGMENTS

Thanks are due to Dr. D. C. Forster and his staff of the Haematology Department, and to the Honorary Medical Staff of Prince Henry's Hospital, for permission to investigate patients under their care. Particularly, the assistance was greatly valued of Dr. J. Hirsh, and Dr. A. Gallus, both of the Department of Medicine, St. Vincent's Hospital, Melbourne. Mrs. P. Graves of the Department of Mental Health, Victoria, provided the statistical analyses. This study was conducted during the tenure by one of us (J.B.M.) of a Fellowship in Medicine, Neurology, of Prince Henry's Hospital.

DISCUSSION

Dr. Rischbieth: Recently, a male came under my care with an abrupt onset of mild dysphasia. Assuming that this was of ischaemic origin, carotid angiography was performed, and found to be normal. However, it was noted subsequently that he developed a sizable haematoma at the site of the percutaneous injection. Subsequently, preparatory to commencing anticoagulant therapy, we found that his prothrombin time was only 30% and remained at that level for several days. At that time, I wondered whether this finding was related to the patient having just had an angiogram, although after hearing Dr. Morley's paper I am less inclined to this view. Nevertheless, I wondered how many of the patients in this study may have had angiograms performed.

Dr. Morley: I regret that I cannot give you actual figures, but I think I can answer your question in this way. Plainly, only patients with established stroke were included in the survey, thereby excluding those patients with so-called "transient ischaemic attacks". It is our practice usually not to perform angiograms on patients with established stroke, unless the stroke has had time to resolve, and assuming that the angiogram is requisite in determining further management of the patient. This policy holds for all strokes including subarachnoid haemorrhage. Accordingly, a relatively small minority of these patients would have had angiograms performed, and then at least 10-14 days after the onset of the illness, i.e. after the conclusion of the observation period.

Dr. M. Anthony: Does Dr. Morley intend to pursue this investigation further on the basis of this disturbance of prothrombin time, with such means as the thromboplastin generation time test?

Dr. Morley: To date, our terms of reference have been confined to establishing simply whether or not there is a real disturbance of prothrombin time, and if so, whether such disturbance is directly related to the cerebral insult. To extend the scope beyond this, we have deemed outside our competence. Nevertheless, I agree that it is desirable that this should be pursued along lines such as suggested by Dr. Anthony.

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OXIDATIVE ENZYME ACTIVITY IN SINGLE NEURONES IN RELATION TO SELECTIVE VULNERABILITY TO ISCHAEMIA*

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INTRODUCTION

In ischaemia and hypoxaemia it is known that neurones in certain cerebral regions are more likely to sustain damage than are neurones in other regions. Two hypotheses have been put forward to explain such selective ischaemic vulnerability. The vascular hypothesis of Spielmeyer (1928) attempted to account for the phenomenon purely in terms of differences in local blood supply, whereas the Vogts (1922) suggested that a "pathoclisis", a postulated regional pecularity in the physico-chemical constitution of the brain, was responsible. There is agreement that, at least in the brain stem, vascular factors alone cannot adequately account for selective vulnerability to ischaemia (Scholz, 1953). On the other hand the actual factors responsible for pathoclisis do not seem to have been defined.

We have carried out studies to try to determine whether patterns of neuronal metabolism in different brain regions can be related to selective ischaemic vulnerability. The problem has been investigated in the cerebellar cortex and in certain brain-stem nuclei. In the cerebellar cortex it is known that the Purkinje cells are more prone to ischaemic injury than the neighbouring granule cells (Scholz, 1953; Meyer, 1958). In brain stem ischaemia due to vertebro-basilar arterial insufficiency and basilar artery migraine, disturbance of equilibrium is more frequent than disturbance of hearing (Williams and Wilson, 1962; Bradshaw and McQuaid, 1963; Eadie and Tyrer, 1968). This suggests that the vestibular nuclei are more susceptible to ischaemic disturbance than the cochlear nuclei as neither the pattern of blood supply to the inner ear, with its origin from a single vessel and its anastomoses between terminal arteries (Alpers, 1958), nor the oxidative enzyme constitution of the vestibular end organ (Nomura and Balogh, 1964) would suggest that the selective vulnerability was determined peripherally. It is difficult to conceive how vascular factors alone could account for the vulnerability to ischaemia of the whole layer of Purkinje cells in the cerebellar cortex, and Kety's (1963) measurements show that the vestibular and cochlear nuclei of the brain stem have similar perfusions, so that ischaemic vulnerability in these regions of brain stem and cerebellum seems likely to involve a factor of pathoclisis.

* This work was supported by a grant from the National Health and Medical Research Council of Australia.

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THE NEURONE IN ISCHAEMIA

A prime funcion of the neurone is to conduct impulses, and it does this by virtue of producing an ionic concentration gradient across its cell membrane. The energy to maintain this concentration gradient is obtained from the metabolism of glucose, which breaks down anaerobically to pyruvate, which then breaks down in the absence of oxygen to lactate, and in the presence of oxygen, via the Krebs cycle, to CO_2 and water (Fig. 1). The aerobic metabolism of glucose yields about 11 times as much energy as its anaerobic metabolism (Dixon, 1965). As well as the usual Embden-Myerhoff pathway for anaerobic oxidation of glucose there is an alternative parallel anaerobic pathway ending in lactate though passing through the pentose phosphate shunt. As a neurone at any time has some minutes' reserve

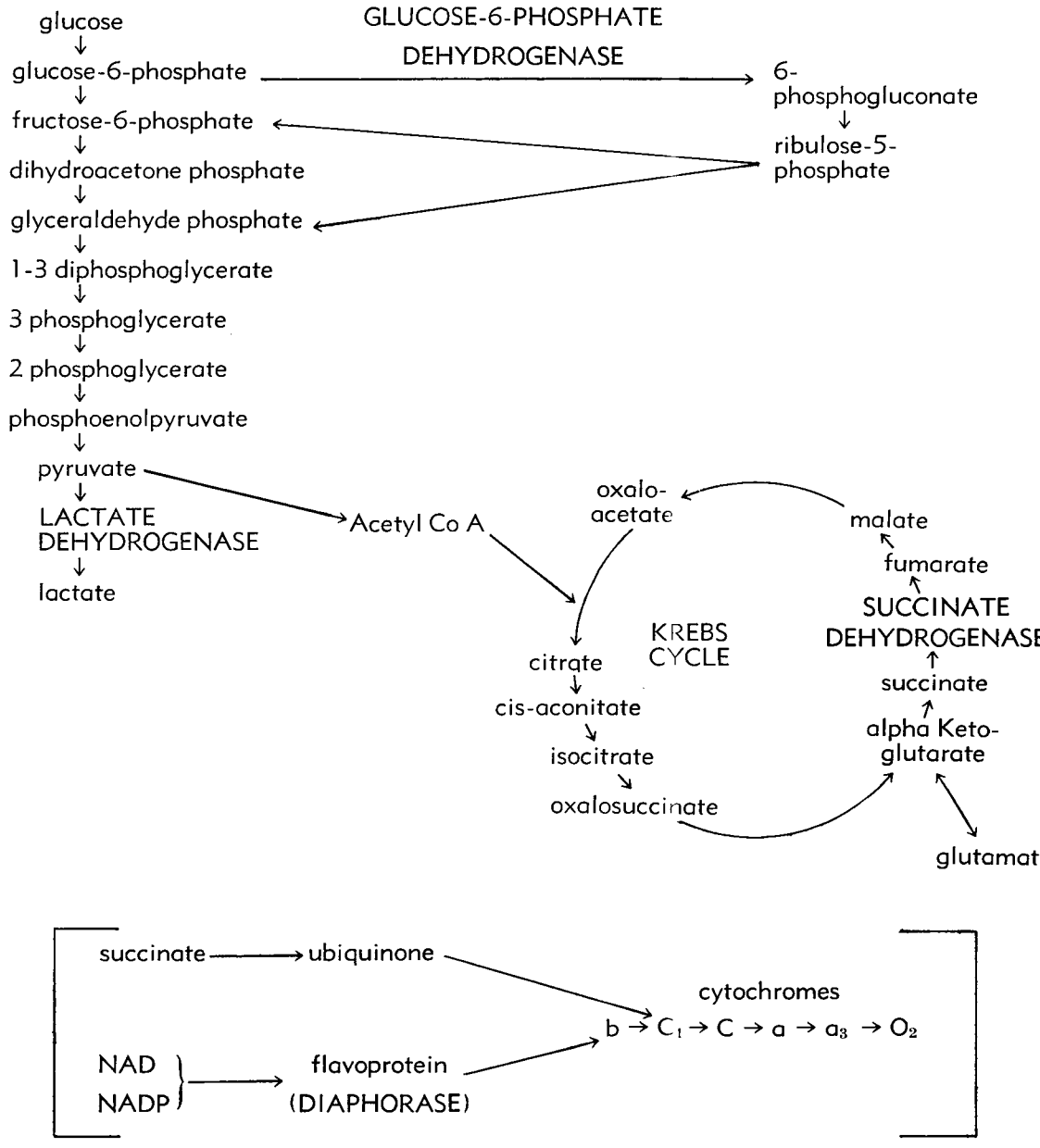

FIG. 1—An abbreviated outline of the pathway for neuronal metabolism of glucose. The enzymes studied in the present histochemical work are shown in capitals. The other enzymes involved in the pathways are not indicated. The electron transport pathway is shown at the bottom of the page.

supply of glucose, but only about 10 seconds reserve of oxygen (Dixon, 1965), oxygen lack is the determinant of ischaemic damage. The more highly developed the Embden-Myerhoff or pentose phosphate pathway is relative to the Krebs cycle in a neurone, the better equipped is that neurone to keep functioning in the presence of ischaemia. The concentration in neurones of enzymes of anaerobic Embden-Myerhoff glycolysis (e.g., lactate dehydrogenase —L.D.H.) and of the pentose phosphate shunt (e.g., glucose-6-phosphate dehydrogenase—G-6-P.D.H.), relative to their concentrations of aerobic Krebs cycle enzymes (e.g., succinate dehydrogenase—S.D.H., and for the most part N.A.D. diaphorase), can thus serve as a measure of ischaemic vulnerability.

However, a consideration of ischaemic vulnerability at the cellular level must also take into account local tissue geometry. Neurones are not all of the same size and shape, and hence, in ischaemia, all other things being equal, the larger the cell body the greater the potential vulnerability because of surface/mass considerations. Therefore not only enzyme concentration, but total enzyme content of neuronal cell body, provides a measure of susceptibility to ischaemia. High concentrations, and high total contents of Krebs cycle enzymes, should correlate with vulnerability to ischaemia, and high concentrations of enzymes of anaerobic glycolysis and of the pentose-phosphate shunt with resistance to ischaemia. However, high total contents of the latter anaerobic enzymes may correlate with susceptibility to ischaemia, rather than with resistance to it, because the anaerobic breakdown of glucose produces lactic acid, and as in the presence of an inadequate circulation this will diffuse less adequately from larger than from smaller cells, there is more risk of altered cell pH interfering with neuronal function in larger cells.

In the light of the above considerations, the potential ischaemic vulnerability of structures in the cerebellar cortex and in vestibular and cochlear nuclei was studied by measuring the mean cytoplasmic concentrations, and total contents in a 20μ thick segment of cell body cytoplasm, of the following enzymes:—

N.A.D. diaphorase, succinate dehydrogenase (Krebs cycle), lactate dehydrogenase (anaerobic glycolysis) and glucose-6-phosphate dehydrogenase (pentose phosphate pathway). The measurements were made on neurones in the medial and lateral (Deiters') vestibular nuclei, the dorsal and ventral cochlear nuclei, the abducens nucleus, and the Purkinje cells of the cerebellum. Measurements also were made on fixed volumes of cerebellar cortical granular and molecular layers, in which regions the enzymes are present in the neuropil.

Material Studied

The enzymes mentioned above were studied in one three-month-old human infant brain, and in two adult human brains, both over the age of 60 years. Portions of brain were quenched in liquid nitrogen within 8 hours of death for the adult material, and parts of the infant brain were quenched between 6 and 12 hours after death.

Methods

The four enzymes studied were demonstrated in 20μ thick cryostat microtome sections by standard tetrazolium histochemical methods as described by Pearse (1960), with minor modifications. The reactions were calibrated in accordance with principles defined by Eadie *et al.* (1970) so that all results were expressed in arbitrary units which were directly proportional to the amount of H^+ transferred by enzyme activity in unit time.

The histochemical system used is based on the following principle, and results in the production of blue insoluble particulate deposits of the diformazan of Nitro-blue tetrazolium at sites of enzyme activity in tissue.

$$\text{substrate} \xrightarrow{\text{DEHYDROGENASE}} \text{product} + H^+$$

$$H^+ + \text{NAD(P)} \longrightarrow \text{NAD(P)H}$$

$$\text{NAD(P)H} + \text{flavoprotein (DIAPHORASE)} \rightarrow \text{NAD} + \text{reduced flavoprotein (FADH)}$$

$$\text{FADH} + \text{tetrazolium salt} \rightarrow \text{FAD} + \text{formazan}$$

The light absorbances of the formazan deposits in individual neurones were measured by the Ornstein-Patau (1952) two-wave length method of cytophotometry, using a Leitz MPV microspectrophotometer. This instrument contains an adjustable diaphragm which permits optical isolation and measurement of the dimensions of a portion of a microscope field in which one is interested, and then measurement of the intensity of the light passing through that portion of the field delimited by the diaphragm.

TABLE 1

Mean concentrations of enzyme activity (expressed in arbitrary units) in single neurones of different brain regions. For simplicity standard deviations are not shown, and sets of data within which there are statistically significant differences ($P < 0.05$) are marked with an *. Findings for the brain stem structures were analysed separately from findings for the cerebellum. The ventral cochlear nucleus was lost from one adult brain.

	Enzyme			
	N.A.D. Diaphorase	*S.D.H.*	*L.D.H.*	*G-6-P.D.H.*
		ADULT		
Lateral vestibular Nc.	0·087*	0·018	0·055	0·011
Medial vestibular Nc.	0·071*	0·016	0·049	0·008
Dorsal cochlear Nc.	0·049*	0·013	0·046	0·006
Ventral cochlear Nc	0·107*	0·025	0·055	0·014
Abducens nucleus	0·093*	0·018	0·056	0·010
Cerebellar cortex				
Purkinje cells	0·088*	0·019	0·056	0·006
Granular layer	0·030*	0·014	0·029	0·005
Molecular layer	0·030*	0·011	0·028	0·004
		INFANT		
Lateral vestibular Nc.	0·062*	0·011*	0·016*	±
Medial vestibular Nc.	0·052*	0·009*	0·006*	±
Dorsal cochlear Nc.	0·038*	0·008*	0·010*	±
Ventral cochlear Nc.	0·064*	0·019*	0·019*	±
Abducens nucleus	0·087*	0·011*	0·025*	±
Cerebellar cortex				
Purkinje cells	0·050*	0·008*	0·021*	±
Granular layer	0·023*	0·006*	0·011*	±
Molecular layer	0·021*	0·007*	0·010*	±

± Too little enzyme to be measured with acceptable accuracy.

TABLE 2

Mean content of enzyme activity in cell bodies in 20 μ thick segments of different brain regions, expressed in arbitrary units. Standard deviations are omitted. The findings for the brain stem structures were analysed statistically, and sets of data containing significant differences ($P < 0.05$) are marked with an *. The ventral cochlear nucleus was lost from one adult brain.

	Enzyme			
	N.A.D. Diaphorase	*S.D.H.*	*L.D.H.*	*G-6-P.D.H.*
		ADULT		
Lateral vestibular Nc.	74·3*	14·8*	49·2*	9·3*
Medial vestibular Nc.	25·0*	5·7*	21·8*	2·8*
Dorsal cochlear Nc.	9·7*	2·8*	10·9*	1·3*
Ventral cochlear Nc.	37·6*	9·4*	30·6*	5·6*
Abducens nucleus	66·4*	14·1*	47·4*	6·5*
Cerebellar cortex				
Purkinje cells	40'1	8·6	32·6	3·1
		INFANT		
Lateral vestibular Nc	26·0*	6·9*	12·0*	±
Medial vestibular Nc.	15·6*	2·6*	1·7*	±
Dorsal cochlear Nc.	6·4*	1·3*	3·0*	±
Ventral cochlear Nc.	16·8*	6·3*	8·8*	±
Abducens nucleus	33·2*	4·9*	12·8*	±
Cerebellar cortex				
Purkinje cells	15·0	2·6	8·4	±

± Too little enzyme to be measured with acceptable accuracy.

Results

Enzyme concentrations measured in brain stem and cerebellar neurones and cortical laminae are set out in Table 1, the results for the infant and adult brains being analysed separately. Findings for total enzyme content of 20μ thick segments of neurones in these structures are set out in Table 2.

Discussion

If one accepts the criteria of ischaemic vulnerability delineated earlier in the present paper, the results of the study would suggest on the whole that the Purkinje cells of the cerebellum should tolerate ischaemia less well than would the adjacent granular and molecular layers. Although lactate dehydrogenase concentration data could imply that the Purkinje cells would be less vulnerable than granule cells it must be remembered that aerobic metabolism is so much more efficient in producing energy than anaerobic glycolysis that it would take a many times larger increase in lactate dehydrogenase activity to compensate for any excess of succinate dehydrogenase activity. Moreover, the fact that several capillaries would be expected to occur in a volume of granular layer equal to the volume of the perikaryon of a single Purkinje cell (Lierse, 1963) is an additional factor favouring Purkinje cell vulnerability to ischaemia.

In the vestibular and cochlear nuclei the data for total enzyme content of cell body would tend to suggest that the giant Deiters' cells should be more susceptible to ischaemia than cochlear neurones. This would be in keeping with the clinical observation that vertigo occurs more readily than hearing disturbance in brain stem ischaemia.

Thus chemical factors which could explain pathoclisis have been determined for the cerebellar cortex and for certain brain stem nuclei. Further study will be necessary before a general explanation for pathoclisis can be provided, but it is interesting that pathoclisis in the situations studied may be less a matter of major differences in patterns of metabolism between different cell groups, than of differing cell body dimensions in the presence of generally similar patterns of neuronal metabolism.

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Discussion

Dr. Burns: I wonder if you have considered the possibility that the vertigo of basilar artery disease might not arise from the brain stem or inner ear? The basilar artery supplies part of the blood that goes to the temporal lobes and the giddiness might originate here.

Dr. Eadie: I have to admit that that is a possibility though I would have thought that the brain stem was the more likely site of origin of the vertigo. We have not looked at oxidative

enzyme activity in the temporal lobe yet, though we hope to.

Dr. Alpers: I think I have read that the Purkinje cells are tetraploid and I wonder if lateral vestibular cells also might be tetraploid?

Dr. Eadie: It would be quite feasible to measure the D.N.A. content of the cells with the microspectrophotometer but I cannot see how this information would help one assess the problem of ischaemic vulnerability.

CLINICAL AND PATHOLOGIC ASPECTS OF CENTRAL NERVOUS SYSTEM INVOLVEMENT IN THE HAEMOLYTIC URAEMIC SYNDROME

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INTRODUCTION

The clinical and pathologic features of the Haemolytic Uraemic Syndrome (H.U.S.) have been recognized and reported with increasing frequency in the past decade. Although most reports acknowledge that cerebral involvement is common, the incidence and variability of neurological features are discussed in detail only in the papers of Gianantonio *et al.* (1964), Habib *et al.* (1967) and Mathieu *et al.* (1969). Barnard and Kibel (1965) briefly review neuropathological findings in five cases, but no detailed study of the neuropathology of the H.U.S. is known to the authors. It is the purpose of this report to describe the incidence and type of clinical neurological involvement in a consecutive series of patients with the H.U.S. and report further details of neuropathology in this condition.

CLINICAL FEATURES OF CENTRAL NERVOUS SYSTEM INVOLVEMENT

These were studied in a consecutive series of 25 patients (Table 1) seen between January 1963 and June 1969 in a large children's hospital; eleven of the patients died during the acute phase of the illness. All patients satisfied the usual clinical criteria for diagnosis of the H.U.S., i.e. acute haemolytic anaemia with typical fragmentation and distortion of red blood cells, thrombocytopenia and renal impairment. Two patients presented with neurological features; the remainder had vomiting and/or diarrhoea for several days as the initial manifestation. Pallor was noted in all patients. The platelet count was 70,000 per cmm or less in 24 patients, and 102,000 per cmm in the remaining patient. All patients had a blood urea of 70 mg per 100 ml or greater at some stage during the acute illness. To assist in clinico-pathological correlation a brief summary of the clinical history is provided in the section dealing with pathology of those patients who died and whose brains were available for examination.

The neurological symptoms and signs noted may conveniently be divided into minor and major syndromes.

Minor Central Nervous System Disturbance

Irritability and drowsiness were noted in most cases. These symptoms frequently preceded more serious manifestations, but in eight instances provided the only evidence of nervous system dysfunction. Minor twitching was present in two cases. Three of the older patients had headaches, but in each case this was a prelude to more severe central nervous system disturbance.

Major Central Nervous System Disturbance

Thirteen of the 25 patients had evidence of severe central nervous system dysfunction. Eleven of these had convulsions, and in six cases these were at times of focal or multifocal type. Four patients had generalized tonic spasms of decerebrate type. All patients who had such spasms died. Further details of these are included in a subsequent case history (Case 22). Four patients had transient hemiplegia. Marked depression of conscious state (coma or semi-coma) was present in 10 patients and in one infant this persisted for six weeks. However, this patient survived with no neurological

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sequelae. The cerebrospinal fluid was examined in three patients. In one case the cell count was abnormal, containing 37 polymorphonuclears and 16 lymphocytes per cmm, protein 40 mg per 100 ml and sugar 40 mg per 100 ml. Later examination of the brain (Case 11) revealed a cerebral infarct as the probable cause of this cellular response. Another patient had raised protein in the cerebrospinal fluid (Case 16). Electroencephalograms were obtained in eight patients, and in five showed an excess of slow wave activity, often of marked degree.

Neurologic Manifestations as Presenting and Predominant Features

Of special interest are two children in whom neurologic disturbance was the initial and major clinical manifestation. The case histories of these patients are summarized in some detail.

CASE 16: S.M., a five-month-old male infant had two left-sided convulsions in the 24 hours prior to admission, each lasting about 20 minutes. There were no urinary or gastro-intestinal symptoms. Pregnancy, labour and delivery were uneventful, general health was good, and development normal. When seen the baby was alert and contented. Blood pressure was not recorded at this stage. Pallor was not marked and bruising was not seen. Twenty-four hours after admission a generalized but predominantly right-sided convulsion lasting 10 minutes was observed. The blood sugar (Dextrostix) was 90 mg per 100 ml and blood pressure immediately postictal 130/100. A few fine petechiae on the limbs and trunk were then noted for the first time. Over the next two days two further convulsions occurred, each lasting about 15 minutes. On the first day in hospital results of investigations were: haemoglobin 10·0 gm per 100 ml with a normal peripheral blood film; the cerebrospinal fluid contained 2 lymphocytes per cmm, 2 red blood cells per cmm, protein 55 mg per 100 ml and sugar 40 mg per 100 ml; skull x-ray and chest x-ray were normal; micro urine showed 10 white blood cells per cmm and 130 red blood cells per cmm, and Albustix testing gave +++ reaction for urinary protein. On the fourth day there was increased pallor, he vomited several times and became disinterested in feeding.

TABLE I

Central nervous system in 25 children suffering from the Haemolytic Uraemic Syndrome

Case No.	*Year*	*Age*	*Sex*	*Main Neurological Features*	*Outcome*
1	1963	3 mo.	M	Nil	R
2	1963	6 mo.	F	Listless, irritable and "jumpy"	D
3	1963	1 yr.	M	* Status epilepticus, transient hemiparesis, decerebrate spasms	D
4	1964	2½ yr.	F	Drowsiness and irritability	R
5	1964	10 mo.	F	Neck retraction and full fontanelle	R
6	1964	7½ yr.	M	* Headache, G.M. convulsions, coma, and opisthotonic spasms	D**
7	1964	5 mo.	M	* Status epilepticus and coma	D**
8	1964	3½ yr.	F	Nil	R
9	1964	8 mo.	F	Nil	R
10	1964	10 mo.	M	* G.M. convulsions, decerebrate spasms, coma, transient L. hemiparesis	D
11	1965	8 mo.	F	* L. focal seizures, semi-coma	D**
12	1967	3 mo.	F	Irritable	R
13	1967	2½ yr.	M	* R. focal and generalised convulsion and coma	D
14	1968	3½ yr.	F	Irritability	R
15	1968	7 mo.	F	Drowsiness	R
16	1968	5 mo.	M	* Multi-focal and generalised convulsions	R
17	1968	4 mo.	F	* G.M. convulsions	R
18	1968	6 yr.	F	* Aphasia, R. hemiparesis, coma and decerebrate spasms	D**
19	1968	10 mo.	F	* G.M. and focal R. convulsions, semi-coma, L. hemiparesis	R
20	1968	6 mo.	M	Nil	R
21	1968	16 mo.	F	Irritable and jittery	D**
22	1969	5 yr.	F	* Headache, strabismus, dystonic and decerebrate spasms	D**
23	1969	9 yr.	M	* R. focal convulsions, hallucinations, transient L. hemiparesis transient R. hemiparesis, cortical blindness, coma, retinal haemorrhages	D**
24	1969	6 mo.	M	Nil	R
25	1969	7 mo.	F	* G.M. convulsions	R

* Major neurological disturbance
** Brain examined (see appendix)

D Death during acute illness
R Recovered from acute illness

Purpura was present on both legs and the upper chest. Blood pressure was 160/100 and haemoglobin 5·0 gm per 100 ml. A peripheral blood film now showed characteristic changes of the H.U.S. with fragmentation of red cells, anisocytosis, poikilocytosis and microcytosis. The platelet count was 47,000 per cmm. The blood urea was 80 mg per 100 ml. Blood transfusion raised the haemoglobin to 12·9 gm per 100 ml. No further convulsions occurred, he improved progressively and was discharged well 10 days later. When last seen five months after the acute episode developmental progress was normal. Blood pressure, micro urine and blood urea were normal, and there was no proteinuria.

CASE 22: J.B., a five-year-old girl was referred with a provisional diagnosis of cerebral tumour. Her past history was uneventful, and development normal. Three months prior to admission a minor fall was followed by a brief episode of vomiting. Mild headaches and vomiting occurred infrequently over the following weeks. Four days before admission headache and vomiting became more severe. A previously noted mild left squint became more marked. Initial examination revealed a pale, listless but cooperative and alert child. She was afebrile and blood pressure was 120/80. A soft ejection systolic murmur was heard at the cardiac apex. There was general hypotonia but deep tendon reflexes were brisk and symmetrical. The fundi were normal. A left nonparalytic internal strabismus was present. Lumbar puncture revealed pressure of 100 mm of cerebrospinal fluid; there were no cells, protein was 25 mg per 100 ml and sugar 50 mg per 100 ml. Twelve hours later pallor was more marked and a few scattered petechiae were noted on the trunk and upper limbs. Conscious state was now depressed and intermittent dystonic spasms of limbs with athetoid posturing of hands and decerebrate posturing of legs appeared. The spasms were associated with tongue protrusion, opening of the mouth and emission of a strange cry. Bilateral ankle clonus and extensor plantar responses were present. Urine had not been passed since admission. Blood examination showed haemoglobin 6·0 gm per 100 ml and platelet count of 27,000 per cmm. The red cells demonstrated marked anisocytosis, microcytosis, poikilocytosis, and numerous burr cells and fragmented cells were seen. Blood urea was 370 mg per 100 ml. Serum electrolytes—sodium 136 mEq per litre; potassium 7·9 mEq per litre and chloride 91 mEq per litre. Bleeding time, 6 minutes; capillary clotting time, 3½ minutes; fibrinogen (Fibrindex test) normal. Skull x-ray normal. An E.E.G. revealed generalized slow delta activity. Blood cultures were negative, and virus studies from throat swab, faeces and cerebrospinal fluid were negative. The patient died less than 24 hours after admission as dialysis and heparinization were being commenced. Autopsy was performed and showed changes in the kidney typical of H.U.S. The cerebral pathology is described below.

PATHOLOGICAL FEATURES OF CENTRAL NERVOUS SYSTEM INVOLVEMENT

The brains of seven patients who died were available for neuropathological examination. After fixation in formalin, surface structures and coronal sections were examined macroscopically and tissue was taken for histological examination from frontal, temporal, and occipital cortex, basal ganglia and thalamus, midbrain, pons, medulla and cerebellum.

CASE 6: I.H., aged 7 years, was admitted with a 3-day history of diarrhoea, abdominal pain and the passage of blood and mucous per rectum. Abdominal tenderness was the only abnormal physical sign noted initially, and blood pressure was normal. Haemoglobin was 14 gm per 100 ml, platelet count 25,000 per cmm, and peripheral blood smear contained numerous distorted and fragmented cells. The blood urea was 260 mg per 100 ml. On the second day in hospital, headache, convulsions and coma ensued, and he died 3 days after admission.

No major abnormality was present on macroscopic examination of the brain, although there was some congestion of pial vessels, and vessels seen on the cut surface of coronal sections of the brain. A small slit haemorrhage was present in the cortex of one occipital lobe. Microscopic examination revealed some small perivascular haemorrhage adjacent to this slit haemorrhage, but the absence of any associated inflammatory reaction suggested they were terminal. There was no definite cerebral oedema present, and no evidence of vasculitis or eosinophilic thrombi.

Sections of the kidneys showed multiple hyaline eosinophilic capillary thromboses in glomeruli and juxta-glomerular arterioles. Coagulative necrosis, exfoliation and vacuolation of tubular cells was also present. The rectal mucosa also showed extensive necrosis with prominent thrombosis of vessels in submucosa, muscularis, and adventitial layers.

Comment: The outstanding feature was the paucity of neuropathological findings to correlate with the history of headache, grand mal convulsions, coma and opisthotonic spasms that had occurred during the six-day illness.

CASE 7: B.M., aged 5 months, had diarrhoea, vomiting and pallor for 6 days before admission to a country hospital where a provisional diagnosis of megaloblastic anaemia was made. He was transferred for further investigation when he failed to respond to folic acid. Prolonged convulsions occurred on the way to hospital and he was pale and comatose when admitted. Haemoglobin was 3·6 gm per 100 ml with marked anisocytosis, poikilocytosis and fragmentation of red cells. The platelet count was 20,000 per cmm and blood urea 263 mg per 100 ml. In spite of resuscitative measures including blood transfusion he died 17 hours later.

No significant abnormality was present on macroscopic examination of the brain. Microscopic ex-

amination of cerebral cortex and white matter revealed patchy anoxic damage with chromatolytic neurons, particularly in the occipital cortex and some swollen vacuolated astrocytic nuclei in adjacent white matter. Occasional vessels contained pink hyaline material, but there was no visible abnormality of vessel walls. The cerebellum showed congestion of vessels and small perivascular haemorrhages, but Purkinje cells appeared normal. Examination of other areas revealed no definite pathology of neural structures or associated blood vessels.

Sections of the kidney showed marked glomerular crowding, with avascularity of glomerular loops due to increased ground substance and intracapillary thrombosis and endothelial cell hyperplasia. Tubules showed patchy necrosis, dilatation, vacuolation and cast formation.

Comment: The significance of occasional cerebral vessels containing pink hyaline material is doubtful as it is our impression that this finding is not infrequent as an unexplained incidental finding in a variety of unrelated conditions. There was no evidence of vasculitis. The recent changes were thought to be due to hypoxic episodes during prolonged convulsions that occurred during a 300-mile ambulance journey to hospital.

CASE 11: K.L., aged 8 months, had diarrhoea, vomiting, fever, pallor and bruising noted for 2 weeks prior to admission. Investigation elsewhere revealed a haemoglobin 4·2 gm per 100 ml, platelet count 85,000 per cmm, and blood film showing numerous fragmented cells and 52 normoblasts per 100 white cells. Blood urea was 110 mg per 100 ml. She was transferred for further management and, although initially normotensive, marked hypertension was soon noted with blood pressure up to 230/130. The hypertension persisted intermittently and was not satisfactorily controlled with hypotensive drugs. Two months after admission onset of semi-coma and left focal seizures was noted. Although she then showed temporary improvement she remained lethargic and died 3 months after admission to hospital.

The cerebral hemispheres showed evidence of mild generalized atrophy with widened sulci. Coronal sections revealed mild dilatation of the lateral ventricles. Two small areas of cystic degeneration were present in the left internal capsule and right parietal region. The outstanding feature on microscopic examination of the brain was an area of infarction in the lower pons adjacent to the floor of the fourth ventricle. In this region swollen astrocytes and axons were conspicuous. No abnormality of vessels was evident and no hyaline thrombi were present. A mild accumulation of small round cells was present in the leptomeninges. Cerebral cortex was generally normal, but white matter showed an increase in astroglia cells and scattered small calcifications. An old infarct, probably of two to three months' duration, with dense gliosis of white matter and virtual absence of neurons in adjacent cortex was present in the occipital cortex. Some endothelial thickening was present in surface vessels. The cystic lesions in the region of the left internal capsule and right parietal region were infarcts. They were thought to be of approximately three to four weeks' duration. The cerebellum was normal.

The renal cortex was reduced in width and glomeruli were crowded. A large number of capillary loops were obliterated by intra- and extra-cellular P.A.S. positive material and remnants of hyaline intracapillary thrombi and foci of endothelial hyperplasia were present. Arteries showed prominent internal and external elastic lamina, and fibrinoid changes.

Comment: During this child's three-month illness there were prolonged periods of uncontrolled and marked hypertension and it is likely that the ischaemic and hypoxic lesions were related to vasospasm associated with hypertensive encephalopathy. It is probable that the left focal seizures approximately three weeks before death were due to the infarct on the right cerebral hemisphere, as a cerebrospinal fluid pleocytosis was present at that time.

CASE 18: G.T., 6 years, had onset of anorexia, vomiting, oliguria and passage of dark urine several days prior to admission to hospital. Examination elsewhere revealed mild generalized oedema and marked pallor. Blood pressure was normal. The haemoglobin was 6·1 gm per 100 ml, and blood urea 268 mg per 100 ml. She was subsequently transferred for further assessment and on admission to this hospital haemoglobin had fallen to 3·4 gm per 100 ml and blood smear showed many fragmented red cells. The blood

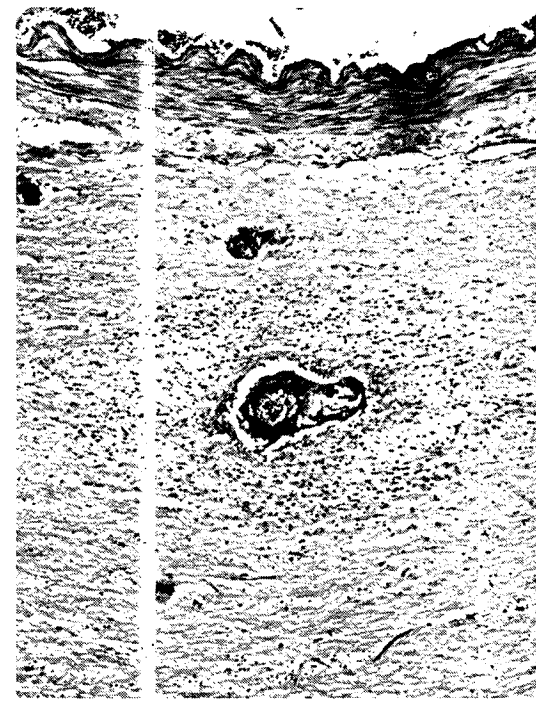

FIG. 1—Case 18. Section of anterior pons showing fibrinoid degeneration in perforating artery with surrounding ischaemic lesion and cellular infiltration.

urea was 355 mg per 100 ml. Blood transfusion was given and treatment commenced with high dosage cortico-steroids. After temporary improvement vertigo, aphasia and right hemiparesis appeared 10 days after admission. The following day she became comatose with decerebrate spasms, pin-point pupils and extensor plantar responses. She remained comatose and over the week prior to her death had fluctuating hypertension.

Macroscopic examination revealed generalized softening of the brain, Areas of congestion were present in the basal ganglia on the right, and a small haemorrhage was present in the cortex of the right lateral lobe of cerebellum. The outstanding feature on microscopic examination was fibrinoid necrosis of small vessels in the anterior pons with infarction of adjacent tissue in the pons (Fig. 1). Eosinophilic thrombi were not present in these vessels. In the cerebral cortex there was recent patchy anoxic damage with oedema, and chromatolysis of neurons. Small vessels in the cerebral cortex appeared collapsed and had prominent perivascular spaces of Held. In contrast, vessels in the white matter, which was oedematous, were dilated and congested. Hypoxic lesions, with pyknotic neurons and patchy oedema, were also present in basal ganglia and cerebellum, where there was moderate fallout of Purkinje cells and several small haemorrhages.

The renal histology showed focal hypercellularity, necrosis and fibrosis of glomeruli with numerous hyaline thrombi in capillary and small arterioles.

Comment: It was concluded that the ischaemic lesion of the pons was the basis of the decerebrate spasms, coma and pin-point pupils which occurred one week prior to death. Although there was fibrinoid necrosis of small pontine arteries it is uncertain whether this was the cause or effect of the infarct. Mild hypertension was present at the time of the episode. The widespread hypoxic damage was thought to be associated with terminal intermittent positive pressure artificial respiration.

CASE 21: A.S., aged 2 years, had diarrhoea and vomiting for several days prior to admission. Pallor was the main finding on examination. Haemoglobin was 8·9 gm per 100 ml, platelets 37,000 per cmm and blood smear showed marked fragmentation of red blood cells. Blood urea was 192 mg per 100 ml. Although she was irritable and jittery no specific neurological findings were noted. Urinary output was poor and blood pressure became elevated to 150/90. She became febrile and septicaemia was suspected. Peritoneal dialysis was employed but she died 10 days after the onset of symptoms. Aerobacter aerogenes was isolated from both antemortem and postmortem blood cultures.

Some petechial haemorrhages in the cerebral white matter were the only abnormalities noted on macroscopic examination. Histological study revealed fibrinoid necrosis of some small arterial vessels in the regions of petechial haemorrhage. Bacterial colonies were present in the walls of some vessels in the same areas of haemorrhage. No hypoxic changes were noted. Apart from congestion and a small amount of subarachnoid haemorrhage in the region of the mid-brain, no other abnormality was present.

Sections of the kidneys showed proliferation of endothelium and increased cellularity of glomeruli with basement membrane thickening. One glomerulus contained fibrin. Patchy lymphocytic macrophage and polymorph infiltration was present in the medulla.

Comment: It was established that this infant had terminal septicaemia, and it is likely that the colonies of bacteria noted on microscopic examination were due to postmortem proliferation of organisms. The petechial haemorrhages and fibrinoid necrosis of vessels in the areas of haemorrhage are probably due to the septicaemia.

CASE 22: Details of the clinical features are given previously.

No abnormality was apparent on macroscopic examination of the brain. Microscopic examination revealed some patchy oedema, especially of the frontal cortex and white matter with chromatolysis of some neurons in the insular region. No abnormality of the basal ganglia or cerebellum was present. Occasional chromatolytic neurons were present in the peri-aqueductal grey matter of the mid-brain. Congestion of some vessels and collapse of others, with surrounding oedema, was also present in the medulla. A similar change was present in the tegmentum of the pons. No primary vascular pathology was present.

Microscopic examination of kidneys revealed thrombi in glomerular capillaries and afferent arterioles. Patchy and extensive tubular necrosis was also present.

Comment: It is difficult to correlate the pathological findings of minimal hypoxic damage with the prominent neurological features described previously. It is possible that this child did not live long enough for more conspicuous pathological processes to become apparent, as the major neurological illness was of little more than 24 hours' duration.

CASE 23: R.C., aged 9 years, presented with a 10-day history of anorexia and vomiting, jaundice for one week, and haematemesis and epistaxis shortly before admission. Examination revealed pallor and lethargy but blood pressure was normal initially. Haemoglobin was 5·0 gm per 100 ml, platelet count 28.000 per cmm and blood urea 275 mg per 100 ml. Fluctuating neurological signs with convulsions, and alternating hemiparesis ensued. Blood pressure was intermittently elevated, particularly at night. Cortical blindness was apparent shortly before death which occurred 7 weeks after the onset of symptoms.

The cerebral hemispheres appeared moderately swollen, and there was evidence of tentorial coning with bilateral hippocampal grooving. Subarachnoid blood was present over the inferior and lateral surfaces of temporal and occipital lobes, and over the cerebellum. Coronal cuts through the cerebral hemispheres showed scattered haemorrhages in the grey matter of both occipital lobes and the inferomedial cortex of the temporal lobe. The largest such haemorrhage (2 cm in diameter) was present in the right visual cortex (Fig. 3) and smaller haemorrhages were

FIG. 2—Case 23. Hippocampus showing loss of pyramidal cells in Sommer's sector.

present in the left visual cortex. Several smaller haemorrhages were present in the cortex of the cerebellum. All macroscopic vascular lesions were in the territory of the vertebrobasilar artery. However, no lesions of these or other major arteries were visible. Microscopic examination revealed changes of varying age. In the white matter of occipital and temporal lobes there was marked gliosis with loss of myelin and predominant reactive astrocytes. This change was thought to be of several weeks' duration. Some small vessels in the areas of gliosis showed fibrous thickening of the media. Similar lesions were also evident in the hippocampus, where there was gliosis and almost complete loss of neurones from Sommer's sector (Fig. 2). Recent lesions consisted mainly of the multiple haemorrhages noted above and some areas of patchy hypoxic damage with chromatolysis of neurones and oedema. The latter were not confined to the area of pathology noted macroscopically, as they were seen in the frontal lobes as well. No pathology of the basal ganglia, mid-brain, pons or medulla was noted. The cerebellum showed recent pathology only, with patchy areas of cortical necrosis associated with haemorrhage. In the middle of the haemorrhages some vessels showed fibrinoid necrosis.

Renal histology showed glomeruli with increased cellularity and fibrin deposits. Afferent arterioles showed fibrin deposits in their walls and some were occluded by thrombi. There was patchy dilatation and loss of tubules.

Comment: The older lesions of hypoxic damage and gliosis correlate with neurological episodes such as the transient hemiplegias that occurred several weeks prior to death. The pathogenesis of these lesions is obscure, although fluctuating hypertension of severe degree is the possible explanation, i.e. hypertensive encephalopathy. The recent changes, consisting of haemorrhages in the occipital lobes, undoubtedly produced the cortical blindness noted in the terminal stages. These also may have been of hypertensive origin.

In summary it is seen that ischaemic and/or hypoxic lesions were present in the five cases and they correlated reasonably well in age and distribution of the clinical findings. They were of variable extent and distribution although the three cases in which changes were most marked the lesions were in the territory of the vertebro-basilar arterial system, i.e. the pons (Fig. 1), hippocampus (Fig. 2) and occipital cortex. Although fibrinoid necrosis of the walls of the vessels was present in three cases it was

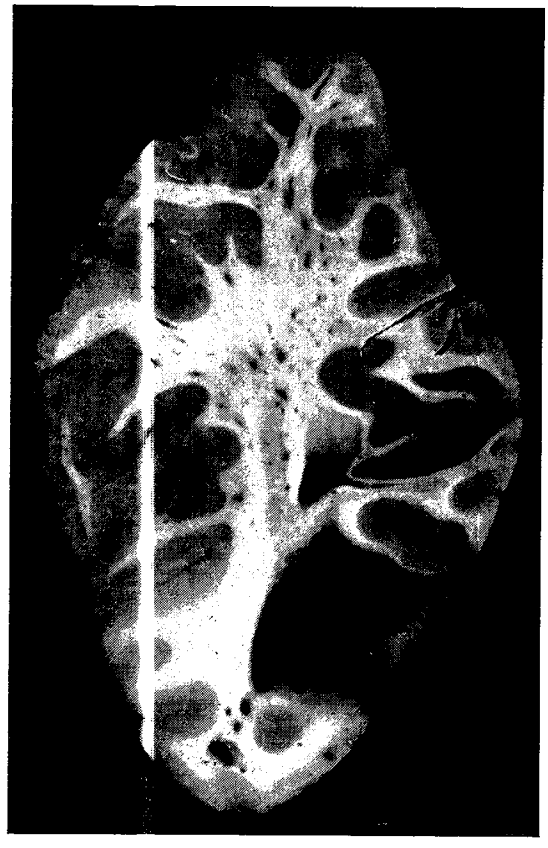

FIG. 3—Case 23. Multiple haemorrhages in the right occipital lobe.

seen only in areas of haemorrhage and infarction. It is possible that the fibrinoid necrosis may have been a sequel of these changes rather than their cause. In Case 21 these vascular lesions may have been due to septicaemia rather than Haemolytic Uraemic Syndrome. Occlusion of cerebral vessels with eosinophilic thrombi was seen in Case 7 but only in occasional small arteries. Endothelial proliferation was also seen in only one instance, as were multiple haemorrhages. Macroscopic cerebral swelling was present in two cases, and in another three there was patchy oedema present on microscopic examination.

Discussion

In the H.U.S. it is suggested that both haemolysis and renal impairment may be a sequel of changes in the small blood vessels in the kidney, i.e. a renal microangiopathy (Brain, 1968). The similarity of the renal pathology to that seen in the Schwartzman reaction has been pointed out frequently. However, the aetiology of the syndrome remains obscure and may be multiple.

Of importance to a consideration of the neurological features is the relationship of H.U.S. to thrombotic thrombocytopenic purpura (T.T.P.). The grounds for clearly separating these conditions are tenuous. From published reviews of T.T.P. (Ambrosi and Ultmann, 1966; Singer, 1954) it would seem that the main clinical features distinguishing T.T.P. from H.U.S. are threefold: in T.T.P. the patients are usually adults, central nervous system involvement is almost always present, and death occurs in almost every case. Thus it could be argued that, on clinical grounds, several of the older patients reported here, e.g., Cases 18, 22 and 23 might be examples of T.T.P. in childhood. The differentiation on pathological grounds is also poorly defined, and in some reports rests mainly on the more limited distribution of the microangiopathy in the H.U.S. compared with widespread distribution of vacular lesions in T.T.P. (Giromini and Laperrouza, 1969). In particular the few reports available of neuropatholgy in T.T.P. (Adams *et al.*, 1948, and Ladwig, 1953) have described widespread occurrence of cerebral vascular occlusions with eosinophilic hyaline material, and endothelial proliferation. No case reported in this paper would qualify for the diagnosis of T.T.P. on these grounds. However, we remain somewhat sceptical of the separation of these entities on the clinical and pathological features mentioned above.

It is difficult to assess the incidence of neurological involvement in H.U.S. from a study of the literature. Many papers, including reviews (Piel and Phibbs, 1966) and clinico-pathologic studies (Liebermann *et al.*, 1966) devote little more than a brief mention to neurological features. In reports containing details of case histories, or a specific statement as to the incidence of significant neurological involvement, it varies from just under 60% (Gianantonio *et al.*, 1964) to 33% (McQuiggan *et al.*, 1965). In the present study the incidence of major neurological involvement is just over 50%. Although it is likely that there is a true difference in the incidence of neurological features in the different series, the very low incidence in some reports is probably an apparent difference due to a predominant interest in the renal and haematologic aspects of the syndrome. The importance of cerebral involvement appears to have been underestimated. Gianantonio *et al.* (1964) pointed out that the severity of neurologic involvement provided the only statistically significant clinical correlation with mortality rate. This is confirmed in the present study. Nine of 13 patients who had major central nervous system disturbance died; this contrasts with only two deaths in the remaining 12 patients. The correlation is even more striking if patients who had evidence of more severe nervous system dysfunction are considered separately from those having convulsions alone. If hemiplegia, coma, or decerebrate spasms ensued the prognosis was very poor as nine of 10 such patients died; in contrast three patients who had convulsions as the only evidence of central nervous system disturbance recovered.

A study of neuropathological features in seven cases who died has not produced definite evidence as to the pathogenesis of the central nervous system involvement. No distinctive pathologic process that can be considered specific for H.U.S. was seen in the brains or in cerebral vessels. The changes seen most frequently were those of hypoxia and/or ischaemia. Although it seems that most of these changes correlated with hypertension that was often severe and prolonged, or markedly fluc-

tuating, this was not always so. For instance, in Case 18 in which aphasia, right hemiparesis, coma and decerebrate spasms occurred progressively over a period of 10 days prior to death, there was only mild hypertension present when neurological disturbance appeared. Although fibrinoid necrosis of small arteries in the areas of infarction was noted in this case, and in Case 23 in an area of terminal haemorrhage, this change was not thought to be specific. Areas of cerebral infarction or haemorrhage from many different causes may be associated with such fibrinoid necrosis of vessels within the lesion. Capillary endothelial proliferation with occlusion, as described by Barnard and Kibel (1965), was not a feature in the present series.

It is likely that multiple factors are involved in the pathogenesis of the convulsions. Hypertension seemed the most likely factor in some cases, but the infrequency of recording, and the difficulty experienced in accurately recording blood pressure in small infants in this series does not allow an adequate assessment of the role of hypertension in causing convulsions. There was no particular metabolic disturbance seen in patients with convulsions that was not present in other patients. In a minority of cases there was a clear-cut structural basis for convulsions, e.g., Case 10, where a cerebral infarct correlated well in age and location with a previous episode of focal convulsions.

It is not proposed to enter into a detailed discussion of treatment of the H.U.S. In the present series it was largely symptomatic. In several patients it proved difficult to control hypertension with hypotensive drugs. From a study of the neuropathological features, it is difficult to envisage heparinization having a direct beneficial effect on cerebral symptomatology as cerebro-vascular occlusions by platelet or other thrombi were not conspicuous. This does not, of course, mean that heparinization may not improve cerebral involvement by modifying the disease process elsewhere, e.g., in the kidneys, or by diminishing the risk of cerebral haemorrhage due to thrombocytopenia. Heparin was used in only two patients in the present series (Cases 10 and 23) and they progressively deteriorated during a short period of heparinization. Further experience is required to verify the impression of Brain *et al.* (1968) and Gilchrist *et al.* (1969) that heparinization is of value, especially as at least one report to the contrary has been published (Katz *et al.*, 1969). Three cases received treatment with cortico-steroids without clear-cut benefit.

Cases of H.U.S. have not been reported previously in which neurological features have been the presenting and predominant clinical abnormality. From our experience it appears that this condition should enter into the differential diagnosis of acute and subacute encephalopathies in childhood. Examination of a blood smear for the characteristic morphological changes of red blood cells, platelet count and blood urea estimation should be carried out in any severe acute or subacute neurological disturance of obscure origin in childhood.

Summary

Major clinical neurological features were seen in 13 of 25 consecutive patients with the H.U.S. convulsions were present in most of the 13 cases. Hemiparesis, aphasia, coma and decerebrate spasms were other manifestations, and these were associated with a mortality of 90% compared with an overall mortality of 45%. Two case histories are given in which neurological involvement was the presenting and predominant clinical feature. Neuropathological examination showed hypoxic and/or ischaemic lesions and cerebral oedema as the most frequent changes but did not reveal distinctive vascular lesions similar to those seen in the kidneys of these patients. The diagnosis of H.U.S. should be considered in severe acute and subacute encephalopathies of obscure origin in childhood.

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THE USE OF IMMUNOSUPPRESSIVE AGENTS IN PERIPHERAL NERVE HOMOGRAFT SURGERY: AN EXPERIMENTAL STUDY

J. D. POLLARD, J. G. MCLEOD and R. S. GYE*

INTRODUCTION

The treatment of peripheral nerve defects following severe injury to a limb is often extremely difficult. The relatively simple procedure of nerve suture often yields disappointing results; moreover, if the defect is large, sufficient autologous nerve tissue may not be obtainable.

During the last century attempts to bridge large peripheral nerve defects in man with simple homografts (cadaver allografts) produced disappointing results (Barnes *et al.*, 1945; Sanders and Young, 1942). Failure of regeneration has been attributed to both immune reactions (Sanders, 1942; Seddon, 1963) and inadequate graft vascularization (Tarlov and Epstein, 1945). Methods of treating donor nerves, which probably alter their antigenicity, have produced some successful results (Marmor, 1967; Klemmer, Woolsey and De Rezende, 1943). Such methods include storage in Ringer's solution or normal saline, formalin fixation, freeze-drying and irradiation (Fricke, Leone and Landmann, 1957).

We considered that immunosuppressive agents used in conjunction with previously treated grafts may improve the results of nerve allograft surgery. This has proved to be the case in a study performed on Wistar rats and Rhesus monkeys.

METHODS

Nerve allografts have been inserted into peripheral nerves of 42 Wistar rats and 11 Rhesus monkeys. The rats were anaesthetized with intraperitoneal pentobarbitone sodium (1·5 mg/Kg) and the monkeys with intramuscular phencyclidine hydrochloride (0·15 mg/Kg).

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Approximately two centimetres of the sciatic or posterior tibial nerve was excised from the rats and replaced by a slightly larger fresh or freeze-dried nerve allograft. Twenty-one of these animals received a daily dose of azathioprine (Imuran) intraperitoneally. Twenty-one received no immunosuppressive therapy.

In the case of the monkeys, approximately five centimetres was excised from the median nerve immediately above the wrist and replaced by a freeze-dried and irradiated nerve allograft. Five animals received immunosuppressive therapy, either azathioprine orally or antilymphocyte globulin intramuscularly, and six control animals were untreated.

Preparation of Grafts

Sections of nerve to be used as allografts were obtained from animals within 24 hours of death and then stored in Ringer's solution or normal saline for about 56 hours at 10°C. The grafts were then freeze-dried and stored at −10°C for at least four weeks prior to use. Grafts were irradiated in the freeze-dried state with 2×10^6 r.e.p.

Grafting Techniques

The nerve graft was held in position against the divided ends of the peripheral nerve by cuffs of two closely fitting sleeves of vein or artery allograft (Weiss and Taylor, 1943). A small amount of alpha ethyl cyanoacrylate (Aron Alpha A "Sanyo") (Yodh and Wright, 1967) placed at the margin of the cuff made firm the union. The graft with the two cuffs attached was inserted in the freeze-dried state. In this condition it is firm and easily manoeuvred; morover, the cuffs are transparent, allowing close apposition of graft to the nerve endings.

Immune Suppressive Therapy

Azathioprine was administered to the rats by daily intraperitoneal injection. The initial dosage of 5 mg/Kg was commenced the day prior to surgery and reduced to a daily maintenance dose of 3 mg/Kg by one week. Azathioprine was administered to the monkeys orally in the same dosage, commencing two days before graft insertion. Antilymphocyte globulin was given by intramuscular injection in a dosage of 10 ml daily, commencing two days before insertion of the graft, until one week post-operatively and then 5 ml thrice weekly for three months.

Electrophysiological Studies

Motor conduction velocity was determined in the appropriate nerve before graft insertion and at weekly intervals thereafter with techniques similar to those employed by McLeod and Wray (1967).

Histological Studies

In nerves removed for histological examination, the density and fibre diameter of myelinated fibres was measured in transverse sections taken through, proximal and distal to the graft. Single nerve fibres were teased from the nerve and measurements were made of internodal length and fibre diameter. The histological techniques used were those described by McLeod and Penny (1969).

Results

Rats

The results are summarized in Table 1.

Of the 21 rats which received azathioprine therapy, 20 exhibited clinical and electrophysiological evidence of regeneration 12 weeks after graft insertion; in some animals electrophysiological evidence of muscle re-innervation was seen as early as five weeks. By contrast, in only nine control animals was similar evidence of regeneration seen at 12 weeks.

Table 2 compares the motor conduction velocity of grafted sciatic nerves and the amplitude of the muscle action potential obtained from foot muscles in the 20 rats from Groups I and II in Table 1.

Histological examination was performed at six months after graft insertion. In 20 azathioprine-treated animals the grafts had re-innervated well, and in one re-innervation was poor. In the control group, myelinated fibres

TABLE 1

Wistar Rats

Type of Graft	*No. of Animals*	*Electrophysiological Evidence of Regeneration at 12 Weeks*	*Histological Evidence of Re-innervation at Six Months*
Group I Fresh nerve	10	4	6
Group II Fresh nerve + Imuran	10	9	10
Group III Freeze-dried nerve	11	5	9
Group IV Freeze-dried nerve + Imuran	11	11	11
Total without Imuran	21	9	15
Total with Imuran	21	20	21

TABLE 2

Wistar Rats—A comparison of post-operative motor conduction velocity and amplitude of muscle action potential in azathioprine-treated rats (Group I) and control rats (Group II)

No. of Animals	*Post. Op. Conduction Vel.*		*Amplitude of M.A.P.*	
	Group I	*Group II*	*Group I*	*Group II*
1	22·5 m/sec	17 m/sec	2·7 mV	0·5 mV
2	20 „	17 „	2·8 „	0·35 „
3	33 „	19 „	5·0 „	1·4 „
4	31 „	20 „	3·0 „	3·5 „
5	30 „	—	5·5 „	—
6	16 „	—	4·0 „	—
7	27 „	—	5·0 „	—
8	25 „	—	3·4 „	—
9	28 „	—	3·0 „	—
10	—	—	—	—
Mean	25·8 m/sec S.D. 5·5	18·25 m/sec S.D. 1·5	3·8 mV S.D. 1·1	1·43 mV S.D. 1·4

Mean Normal Conduction Velocity in Rat Sciatic Nerve 47·6 m/sec S.D. 6·1

Mean Normal Amplitude of M.A.P. 15·2 mV S.D. 3·3

S.D. = Standard Deviation.

were seen in 15 animals, including the nine animals in whom definite muscle action potentials had been obtained.

The fibre density in the control animals was considerably reduced when compared to the azathioprine-treated animals, especially in the six animals in which no electrophysiological responses had been recorded. In Figure 1 this finding is illustrated by the comparison of transverse sections from the grafts of control and azathioprine-treated animals with a section from a normal sciatic nerve. Figure 2 makes the same comparison by means of histograms constructed from nerve fibre counts.

As shown by Figure 3, in all regenerating nerves, there was, in comparison with normal nerves, a reduction in the number of large diameter fibres and a preponderance of fibres of small diameter and short internodal length. When regeneration was judged to be well advanced by clinical and electrophysiological means, azathioprine therapy was suspended and the animals were observed for a further two months. No evidence of regression in nerve function was observed. The rates of regeneration through grafts in the rats has varied between 1·5 to 2 millimetres daily.

Monkeys

The results are summarized in Table 3.

Of 20 monkeys given peripheral nerve allografts, 11 have been followed for a period of six months. Five animals treated with immunosuppressive agents have shown electrophysiological evidence of regeneration at six months compared to two of six control animals. Two further monkeys treated with antilymphocyte globulin died during anaesthesia at four and six weeks post-operatively. Silver stains performed on the grafts from these animals have shown a high density of axons growing through these grafts.

TABLE 3

Rhesus Monkeys

Type of Graft	*No. of Animals*	*Electrophysiological Evidence of Regeneration at Six Months*
Group I Freeze-dried, irradiated nerve	6	2
Group II Freeze-dried, irradiated nerve + Atilymphocyte gnlobulin	3	3
Group III Freeze-dried, irradiated nerve + Imuran	2	2
Total without suppression	6	2
Total with suppression	5	5

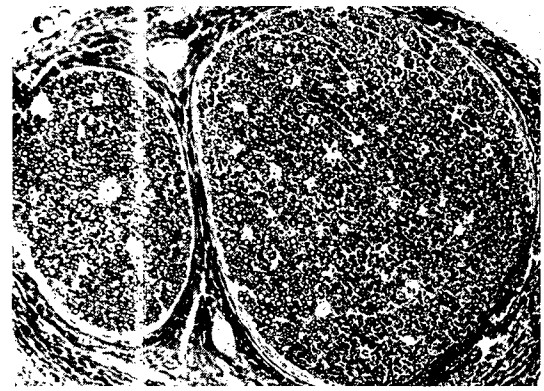
The rate of regeneration through grafts in the monkeys has been about 2 millimetres daily.

DISCUSSION

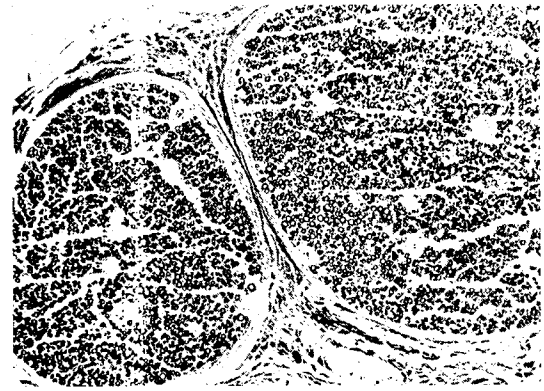
The preliminary results of this investigation indicate that immunosuppressive agents assist regeneration in peripheral nerve allografts.

Clearly to destroy the antigencity of the nerve graft prior to insertion would be preferable to the administration of immune suppressive agents to the host. However, the methods which are currently used in an attempt to alter nerve antigenicity achieve only partial success both in the experimental animals and in man (Tucker and Hayes, 1970). We feel, therefore, in the light of this study, that the insertion of nerve allografts in man may be improved by the use of immunosuppressive agents.

It is highly likely that myelin is the chief antigen in peripheral nerves (Das Gupta, 1967). The ideal material for a graft should, therefore, be "pre-degenerated" nerve in which no myelin remains. Though successful in animal experiments, such grafts are not feasible in the human situation. Of the methods which



(a)



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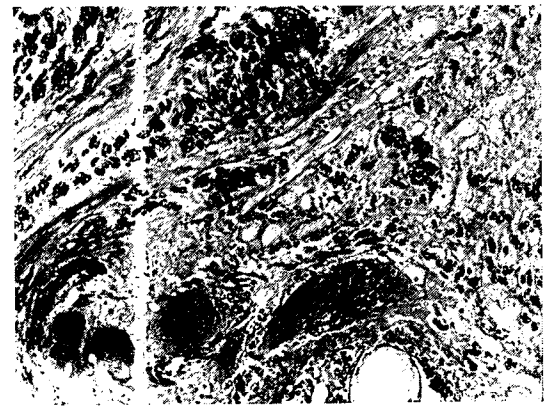


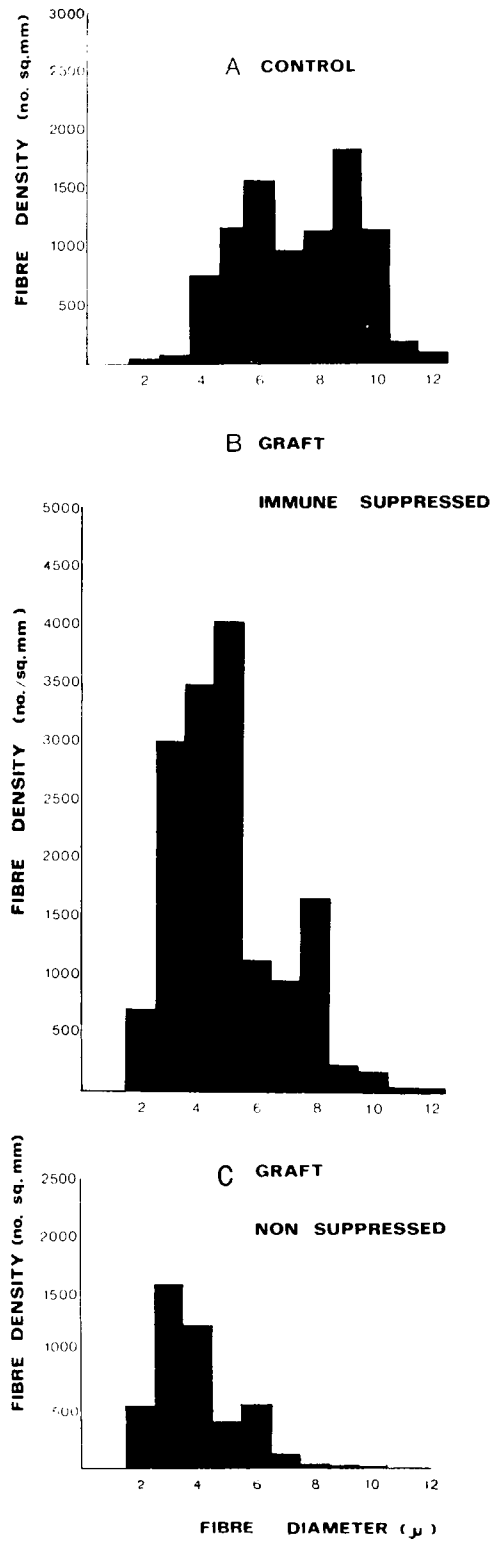
(c)

FIG. 1—Transverse sections stained with osmic acid × 80.

1 (a) Normal rat sciatic nerve.

1 (b) Allograft from azathioprine-treated rat.

1 (c) Allograft from control rat.



alter the nerve antigen, irradiation appears to be the most effective. However, there is evidence (Roberts, 1967) which is further confirmed by some preliminary findings from our work, to suggest that irradiation so alters the graft that vascularization and removal of myelin fragments are delayed. Better regeneration may then be achieved by the use of stored non-irradiated graft tissue and immunosuppressive agents.

It is well known that technical considerations are of great importance in peripheral nerve surgery. The satisfactory results obtained in this experimental study may be partly attributable to the technique of insertion. There has been no evidence of neuroma formation at the site of union which is commonly seen when sutures are employed.

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FIG. 2—Distribution of diameters of myelinated fibres in:
(a) normal rat sciatic nerve,
(b) allograft from azathioprine-treated rat, and
(c) control rat.

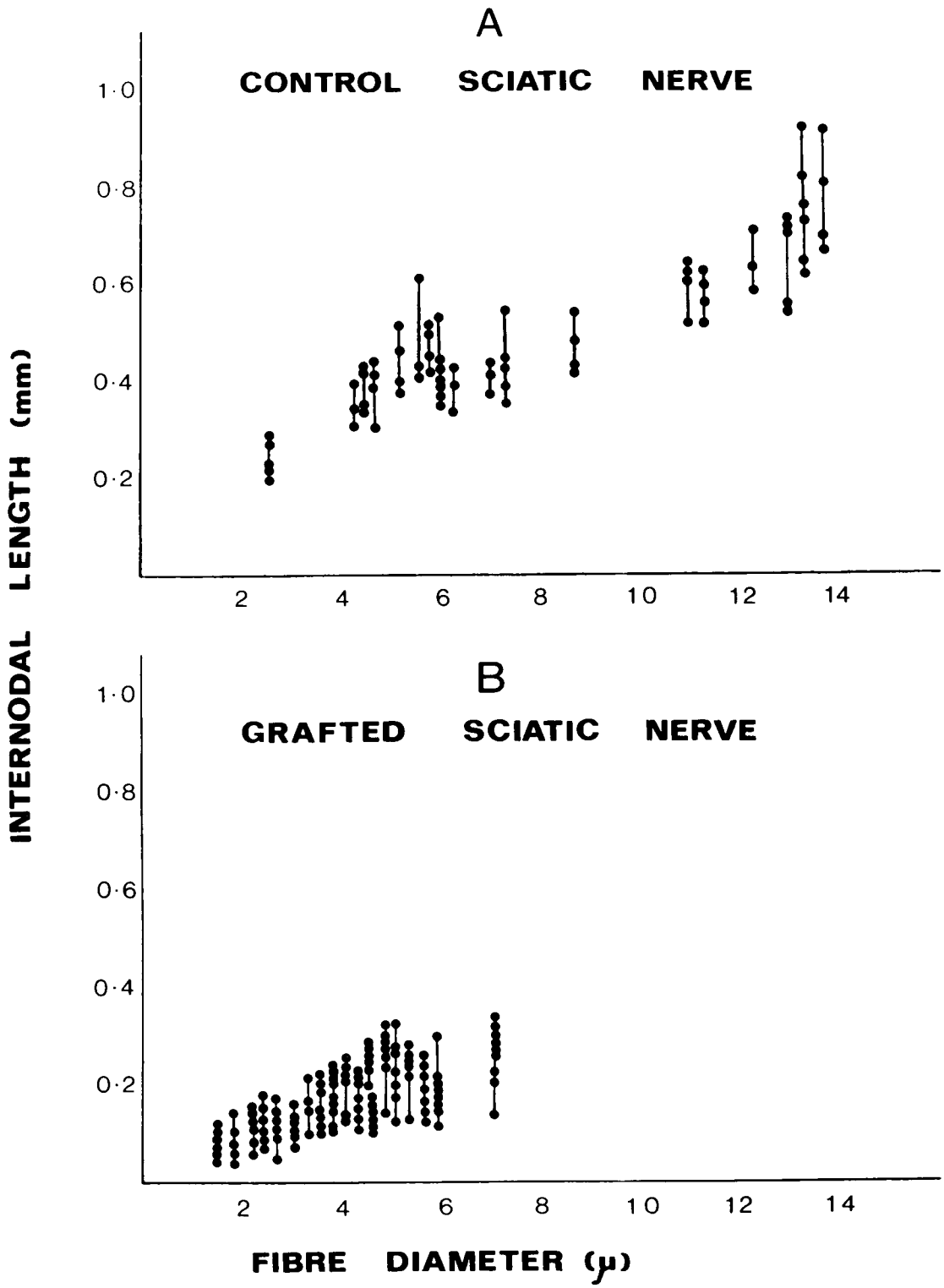

FIG. 3—Distribution of internodal length in single fibres teased from:
(a) normal rat sciatic nerve,
(b) from a graft in an azathioprine-treated rat.

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ACKNOWLEDGMENTS

We wish to thank the National Health and Medical Research Council for supporting this work. The antilymphocyte globulin and the monkeys were made available through the helpful co-operation of Associate Professor A. G. R. Sheil of Sydney University. We are particularly grateful to Miss Julia Baverstock for technical assistance.

IN VITRO DESTRUCTION OF HUMAN FOETAL MUSCLE CULTURES BY PERIPHERAL BLOOD LYMPHOCYTES FROM PATIENTS WITH POLYMYOSITIS AND LUPUS ERYTHEMATOSUS

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INTRODUCTION

Polymyositis has been known for more than 100 years (Wagner, 1863, 1887; Unverricht, 1887; Jackson, 1887) as an important cause of non-hereditary progressive muscular weakness. Clinically, about two-thirds of patients have cutaneous lesions (Barwick, 1963) in which case the disease is designated dermatomyositis. A remarkable feature is the high incidence of associated visceral malignancy (Arundell, Wilkinson and Haserick, 1960). Histologically, the muscle lesions consist of segmental muscle fibre necrosis with phagocytosis of necrotic sarcoplasm and regenerative changes. Cellular infiltration is variable and consists of lymphocytes, mononuclear cells and plasmacytes with fewer neutrophils and eosinophils. Polymyositis may be acute or chronic and often occurs in association with connective tissue diseases such as rheumatoid arthritis, polyarteritis nodosa and systemic lupus erythematosus. In this case, the clinical manifestations of the associated connective tissue disease may be minor or dominant (Walton and Adams, 1958).

In recent years, the suggestion has been advanced that polymyositis is due to a disorder of immune function. In support of this concept much clinical, laboratory and experimental evidence has accumulated. Such evidence includes the presence of elevated serum globulins, the frequent dramatic response to treatment with steroid hormones and the clinical association of the disease with other conditions of suspected immunological origin. In addition, the high incidence of carcinoma occurring in relationship to polymyositis and the sensitivity to tumour extracts (Grace and Dao, 1959; Curtis, Heckaman and Wheeler, 1961) may reflect an immunological disorder.

Early attempts to induce an experimental disease of muscle by immunological methods in a laboratory animal were hampered by technical difficulties (Pearson, 1956; Tal and Liban, 1962). In a later study, Dawkins (1965), succeeded in producing an experimental autoallergic myositis which he showed was associated with hypersensitivity to muscle by skin testing. The muscle lesions in such guinea pigs treated with repeated injections of heterologous skeletal muscle and Freund's adjuvant resembled those which occurred in human polymyositis. In a later series of experiments it was demonstrated that lymph node cells of rats with the immunological disease, when added to tissue cultures of differentiated foetal rat skeletal muscle, caused destruction of the outgrowth in the neighbourhood of the explant (Kakulas, 1966). Pursuing this approach it was later found that cytopathic properties also existed for human peripheral (blood) lymphocytes derived from patients with polymyositis or systemic lupus erythematosus when added to cultures of human foetal muscle (Kakulas, 1969). On this basis it is now possible to bring forward a reasonable formulation of a probable aetiology and pathogenesis for the muscle changes which occur in these conditions.

CLINICAL EVIDENCE

In 1954, Eaton reviewed the problem of polymyositis and provided a clinical classification which was further developed by Walton and Adams (1958). More recently, Pearson

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(1969) carefully documented the clinical features of polymyositis and has surveyed the laboratory and therapeutic evidence in favour of an immune origin for the disease. In this regard, the elevated apha 2 and gamma globulins, the presence of circulating rheumatoid factor and the general, although not universal, clinical improvement when steroid hormones are administered, are the most noteworthy.

Pathological Observations

Considerable variability exists in the histomorphology of biopsy specimens of which the common denominator is muscle fibre necrosis. This manifests as architectural disorganization of the muscle fibre in both light and electron-microscopic preparations. The ultrastructural changes appear first in the myofibrils which are rarified due to loss of myofilaments and Z bands are obscured.

Other changes consist of mitochondrial alterations and dilation of the sarcoplasmic reticulum together with lipid vacuoles and myelin "bodies". It is noteworthy that the sarcolemma remains intact (Rose, Walton and Pearce, 1967). Macrophages are observed in the necrotic areas and regenerating muscle fibres are common. Regeneration which is related to the intact sarcolemmal membrane is an almost invariable element of the lesion (Mastaglia and Kakulas, 1968). Regeneration *ad integro* occurs over a period of time when treatment with prednisone is successful (Mastaglia and Kakulas, 1968). Lymphocytes, plasma cells, mononuclear cells and, less commonly, neutrophils and eosinophils, complete the microscopic picture. The histological changes are therefore those of a necrotizing myopathy and closely resemble the changes which occur in a wide variety of conditions including nutritional disorders in animals (Kakulas, 1966).

Additional microscopic features sometimes encountered in biopsy specimens are vacuolar changes in the sarcoplasm (Pearson, 1969) and the presence of islands of atrophied fibres. Grouped atrophy, when present, suggests complicating peripheral nerve disease. This combination is most frequently observed when carcinoma is associated (Denny-Brown, 1948). A very important feature of the lesions which may only be adequately observed in serial sections of muscle biopsies is the close proximity of the focal necrotic muscle lesions to the perivascular cellular infiltrates (Adams, 1969) (Fig. 1).

Necropsy studies, although less numerous than biopsy examinations, reveal a wide spectrum of changes. The final morphology in fatal cases may be termed "end-stage" muscle disease which is characterized by overall loss of muscle fibres and replacement by connective tissue and fat. The few surviving fibres show excessive variation in size and shape and nuclei are irregularly distributed. Leukocytic infiltrates are generally in the form of focal collections of round cells (Fig. 2). Changes in other organs are the result of intercurrent disease or are due to associated conditions, e.g., Hashimoto's thyroiditis, rheumatoid arthritis, atrophic gastritis and polyarteritis nodosa, have been observed in the one patient (Mastaglia and Kakulas, 1967).

It is also relevant that in a necropsy study of 122 patients with neoplastic disease, 21 showed changes of necrosis and degeneration in the vastus lateralis or deltoid muscles. The findings suggested a high subclinical incidence of polymyositis (Kakulas and Mastaglia, 1966).

Experimental Observations

Although attempts to produce myositis by immunological methods were undertaken as early as 1929 (Kakulas, 1969) it was not until 1956 that further interest was shown when Pearson briefly reported "focal polymyositis"

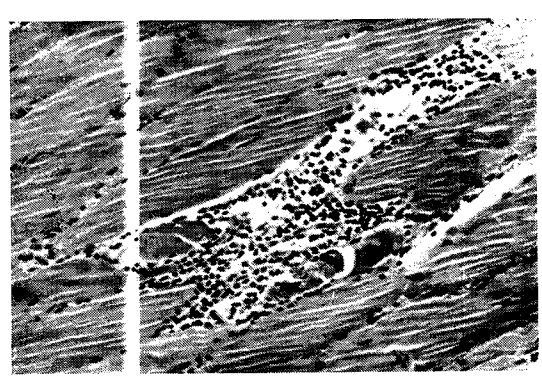

Fig. 1—Muscle biopsy of a patient with polymyositis. In the centre of a field is a small venule surrounded by a cellular exudate consisting predominantly of lymphoid cells. Focally necrotic muscle fibres are in close proximity to both the vessels and the cellular exudate. Haematoxylin and Eosin × 450.

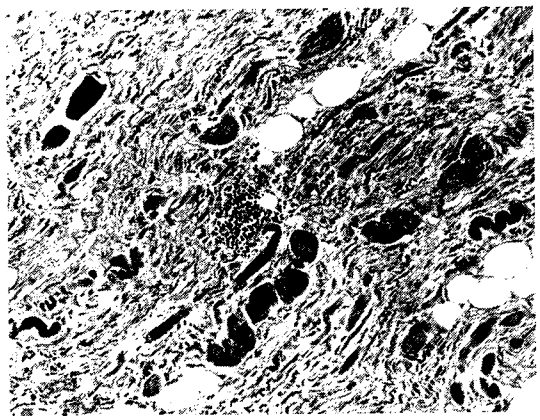

FIG. 2—Psoas muscle in a patient who died of dermatomyositis (A70/25). There is loss of the majority of the muscle fibres, condensation of endomysium and irregular size and shape of surviving muscle fibres ('end-stage' muscle disease). In the centre of this field is a small collection of lymphocytes. Haematoxylin and Eosin × 150.

in rats that had received muscle mixed with Freund's adjuvant (Pearson, 1956). However, more attention was devoted to an associated arthritis which could be produced by the administration of adjuvant alone. A "dystrophy-like" picture in rabbits and guinea pigs receiving homologous and heterologous muscle mixed with Freund's adjuvant was reported in 1962. However, the changes described were primarily atrophic and did not resemble the histological findings in cases of polymyositis (Tal and Liban, 1962).

Dawkins (1963, 1965), in a well-controlled series of experiments undertaken in 1963, conclusively demonstrated that myositis could be induced on an autoallergic basis in the guinea pig.

His work included a study of the "graft versus host" reaction where myopathic changes in immunologically "runted" mice were observed to be widespread. Muscle fibre necrosis was a prominent change in the lesions. Similar muscle lesions did not occur in the muscles of "runted" rats. The mouse "runts" were produced in neonates, less than 24 hours old, by the intraperitoneal injection of about 50×10^6 lymph node or spleen cells from adult donor mice of different strain to the recipient. Necrotic changes occurred in many parenchymal organs and as well as in skeletal muscle.

Lesions similar to those immunologically induced in guinea pigs were also produced in rats by repeated footpad injections of homogenized rabbit muscle and Freund's adjuvant (Kakulas, 1966). Rarely, rats treated in this manner showed signs of muscle weakness (Fig. 3). Histologically, multifocal myositic changes with muscle fibre necrosis and a variable leukocytic infiltrate were consistent (Fig. 4). Careful light microscopic analysis of the development of the muscle changes showed that leukocytic infiltrates which were characteristically perivascular preceded signs of muscle fibre necrosis. The lesions, although generally similar to the changes which occurred in human polymyositis, tended to be more focal. Electronmicroscopy

FIG. 3—Rat with profound muscle weakness after sensitization to rabbit muscle and Freund's adjuvant by repeated footpad injections.

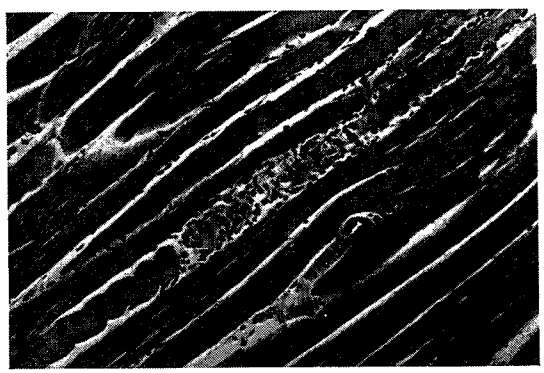

FIG. 4—Focal necrosis of a muscle fibre in the pectoralis major muscle of a rat sensitized to rabbit muscle and Freund's adjuvant by repeated footpad injections. The necrotic focus in the centre of the field is associated with an infiltrate of round cells and macrophages. Haematoxylin and Eosin × 450.

of the experimental disease in rats revealed widspread alterations in the organelle systems, with rarifaction of myofibrils due to loss of myofilaments (Fig. 5), membranous structures (Fig. 6), and dilated vesicles. The ultrastructural changes closely resembled those which occur in biopsy specimens of patients with polymyositis (Rose, Walton and Pearce, 1967).

In vitro studies demonstrated that lymph node cells of rat sensitized to rabbit muscle are cytopathic to tissue cultures of foetal rat skeletal muscle (Kakulas, 1966). In this work, approximately 4×10^6 lymph node cells derived from rats given 3 injections of homogenized rabbit muscle and adjuvant were added to each tissue culture. Thirty-eight of forty cultures treated with cells from rats sensitized to muscle were destroyed within 2 to 6 days (Fig. 7). In contrast, in control studies in which tissue cultures were treated with lymph node cells of animals injected with adjuvant only, or which were sensitized to rabbit kidney as well as cells of untreated rats, showed destructive changes in a much smaller number (Fig. 8). The results indicated that suitably sensitized cells are able to destroy muscle *in vitro* and suggested

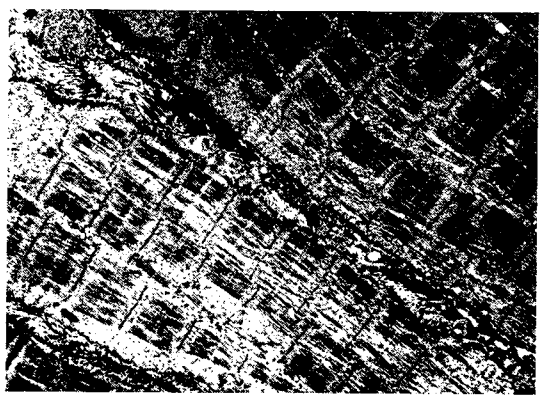

FIG. 5—Electronmicrograph of vastus lateralis muscle of rat sensitized to rabbit muscle showing rarifaction of myofibrils due to loss of myofilaments and disappearance of mitochondria and sarcoplasmic reticulum. The changes represent early necrosis. Uranyl acetate-lead citrate stain × 4,000.

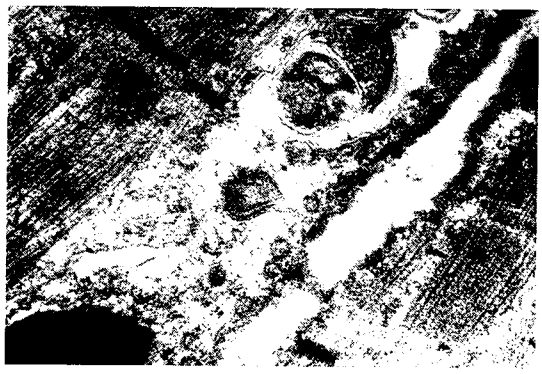

FIG. 6—Electronmicrograph of vastus lateralis muscle of rat sensitized to rabbit muscle showing loss of mitochondria dilatations of the sarcoplasmic reticulum, disappearance of myofilaments and the presence of membranous structures. Uranyl acetate-lead citrate stain × 16,000.

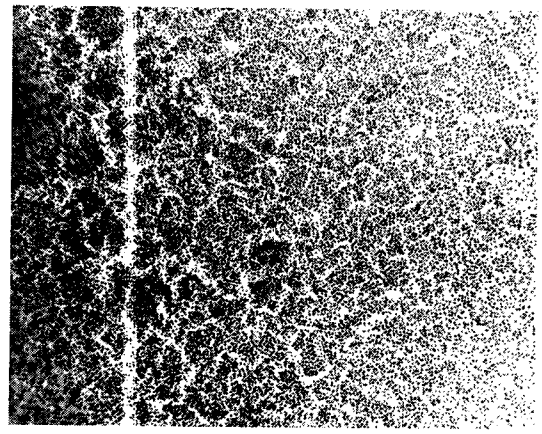

FIG. 7—Destruction of foetal rat skeletal muscle *in vitro* by the addition of lymph node cells of rats sensitized to rabbit muscle. Phosphotungstic acid haematoxylin stain × 150.

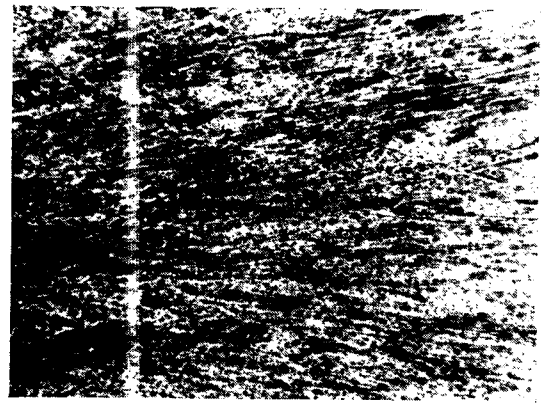

FIG. 8—Foetal rat skeletal muscle fibres *in vitro* remain intact after treatment with lymph node cells of a rat sensitized to rabbit kidney and adjuvant. (Compare with Fig. 7.) Phosphotungstic acid haematoxylin stain × 150.

that a similar pathogenic mechanism may occur in human polymyositis. The results of the animal experiments are summarized in Table 1.

TABLE I RAT EXPERIMENTS

Effect of adding to foetal rat skeletal muscle tissue culture 4 x 10^5 lymph-node cells or 0.02 ml serum from rats sensitized to rabbit muscle or from normal rats.

Donor rats (groups of 10)	Proportion of donor rats showing muscle lesions in : fore-limb	hind limb	Donor material added to culture	Number of cultures destroyed by 6th day	intact on 6th day
Received injections of rabbit muscle plus adjuvant	9/10	10/10	Cells	38	2
			Serum	4	16
Received injections of saline only	0/9	0/9	Cells	7	33
			Serum	2*	18
Received injections of adjuvant only	2/10	3/10	Cells	6	34
			Serum	3	17
Received injections of rabbit kidney plus adjuvant	0/10	1/10	Cells	10**	30
			Serum	3	17

* One of these two cultures was destroyed by bacterial contamination
** Two of these ten cultures were destroyed by bacterial contamination

A logical development of the passive *"in vitro"* transfer work in the rat was the exploration for similar properties of human lymphoid cells obtained from patients with polymyositis and related "autoimmune" diseases. In a large number of such experiments, in which the target tissue was human foetal skeletal muscle, similar destructive changes occurred. The lymphoid cells were concentrated from about 20 ml of blood obtained by venepuncture and were added in lots of approximately $0{\cdot}5 - 1{\cdot}0 \times 10^6$ to each culture. The muscle was grown on collagen coated cover slips in Maximov assemblies and, depending on the supply of satisfactory cultures, approximately 6 were used for the cells of each patient. The cultures were observed microscopically daily and on the sixth day they were fixed and stained with phosphotungstic acid haematoxolin. The cytodestructive effects were essentially similar to those observed in the experimental disorder (Fig. 9). The majority of cultures treated with lymphoid cells from normal subjects serving as controls remained intact (Fig. 10). The cells added to the cultures with cytopathic changes, rapidly underwent enlargement and transformation, whereas the lymphocytes of the normal controls added to the cultures which remained intact generally remained small and inactive. The results are summarized in Table 2.

Discussion

The muscle changes which occurred in the immunologically induced disease in the experimental animal strongly resembled those which arose "spontaneously" in human polymyositis. Differences were minor and included a more focal character to the changes. In some in-

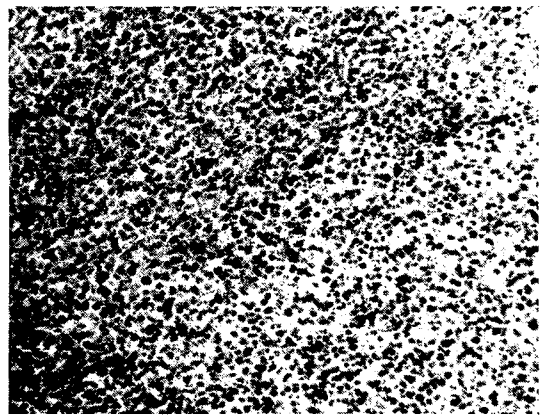

FIG. 9—Human foetal skeletal muscle destroyed by the addition of lymphoid cells from the peripheral blood of a patient with polymyositis. The cells which remain are enlarged and contain heavily staining material. (Compare with Fig. 10.) Phosphotungstic acid haematoxylin stain × 150.

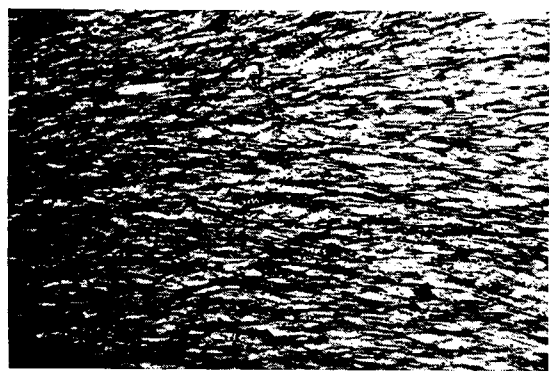

FIG. 10—Human foetal skeletal muscle remains intact after addition with lymphoid cells of a normal subject. Phosphotungstic acid haematoxylin stain × 150.

Table 2

HUMAN CELL EXPERIMENTS

Disorder	Number of patients	Number of cultures Destroyed by 6th day	Intact on 6th day
Polymyositis	7	33	9
Systemic Lupus	6	27	9
Non-specific Muscle Pain	4	8	17
NORMAL CONTROLS	8	6 (5)*	29

TARGET TISSUE : Human foetal skeletal muscle. * *contaminated with fungi*

stances there was a more acute cellular response with polymorphs being more abundant than in the human disease. This laboratory model for human polymyositis was subsequently studied in a number of laboratories (Takayanagi, 1967; Pearson, Peter and Morgan, 1969).

Similarly, the cytopathic properties of peripheral blood lymphocytes of patients with polymyositis were recently corroborated by Currie and Walton (1969). Analogous results were reported for a number of other human diseases, including multiple sclerosis (Koprowski and Fernandes, 1962) and idiopathic polyneuritis (Arneson, Winkler and Hadler, 1969).

In this work, Arneson, Winkler and Hadler (1969) extended their studies of experimental allergic neuritis to include human buffy coat cells of patients with idiopathic polyneuritis. Such cells were myelinodestructive in 48 to 96 hours. It was evident, therefore, that cytopathic mechanisms similar to those demonstrated for polymyositis also existed for other human diseases.

Wilson (1965), Maclennan and Loewi (1968) studied the mechanisms involved in cellular cytotoxicity while Dumonde, Wolstencroft, Panayi, Matthew, Morley and Howson (1969) used the term "lymphokine" for the low molecular weight soluble factors generated by antigen activated lymphocytes, which are believed to be the toxic agents and are different from classical antibodies.

The results of the human lymphocyte work reported here render it possible to formulate an aetiology and pathogenesis for human polymyositis. Thus, in this disease, sensitized lymphoid cells accumulate in the perivascular and interstitial spaces of skeletal muscle and become activated on contact with muscle antigens leading to the secretion of albumen-like factors ("lymphokines" (Dumonde, Wolstencroft, Panayi, Matthew, Morley and Howson (1969)) which cause the muscle fibre necrosis. Other histological elements in the lesions, e.g., regeneration, are an inherent response of muscle to injury (Kakulas, 1969). In the more chronic stages of polymyositis the architectural alterations with loss of fibres, fat and fibrous tissue replacement are due to a continuation of the destructive process and the lesions simply represent "end-stage" muscle disease. As such, the "burnt-out" lesions have much in common with the muscle changes in progressive musuclar dystrophy.

Difficulties in this hypothesis are concerned firstly with differences in the natural history between the experimental and spontaneously occurring diseases and, secondly, with the initiating mechanism in the human disorder. For instance, in the experimental condition the initiating factor is the repeated injection of foreign muscle combined with adjuvant. Such myositic changes which are induced are also self-limiting with recovery occurring on cessation of the injections. In contrast, the naturally occurring disease in man is seemingly self-perpetuating. However, it may be arrested by use of steroids which block the immune mechanism. It is possible that the "triggering" agent for the autoimmune process in man is an infection with "complete" or "incomplete" virus. In support of this suggestion is the observation of Chou (1967) who found virus-like structures in a muscle biopsy of a patient with polymyositis. Virus-like structures are also reported in a patient with systemic lupus erythematosus and suggest that a similar role for a virus exists for this condition (Kawano, Miller and Kimmelstiel, 1969). The experimental findings of Stanley and Walters (1966) with rheovirus 3 support the concept of virus induction of both autoimmune disease and neoplasia.

Another difficulty in the present theory is related to myasthenia gravis which is clinically diverse from polymyositis but for which much evidence suggesting an immunological origin has accumulated (Kakulas, 1969). However, Dawkins (1963), in his experimental work, demonstrated increased curare sensitivity in his guinea pigs sensitized to muscle. Other evidence for a "bridge" between myasthenia gravis and polymyositis exists on clinical grounds as patients with both diseases occurring in association with thymoma have been found (Klein, Gottlieb, Mones, Appel and Osserman, 1964). Associated myasthenia gravis and systemic lupus erythematosus has also been documented (Hausemanowa-Petrusewicz, Chorselski and Strugelska, 1969).

The obviously complex nature of the problem is underlined by the frequent finding of serum antibodies to muscle in patients with a wide variety of disorders and point to the non-

specificity of the findings (Caspary, Gubbay and Stern, 1964; Stern, Rose and Jacobs, 1967). Such reports suggest that cellular immune factors, unassociated with classical antibodies, on the lines presented above, are more important in the pathogenesis of these diseases.

Recent application of the immune theory is the use of immunosuppressive drugs which have been used clinically with good results in patients with polymyositis (Malaviya, Many and Schwartz, 1968; McFarlin and Griggs, 1968). The search for a possible virus underlying polymyositis is currently in progress. Similarly, the cytodestructive properties of peripheral blood lymphocytes in patients with a variety of diseases using the *in vitro* methods outlined above may shed further light on the pathogenesis of these conditions.

SUMMARY

Clinical, morphologic and experimental evidence is presented in support of a possible immune aetiology for human polymyositis. For the first time, direct and objective evidence exists in the human subject for this suggestion on the basis of observed cytodestructive effects *in vitro* of lymphoid cells derived from patients with the disease. Presumably, similar cytopathic effects occur *in vivo* as a result of the perivenular accumulation of the lymphoid cells and are the cause of the necrotic lesions in muscle. Unexplained, at present, is the initiating or "triggering" mechanism for such a process but the recently observed virus particles in polymyositis may be important in this regard. This present report, in addition to providing evidence of an immune aetiology of human polymyositis, describes a method that may be applicable to a wide variety of other human diseases for which the cause is at present unknown.

ACKNOWLEDGMENTS

The original work recorded here was supported by the National Health and Medical Research Council of Australia and by the Muscular Dystrophy Research Association of Western Australia (Inc.).

Gratitude is expressed to Dr. R. D. Adams for discussions, and to Dr. K-E. Astrom for assistance with the electronmicroscopy. Dr. Arneson provided facilities and Mr. H. Upenicks prepared the photographs.

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THROMBOSIS OF THE INTERNAL CAROTID ARTERY AFTER CLOSED HEAD INJURY

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INTRODUCTION

Occlusion of the internal carotid artery may arise in several ways. In adults it is usually the result of thrombosis complicating atherosclerosis (Fisher, 1951, 1954; Luessenhop, 1959), whereas in children and young adults traumatic thrombosis accounts for a greater number of cases (Humphrey and Newton, 1960; Frantzen, Jacobsen, and Therkelsen, 1961; Pitner, 1966). Thrombosis of the internal carotid artery has been reported after penetrating neck injuries (Makins, 1916; Colledge and Dunn, 1917); Caldwell and Hadden, 1948; Lawrence, Shefts, and McDaniel, 1948; Raney, 1948; Hughes and Brownell, 1968), surgical operations on the neck (Fisher, 1951; Ralph, 1954), and intra-oral trauma (Pitner, 1966). In addition, it is being reported with increasing frequency as a complication of closed trauma to the head and neck (Fisher and Friedmann, 1959; Caldwell and Hadden, 1948; Schneider and Lemmen, 1952; Clarke, Dickson and Smith, 1955; Paillas, 1955; Sedzimir, 1955; Murray, 1957; Hockaday, 1959; Luessenhop, 1959; Murphey and Miller, 1959; Verbiest and Calliauw, 1959; Gurdjian, Hardy, Lindner, and Thomas, 1963; Therkelsen and Hornnes, 1963; Thompson, 1963; Houack, Jackson, Odom, and Young, 1964; Toakley and McCaffrey, 1965; Beraud, Bilodeau, Sirois, Reinhardt, and Cote, 1966; Miller and Ayers, 1967; Peerless, Hoffman, and Hendrick, 1967; Hughes and Brownell, 1968).

The majority of these reports are of thrombosis of the vessel in the neck. Although thrombosis of the intracranial portion of the internal carotid artery may occur in association with basal skull fractures (Thompson, 1963), it more commonly follows relatively minor head injuries. Diagnosis is then often difficult and the outcome is usually fatal as the occlusion is surgically inaccessible. The two cases presented illustrate the diagnostic and therapeutic difficulties and the poor prognosis in this situation (Mastaglia, Savas and Kakulas, 1969).

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CASE REPORTS

CASE 1 (R.P.H. no. 131674): A.S. was a 39-year-old woman who was admitted to the Royal Perth Hospital on 29 December 1965 after a collision in a motor vehicle in which she was a passenger. She sustained a head injury and was unconscious for a few minutes. She had no recall for the accident but there was no retrograde amnesia. She had been quite well before the accident and had not been taking oral contraceptives. When examined, a scalp laceration was present in the occipital region and there were several contusions in the tissues of the right arm and left leg. She was unconscious and fully oriented. The pupils reacted normally to light and the fundi were normal. There were no other cranial nerve abnormalities and no lateralizing neurological signs. The pulse was 72 per minute and the blood pressure 120/80 mm Hg. There were no cardiac abnormalities.

On the sixth hospital day she became drowsy, complained of severe headache and nausea, and vomited several times. Over the next 24 hours she became extremely restless and irritable. On the following day a grand-mal seizure occurred and three further seizures ensued in the next three hours. There was right-sided papilloedema and the pupils were equal and reacted normally to light. Post-ictally a flaccid right hemiparesis was present. The deep tendon reflexes were symmetrical and the plantar reflex was flexor on both sides. The pulse rate was 60 per minute and the blood pressure 110/60 mm Hg. Intramuscular phenytoin and paraldehyde were administered and no further seizures occurred. One hour later she was still drowsy and the right pupil was larger than the left and reacted only sluggishly to light. Left carotid arteriography showed complete obstruction of the internal carotid canal (Fig. 1). Her conscious state continued to deteriorate and she became deeply comatose. The pulse rate varied between 40 and 60 per minute. The left pupil became

widely dilated and fixed. The right pupil was small and reacted slightly to light. Extensor spasms of all four limbs occurred. On the eighth hospital day burr holes were placed in the temporal and parietal regions on both sides. On opening the dura the brain was found to be swollen and herniated through the burr holes, particularly on the left side. Despite the administration of intravenous mannitol her condition deteriorated further and she died on the ninth hospital day.

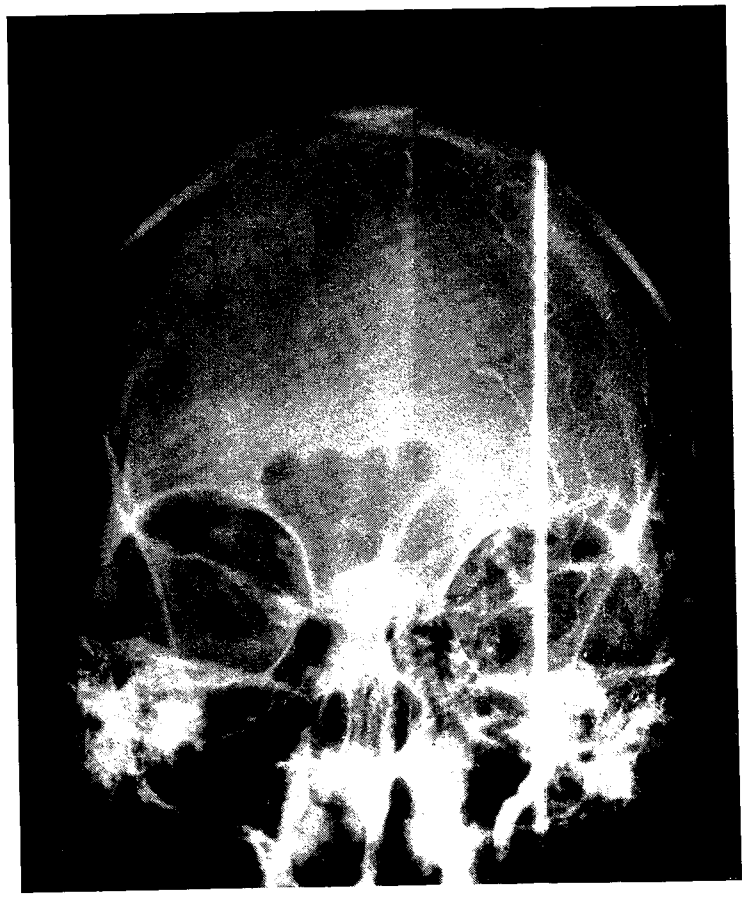

FIG. 1—Early arterial phase of left carotid arteriogram in Case 1 showing complete block of the internal carotid artery in the carotid canal.

Pathological findings (B66.71): The necropsy was performed by the Coroner's medical officer (Dr. William Laurie) who found evidence of purulent bronchitis and basal pulmonary congestion. No evidence of thrombosis was found in the veins of the lower limbs or pelvis or in the heart or aorta.

There was no defect in the atrial septum. No cranial fracture was present. The brain weighed 1,310 g. Both cerebral hemispheres were swollen, the left being larger than the right. There was compression and distortion of the mid-brain with extensive secondary haemorrhages in the mid-brain and upper pons. The tonsils of the cerebellum were deeply grooved. The greater part of the left cerebral hemisphere was softened. Coronal sections through the hemispheres showed swelling and discolouration of the left frontal, temporal and parietal cortex, white matter and basal ganglia in the superficial and deep territories of supply of the left middle cerebral artery. The territory of the anterior cerebral artery was spared (Fig. 2). The inferior temporal and occipital cortex in the territory of supply of the left posterior cerebral artery was also softened and there were multiple punctate haemorrhages throughout the infarcted grey matter. Sections from the infarcted areas showed neuronal loss and the surviving neurons were shrunken and eosinophilic and showed nuclear pyknosis. Pleomorphic microgliacytes were present and small vessels

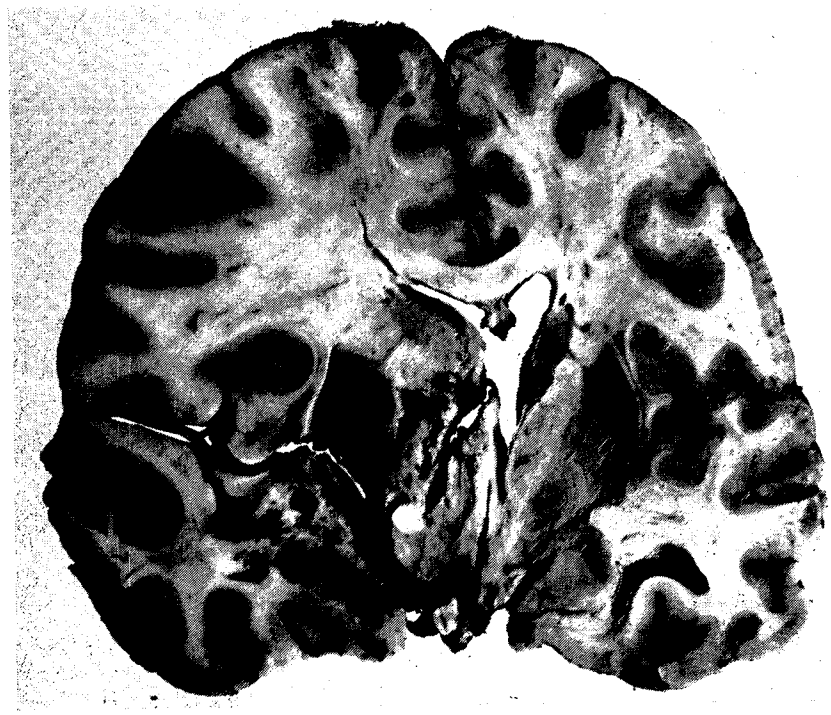

FIG. 2—Coronal section through the cerebral hemispheres in Case 1 showing extensive infarction of the left hemisphere.

were occluded by eosinophilic hyaline material. In the occipital cortex there were also many small perivascular and parenchymal haemorrhages.

Cerebral blood vessels: The internal carotid arteries were patent throughout their course in the neck. However, the left internal carotid artery in the carotid canal was completely occluded by adherent laminated red and yellow thrombus which was propagated distally to the point of bifurcation of the vessel into anterior and middle cerebral arteries (Fig. 3). Microscopic examination of the thrombus showed a typical red cell and fibrin structure.

The vessel wall was normal. The arteries which formed the circle of Willis were free of atheroma and their anatomical pattern was normal. The cerebral veins and venous sinuses were normal.

CASE 2 (R.P.H. no. 89669): J.S. was a previously well 39-year-old man who was admitted to the Royal Perth Hospital on 10 June 1967 after a motor vehicle accident. He was fully conscious but could not give an account of the collision. On physical examination, there was a large haematoma at the angle of the left mandible, a fracture of the left clavicle, and several skin lacerations in the limbs. There were no abnormal neurological signs. His conscious state subsequently deteriorated and three hours after admission (six hours after the injury) he responded to painful stimuli only by moving the left side of the body. The right arm and leg were flaccid and the tendon jerks were diminished. The superficial abdominal reflexes were absent on the right side and the right plantar reflex was extensor. The lower half of the right side of the face failed to move in response to painful stimuli. The right pupil was slightly larger than the left, but both reacted to light both directly and consensually. The pulse rate was 80 per minute and the blood

FIG. 3—Left internal carotid artery from the region of the siphon in Case 1 showing complete occlusion by thrombus.

pressure 170/110 mm Hg. There were no cardiac abnormalities.

Radiographs of the skull showed no cranial fracture, but there was a fracture at the angle of the left mandible. Carotid arteriography performed six hours after admission showed that the left internal carotid artery was fully patent to the level of the cavernous sinus, but only a thin streak of contrast was present beyond this point. There was no filling of the anterior or middle cerebral arteries (Fig. 4). Intramuscular dexamethasone and intravenous heparin were administered at eight-hourly intervals.

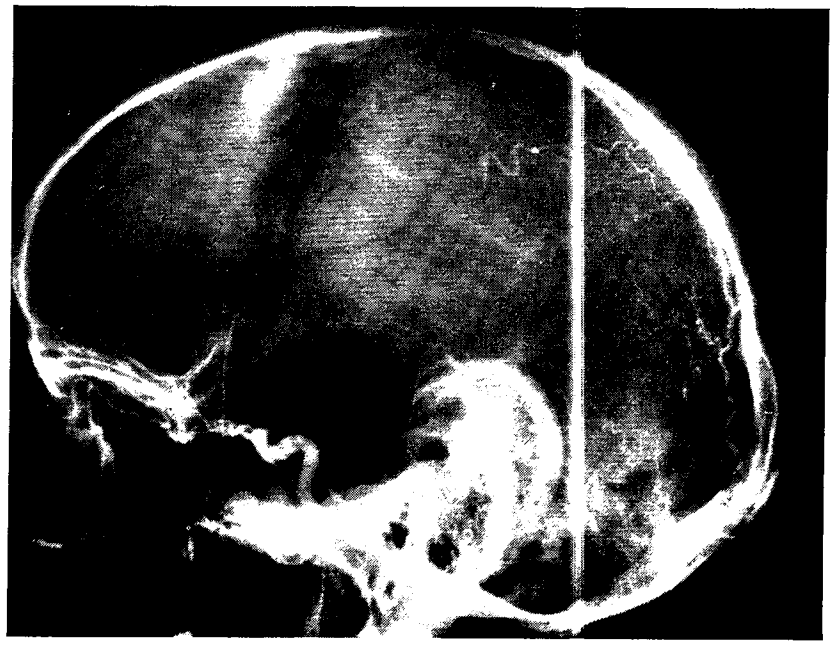

FIG. 4—Arterial phase of left carotid arteriogram in Case 2 showing hold-up of contrast in the carotid siphon immediately beyond the origin of the ophthalmic artery.

Twelve hours later the left pupil was fully dilated and failed to respond to light. The right pupil was constricted but reacted to light. Bilateral papilloedema was present. Shortly afterwards the right pupil also became dilated and unreactive. The blood pressure began to rise, respirations were periodic, and the plantar reflex was extensor on both sides. Burr holes were placed in the left temporal and parietal regions. The brain was found to be swollen and under increased tension. Despite intravenous infusions of mannitol, the patient's condition failed to improve and he died 40 hours after admission.

Pathological findings (B67.1814): The necropsy was performed by the Coroner's medical officer (Dr. Donald Hainsworth). Necropsy findings included fractures of the left mandible and clavicle and basal pulmonary congestion and oedema. No source of emboli was found and the heart was normal. There were no skull fractures. The brain weighed 1,450 g. The left cerebral hemisphere was swollen and there was softening and discolouration of the frontal lobe and greater part of the temporal and parietal lobes. Mid-line structures were displaced to the right. Both unci were deeply grooved, particularly the left. A "Kernohan's notch" was present in the left cerebral peduncle and there were extensive secondary haemorrhages in the mid-brain and upper pons. There was deep grooving of the cerebellar tonsils. Serial coronal sections of the cerebral hemispheres showed swelling and discolouration of the cortex and basal ganglia of the left hemisphere in the anterior and middle cerebral artery territories of supply with sparing of the posterior cerebral artery territory. The infarcted frontal grey matter was haemorrhagic (Fig. 5). Microscopic examination showed excessive eosinophilia and shrinkage of neurones with loss of Nissl's substance and nuclear pyknosis. Small vessels in the infarcted frontal cortex were intensely congested and there were many perivascular and parenchymal haemorrhages. There was early emigration of polymorphonuclear leucocytes into the infarcted tissues but no glial reaction.

Cerebral blood vessels: The internal carotid arteries were patent in the neck but the left internal carotid artery was completely occluded by thrombus at its point of bifurcation into anterior and middle cerebral arteries. The major vessels of the circle of Willis

FIG. 5—Horizontal section through the cerebral hemisphere in Case 2 showing haemorrhagic infarction of left frontal cortex and anaemic infarction of left temporoparietal cortex and basal ganglia.

FIG. 6—Section through the terminal portion of the left internal carotid artery in Case 2 showing occlusion by recent thrombus. Verhoef van Giesen × 75.

were free of atheroma and the communicating arteries were of adequate calibre. Microscopic examination of the internal carotid artery at the bifurcation showed a recent red cell and fibrin thrombus (Fig. 6). The intima and inner part of the media of the vessel wall were intact, but the adventitia and outer media were infiltrated by polymorphonuclear and mononuclear leucocytes.

DISCUSSION

The clinical and pathological features of two patients who died from traumatic occlusion of the intracranial portion of the internal carotid artery are reported. Both were adults in the fourth decade of life. Thrombosis followed a mild concussive head injury in both patients and in neither was there fracture of the skull. After the initial transient loss of consciousness, there was a latent interval of six days in Case 1 and six hours in Case 2, during which time the patient's neurological state was normal. In most of the previously reported cases of thrombosis of the cervical portion of the vessel (Hughes and Brownell, 1968) the occlusion was manifest within 24 hours of the initial injury. However, longer latent intervals of two to five days were described by Sedzimir (1955) in three cases of thrombosis of the intracranial portion of the vessel. Headaches and epileptic seizures were the initial manifestations in Case 1, while in Case 2 deterioration in conscious state was the first indication of the occlusion. In both cases infarction of the dominant hemisphere resulted in hemiplegia, and death due to cerebral swelling and tentorial herniation occurred

three days and 36 hours respectively after the appearance of neurological signs.

Several theories have been advanced to account for the occurrence of ischaemic necrosis after acute carotid occlusion in patients with an anatomically normal circle of Willis uncompromised by atheroma (Paillas, 1955; Beraud *et al.*, 1966). Propagation of the thrombus into the middle and anterior cerebral arteries has been suggested, but this did not occur in either of the present cases. Embolization into the anterior and middle cerebral arteries from the site of primary thrombosis is considered by Pitner (1966) to be the most likely explanation and this could account for the delayed onset of symptoms in most cases. The detection of emboli at post-mortem examination of the cerebral vessels is notoriously difficult, but the finding of haemorrhagic infarction is suggestive of an embolic occlusion even when the embolus is not found. Embolization of thrombotic material from the internal carotid artery may have occurred in Case 2, in which haemorrhagic infarction was present in the frontal cortex.

The other mechanisms postulated are reflex spasm of cerebral vessels due to irritation of the adventitia of the thrombosed carotid, and inadequacy of anastomotic channels. The importance of the first mechanism remains speculative, as the demonstration of arterial spasm in such cases would require contralateral angiography with adequate cross-circulation studies. The most likely mechanism would appear to be an overall inadequacy of collateral blood flow. In contrast with the often fatal outcome in cases of acute carotid occlusion, it is well recognized that gradual occlusion of one or both of these vessels may be completely compensated for by the development of an adequate collateral blood flow. In such cases, well-developed anastomoses between branches of the internal and external carotid arteries are found. In the acute situation such anastomoses are not fully developed and blood flow to the territory of the occluded vessel falls below critical levels.

The mechanism of the thrombosis itself remains obscure in most cases. There are several reports of intimal tears (Schneider and Lemmen, 1952; Verbiest and Calliauw, 1959; Gurdjian *et al.*, 1963; Hughes and Brownell, 1968; Pearce, 1969) with associated damage to the media, curling up of these layers and eventual thrombosis. Pitner (1966) has postulated that a blunt blow to the artery produces a rapid compression and rebound of the vessel which may result in intimal disruption.

Others have stressed the importance of contusion of the vessel wall, subintimal haemorrhage in the presence of atheromatous disease of the vessel, dissection of the vessel wall after intimal damage (Gurdjian *et al.*, 1963), arterial spasm (Schneider and Lemmen, 1952), and compression of the internal carotid artery against the lateral mass of the second cervical vertebra (Peerless *et al.*, 1967). More specifically, with regard to thrombosis of the intracranial portion of the vessel, Sedzimir (1955) has suggested that, when there is relative movement of the brain in an anteroposterior direction within the confines of the skull, the artery is subject to shearing strains at the point where it leaves the cavernous sinus where it is anchored by a firm dural attachment. Such strains may lead to temporary distortion of the vessel wall with intimal damage and spasm, which favours thrombosis. The presence of an inflammatory cellular infiltrate in the adventitia and outer medial of the vessel wall at the site of occlusion in Case 2 may be significant in this regard. The possible contributory role of post-traumatic thrombocytosis and increased platelet adhesiveness has not been studied. It is probable that the formation of the thrombus is a gradual process and that clinical manifestations occur only when there has been significant reduction in size of the lumen of the vessel, thus accounting for the lucid interval.

The gravity of the prognosis in such cases reflects the inadequacy of present therapeutic measures. Best results have been obtained in cases of occlusion of the cervical portion of the vessel when the diagnosis is established early by arteriography and prompt surgical exploration is carried out (Gurdjian *et al.*, 1963; Houck *et al.*, 1964) before the changes in the brain become irreversible. When the occlusion is surgically inaccessible, conservative measures are usually ineffective. These include the use of parenteral vasodilators such as procaine and nicotinic acid, stellate ganglion block aimed at reducing arterial spasm and increasing blood flow through collateral channels. The use of mannitol infusion and parenteral dexamethasone failed to influence the clinical course in

the present cases. In Case 2, anticoagulation was also ineffective. Such therapy may prevent propagation of thrombus into the branches of the internal carotid artery, but Beraud *et al.* (1966) have drawn attention to the risk of cerebral haemorrhage when infarction has already occurred. The anti-coagulants may have contributed to the development of haemorrhagic infarction in Case 2.

Summary

Thrombosis of the internal carotid artery is an uncommon but recognized complication of trauma to the head and neck. The clinical and pathological features of two fatal cases of thrombosis of the intracranial portion of the internal carotid artery after minor concussive head injuries are described. The mechanism of thrombosis and the factors responsible for the development of cerebral infarction are considered.

Acknowledgments

We wish to thank Mr. J. Grummette who prepared the photographs and the Coroner's medical officers—Dr. W. Laurie and Dr. D. Hainsworth—for submitting the brains for examination. Dr. F. L. Mastaglia was in receipt of a Saw Medical Research Fellowship from the University of Western Australia.

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THE VASCULAR LESIONS ASSOCIATED WITH CEREBELLAR INFARCTS

ROLAND RODDA*

INTRODUCTION

Although posterior inferior cerebellar artery thrombosis has received prominence in accounts of ischaemic cerebellar disease (Dow and Moruzzi, 1958), this is a relatively rare phenomenon (Yates, 1957) and indeed Baker (1961) has clearly demonstrated that Wallenburg's syndrome is predominantly the result of unilateral medullary ischaemia due to stenosis or occlusion of a vertebral artery. Nevertheless infarcts of the cerebellum are common (Hutchinson and Yates, 1956) and, further, most of these infarcts are not in the "classical" posterior inferior cerebellar artery territory (Hutchinson and Yates, 1957). In this report the carotid, cerebral, vertebral and cerebellar artery patterns are described in a group of patients in whom cerebellar infarcts were found at necropsy.

MATERIAL AND METHODS

In 21 necropsies in which cerebellar infarcts were found, the vertebral, basilar and cerebellar arteries were specially studied. The 21 cases were aged 41 to 84 with a mean age of 70·6 years. Twelve were male and 9 female. Only 9 cases had presented obvious cerebellar signs which were nystagmus in 4, ataxia in 3, incoordination in 2 and dysarthria in 1 case. Sixteen (76·2%) of the patients were hypertensive as judged by either clinically recorded diastolic blood pressures of at least 110 mm of mercury or, at necropsy, an obvious left ventricular hypertrophy with a heart weight of more than 350 gm for women or 400 gm for men. Twenty of the 21 cases with cerebellar infarcts had massive or focal infarcts elsewhere in the brain. The 21 cases made up about a fifth of a larger group of necropsies on patients selected with a clinical history suggestive of an infarct in the brain in which the carotid and vertebral artery systems were injected *in situ* with a warm-setting gelatin-based radiopaque medium at simulated blood pressure using a multiple nozzle air piston syringe as previously described (Rodda and Dugdale, 1963). The postmortem arteriograms provided a guide to the detail dissection and histological examination of the vascular lesions in the neck and brain.

After fixation of the brain and examination of the Willisian arteries each cerebellar hemisphere was removed and sliced parasagittally. The 5 or 10 mm thick slices were X-rayed with a Baltospot industrial unit using Kodaline standard 2698 high contrast film covered with transparent Nylex 0·004 inch thick. A suitable exposure with an anode-film distance of 28 cm was 35 kV at 5 mA for 6 minutes. Undiluted Kodak D19 high contrast developer was used at 68° for 5 minutes.

The infarcts were readily identified in the slices and then studied by standard neuropathological techniques.

RESULTS

In the 21 cases there were more than 80 separate cerebellar infarcts, some of which comprised areas of recent ischeamic necrosis, but many were represented by old glial scars replacing varying sized areas of cerebellar parenchyma. The larger infarcts were visible on the surface of the cerebellar hemispheres but there were many more cortical infarcts which lay deep in an intralobular sulcus or in an interlobular fissure where the apposing folia of adjacent lobules were often involved (Fig. 1).

Recent massive infarcts. There were recent massive bilateral cerebellar infarcts in 2 cases occurring in association with massive supra-

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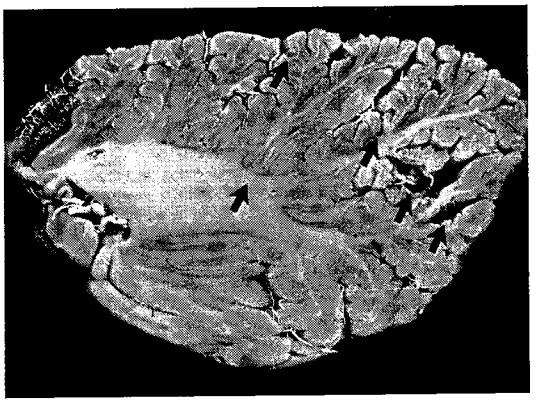

FIG. 1—Photograph of parasagittal slice of right cerebellar hemisphere in Case 36. There are 5 old focal infarcts in the middle depths of the horizontal fissure involving the superior and inferior semilunar lobules, in the upper sulcus of the inferior semilunar lobule, in the middle sulcus of the superior semilunar lobule, in the postlunate fissure involving the superior semilunar lobule and in the medulla beneath the superior semilunar lobule. × 1·4.

tentorial cerebral and brain stem infarction. In each there was a recent thrombus in both posterior cerebral arteries, the basilar artery and both superior cerebellar arteries with large haemorrhagic infarcts involving most of both superior cerebellar artery territories.

Focal cortical infarcts associated with intracerebellar haemorrhage. In 2 hypertensive cases, one of whom died as a result of a massive cerebral infarct and the other from a cerebellar haemorrhage, an old medullary cerebellar haemorrhage was identified with surrounding scar. The puckered scar associated with the larger lesion in the right anterior lobe was quite heavily collagenized but the smaller, represented by a brownish coloured, collapsed and slit-like cavity in the left superior semilunar lobule, showed only a fibrous glial wall. The cerebellar cortex overlying each old haemorrhage showed shrinkage with a loss of nerve cells and astrocytic gliosis but with preservation of the pattern of the folia in the form characteristic of an old healed infarct (Fig. 2). In each case there was severe vertebro-basilar and cerebellar artery stenosis although the occurrence of the medullary haemorrhage undoubtedly contributed to the cortical ischaemic necrosis.

Recent focal cortical infarcts. A recent focal cortical infarct from a few days to a few weeks old was identified in 7 cases (Fig. 3). These infarcts varied in size, the smallest involving only about 2 mm of cerebellar cortex whereas the largest was almost 1·5 cm in diameter and involved the cortex and medulla of several adjacent folia. In 2 cases the recent infarct was in juxtaposition to a very much older infarct.

A recent thrombus was found in only 1 case with a recent focal infarct. This thrombus occluded the stenosed atherosclerotic origin of the right superior cerebellar artery and the haemorrhagic infarct involved some of both anterior lobes and the intervening superior vermis—a boundary zone between the territories of the superior cerebellar arteries. In this case there was a severe stenosis of the left vertebral artery in the neck. In 1 case there was moderate stenosis of the right posterior inferior cerebellar artery but the recent infarct was in the contralateral superior semilunar lobule.

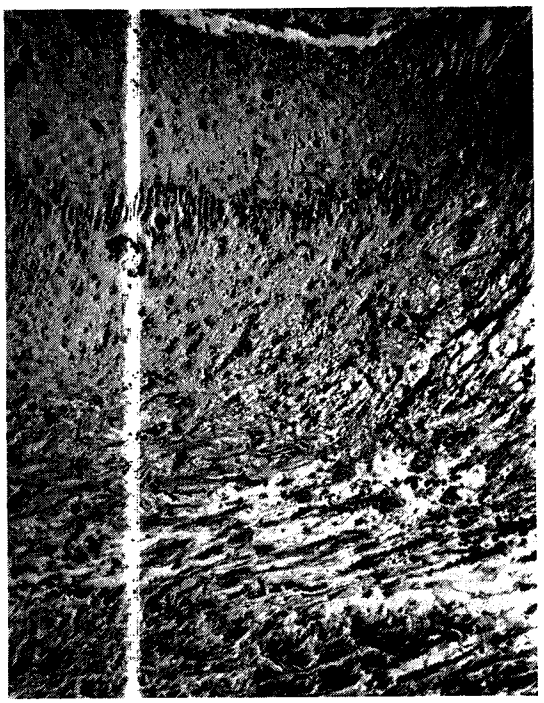

FIG. 2—Photomicrograph showing old ischaemic scar in cerebellar cortex overlying the old intracerebellar haemorrhage in Case 37. The granular layer has entirely disappeared and a zone of proliferated Bergmann astrocytes replaces the lost Purkinje layer. Large amounts of haemosiderin pigment persist in the glial and fibrous haemorrhage scar. L.F.B. × 72.

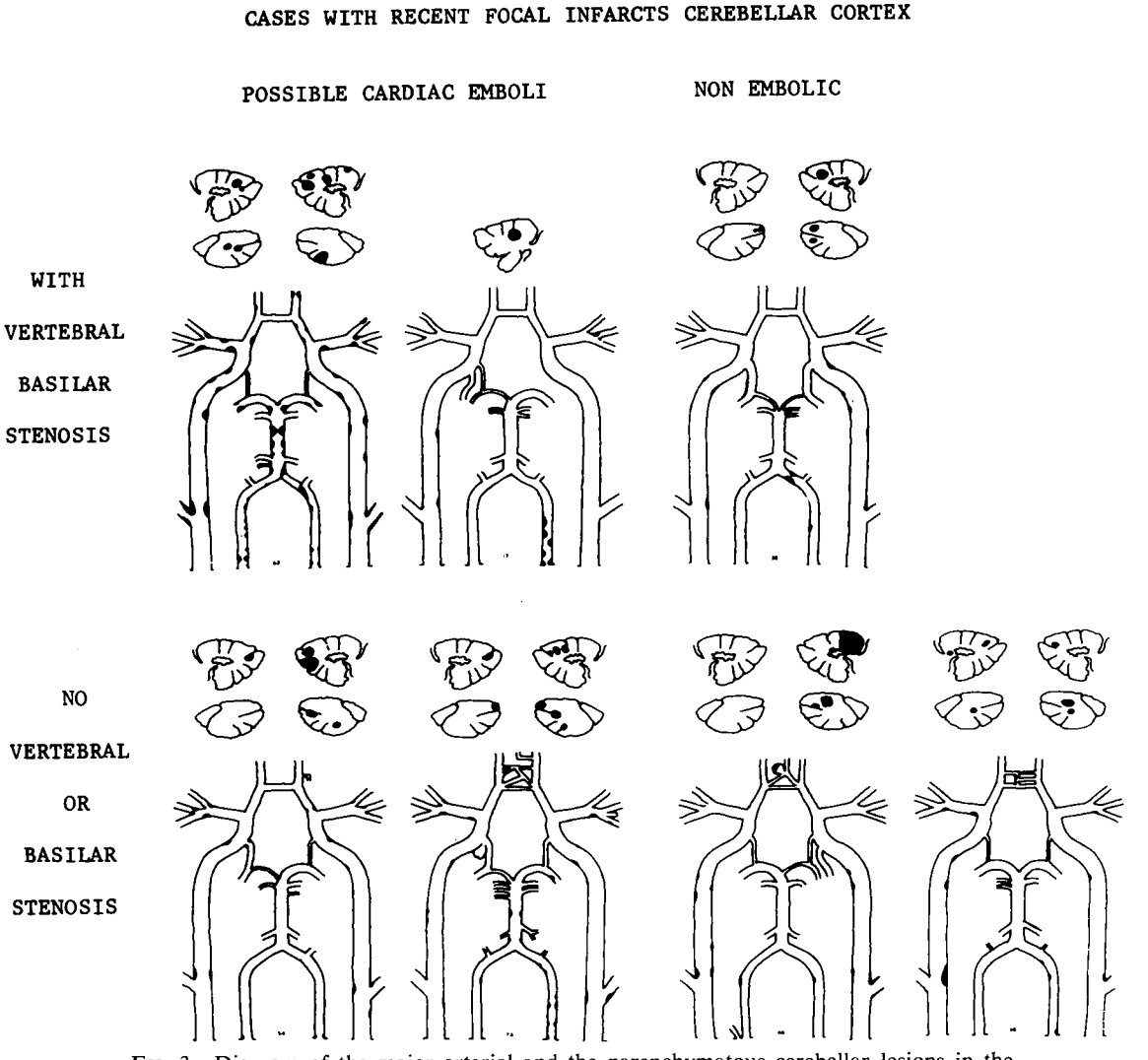

FIG. 3—Diagram of the major arterial and the parenchymatous cerebellar lesions in the 7 cases with recent focal cortical infarcts. Of the 4 cases without vertebro-basilar stenosis, only 2 had possible cardiac emboli.

None of the remaining 5 cases with a recent focal infarct showed any degree of stenosis in the cerebellar arteries. In 3 cases the infarct was also in a superior semilunar lobule, in 1 case in an inferior semilunar lobule adjacent to the horizontal fissure and in 1 case the infarct involved the anterior lobule just to the left of the midline. Thus of the 7 recent focal cortical infarcts, 5 were in the boundary zones of the territories of the superior and posterior inferior cereballar arteries and 2 were in the boundary zones of the left and right superior cerebellar arteries.

Of the 7 cases with a recent focal infarct, 3 showed vertebral or basilar stenosis but in 4 there was no significant stenosis in the vertebro-basilar or even in the carotid artery systems. To consider that there may have been embolism of cardiac origin, the occurrence of atrial fibrillation, the finding of endocardial thrombi or multiple infarcts in other organs (Jörgensen and Torvik, 1966) was regarded as suggestive of embolism. Using these criteria 4 cases could be regarded as possibly embolic but 2 of these were cases with severe vertebral or basilar stenosis. There were therefore 2 cases with

recent focal cortical infarcts without vertebro-basilar stenosis or any evidence of possible embolism from the heart.

Solitary focal cortical infarcts. Single or solitary focal cortical infarcts were found in only 6 cases (Fig. 4). Three of these infarcts were near the midline in the anterior lobe, the other 3 were posterolateral in the semilunar lobules. All these solitary focal infarcts lay in boundary areas of the territories of the superior cerebellar arteries and in 2 cases there was stenosis or occlusion of a superior cerebellar artery. Nevertheless in 3 cases there was only one inferior cereballar artery trunk and in one of these cases that artery was stenosed. In 2 cases there was some stenosis of the posterior inferior cerebellar artery. The one recent solitary focal infarct was associated with a recent right superior cerebellar artery thrombus and left vertebral artery stenosis as indicated above.

Of these 6 cases, 5 showed vertebral stenosis, 3 of which were possibly embolic. The remaining case presented no vertebro-basilar or carotid stenosis but was possibly embolic.

Other focal cortical infarcts. In the remaining 7 cases with focal cortical infarcts (Fig. 5), 5 showed occlusion or stenosis in the vertebro-basilar system but 2 did not. These 2 cases as well as 3 of the 5 cases with stenosis were cases with cardiac disease which was possibly embolic.

Focal medullary infarcts. There were 9 old cystic medullary infarcts in 5 cases. Although 3 of these were stained yellowish by broken down blood pigment, the gross shape and histological appearances were characteristic of old infarcts and not of haemorrhage. Eight were situated near the dentate nucleus and in 3 cases were bilateral but not symmetrically situated. One cyst was in the left brachium pontis. All these lesions were in what may be regarded as boundary zones of the territories of the superior and inferior cerebellar arteries.

Sites of the multiple old focal cortical in-

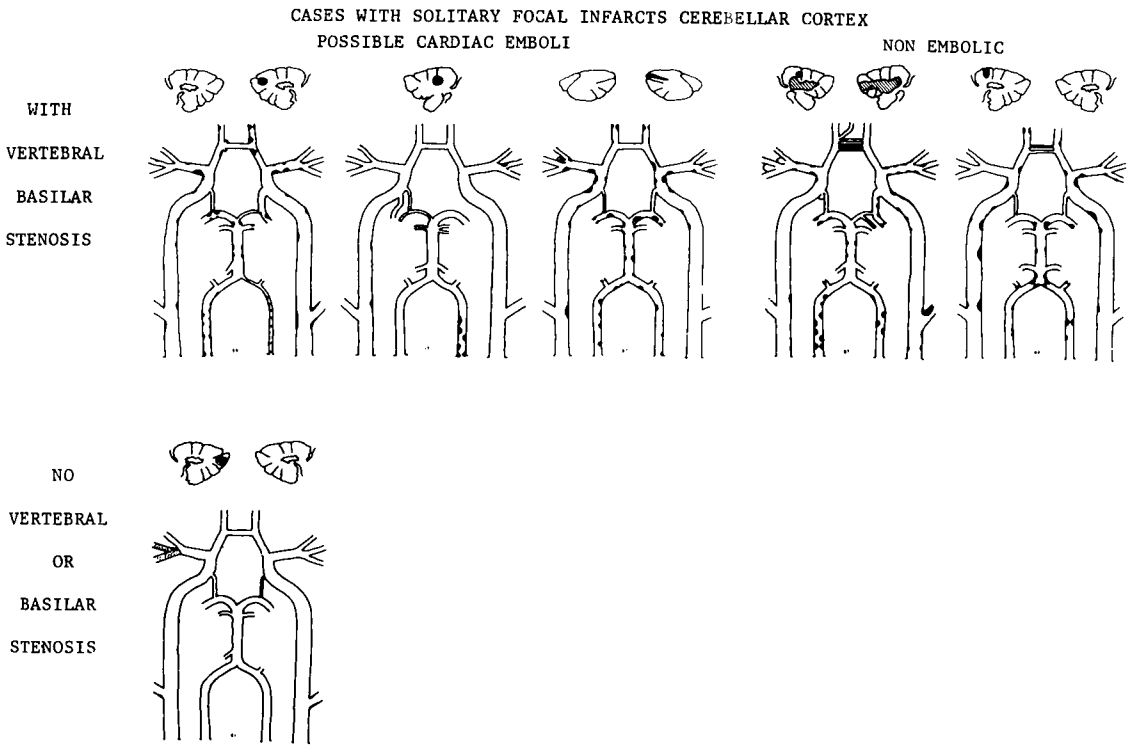

FIG. 4—Diagram of the major arterial and the parenchymatous cerebellar lesions in the 6 cases with solitary focal cortical infarcts including the 2 associated with old intracerebellar haemorrhage. Only 1 case had possible cardiac emboli.

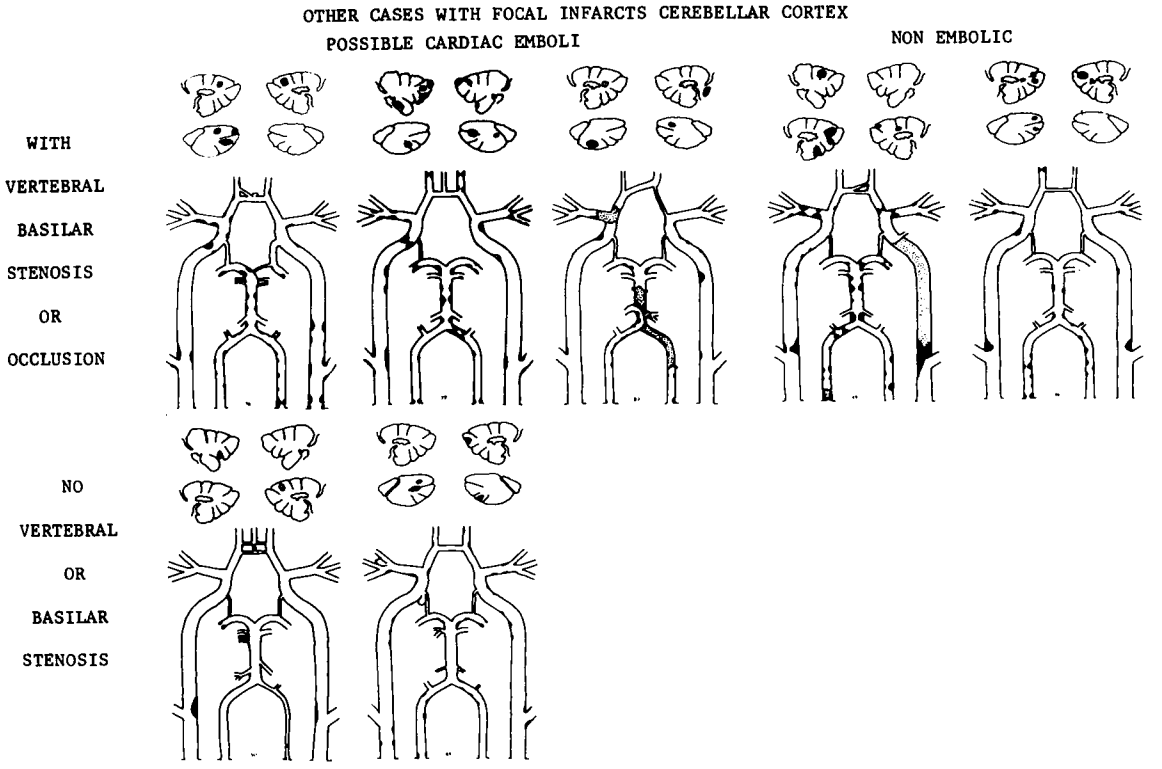

FIG. 5—Diagram of the major arterial and the parenchymatous cerebellar lesions in the other 7 cases with focal infarcts. There were 2 cases without vertebro-basilar stenosis, both of which could have had possible cardiac emboli.

farcts. In 13 cases there were multiple old focal cerebellar cortex infarcts and altogether 63 separate old ischaemic cortical lesions were identified—28 in the right hemisphere, 32 in the left hemisphere and 3 in the superior vermis (Fig. 6). In one case there were 11 old focal infarcts. The multiple infarcts were usually bilateral but seldom symmetrically situated. Only 16 (25·4%) of these infarcts involved the outer surface of the cerebellar hemispheres. Most of the remainder affected folia in the very depths of a fissure or sulcus.

These old focal infarcts were concentrated in the posterolateral parts of the cerebellar hemispheres and 46 (73·0%) were in the ansiform lobes. Of 18 (28·6%) old infarcts in the horizontal fissure, 10 (15·9%) involved both superior and inferior semilunar lobules and there were 32 infarcts (50·8%) affecting the superior semilunar lobules and 24 infarcts (38·1%) affecting the inferior semilunar lobules. The distribution of these infarcts was predominantly in the boundary zones of the territories of the superior and posterior inferior cerebellar arteries.

All the cases with focal cerebellar infarcts. When all the 19 cases with old or recent focal infarcts were considered together (Figs. 3, 4 and 5 combined), 12 showed a marked degree of stenosis in the vertebro-basilar arterial system. In 10 of them there were significant lesions in the cerebellar arteries also, including occlusive thrombosis in 2 cases—1 in the right superior and 1 in the left inferior cerebellar artery. In the other 8 of these 10 cases, severe stenosis was recorded in the main trunks of the superior cerebellar arteries in 7 of them. Stenoses involving more than one cerebellar artery were bilateral in 5 of these 10 cases but they were not symmetrically distributed. In addition to the stenoses in the main trunks of these cerebellar arteries, some of the artery branches in the anastomotic network over the cerebellar cortex were severely stenosed in 5 cases (Fig. 7).

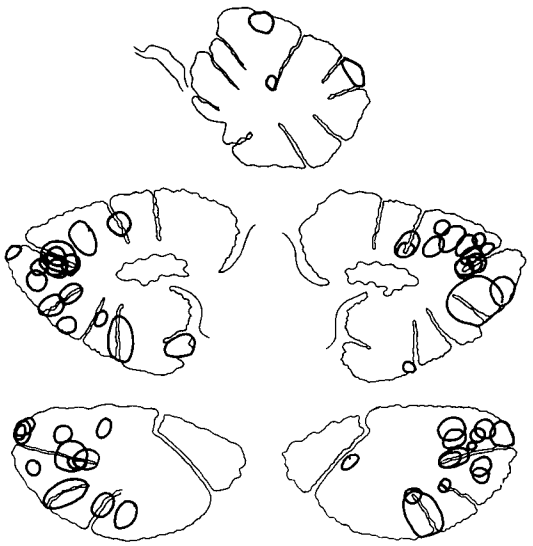

FIG. 6—Composite diagram showing size and situation of 63 old focal cerebellar cortex infarcts in 13 cases. Only 16 infarcts involved the outer surface of the cerebellum. The common localization of focal cortical infarcts around the horizontal fissure is clearly indicated.

Of the 7 cases of focal infarcts without appreciable vertebral or basilar stenosis, 3 showed some narrowing of the internal carotid system but this was not severe although in these 3 cases the posterior communicating arterial pathways were small and of limited possibility as anastomotic channels between the carotid and vertebral artery systems. The remaining 4 cases showed no significant narrowing of the internal carotid arteries or of the vertebro-basilar trunks.

Nature of the arterial lesions. The stenotic lesions in the vertebral, basilar and main cerebellar arteries were all atherosclerotic and many small atherosclerotic plaques were additionally present without producing any significant stenosis. In the anastomotic cortical arteries and in the large penetrating branches to the dentate nucleus irregular lumen narrowing was the result of a fibrotic intimal thickening although occasional lipoid deposits were present in the more severe hypertensives. Many of the meningeal lesions were clearly recanalized thrombi or emboli (Fig. 8).

Although hyaline arteriolosclerosis was not constantly observed in the meningeal vessels, this lesion was found in the penetrating arterioles in every case whether hypertensive or not. It was seldom notable in the meninges or in the cerebellar cortex but was much more prominent in the central medullary white matter. There was severe medullary arteriolosclerosis in 12 hypertensive cases with some arteriolonecrosis in a third of them.

DISCUSSION

The incidence and multiplicity of cerebellar infarcts in this group of patients with ischaemic brain disease is much greater than that reported by Yates and Hutchinson (1961) in a similarly selected group which, however, included more patients with brain haemorrhage. The very high frequency of small focal infarcts in the present cases suggests that these lesions may be found more often if the cerebellum is carefully examined.

The recent massive infarcts resulting from basilar thrombosis involved the superior cerebellar artery territories of both cerebellar hemi-

FIG. 7—Case 77. Radiograph of posterior part of medial parasagittal slice of right cerebellum showing atherosclerotic stenoses in end to end communicating artery between superior and posterior inferior cerebellar systems. There are also stenoses in the smaller superficial cortical anastomotic arteries. × 3.

FIG. 8—Case 76. Photomicrograph showing recanalized old thrombus in small meningeal artery related to an old focal cerebellar cortex infarct. The new lumen contains injection medium. H.E. × 72.

spheres and were similar to those in 2 of the 18 cases described by Kubik and Adams (1946). As Biemond (1951) has also indicated, there may be no ischaemic necrosis of the cerebellum in distal basilar occlusion and this would seem to emphasize the effectiveness of anastomotic pathways when a cerebellar artery is occluded.

Comprehensive accounts of cerebellar haemorrhage (Rey-Bellet, 1960; Dinsdale, 1964) make no reference to any resultant cortical ischaemic lesion although the surrounding scar has been well described (Michael, 1932). The pattern of the overlying cortical scar found in the 2 present cases suggests this scar is essentially the result of the healing of an ischaemic necrosis. Although pressure from a medullary haemorrhage would undoubtedly interfere with meningeal blood flow to the overlying cortex this would be lessened if the arterial flow were already reduced by vertebral stenosis, as seems likely in the 2 cases presented.

The 6 recent focal infarcts were all in superior cerebellar artery boundary zones—often erroneously referred to as "watershed" areas but more aptly likened to the terminal paddocks in a farm irrigation scheme (McHenry and Valsamis, 1966). In only 1 of these cases was there a thrombus in a superior cerebellar artery although it might be expected that a recent occlusive arterial lesion would be more likely in cases with recent infarcts than in the cases with old infarcts. Thompson (1944) has claimed that the superior cerebellar arteries are more susceptible to embolism than either the anterior or posterior inferior cerebellar arteries but in this series no emboli were found even in the cases with recent infarcts. Indeed, 2 of the 6 cases with recent infarcts had neither arterial stenosis nor any likely embolic source.

Of the 7 cases with focal infarcts in which no appreciable stenosis in the vertebro-basilar or even the carotid arterial systems was found (Figs. 3, 4 and 5), it is possible that emboli of cardiac origin may have been responsible for the infarctions in 5 cases. There remain 2 cases, however, in which there was nothing to support such an assumption and it is significant that in both these cases the infarcts were recent, so that if these were the result of embolism, some evidence of the phenomenon could be expected.

Blackwood *et al.* (1969) have recently shown that embolic brain infarcts are generally large and the great majority are supratentorial (Jörgensen and Torvik, 1969). Fisher (1965), in a necropsy study of 114 patients with 376 focal infarcts in the deeper parts of the cerebral hemispheres, has concluded that impaction of emboli in the deep penetrating vessels to these areas is an unlikely cause. It has also been emphasized (Fisher *et al.*, 1965) that occlusion of a large artery does not appear to be an important feature in the causation of these lesions in the cerebrum. Nevertheless the regression of an arteriographically demonstrable severely stenotic vascular lesion in clinical cases of minor stroke is a well recognized phenomenon (Denny-Brown, 1966) and the arterial lesion originally a causative factor in an infarction may not be recognizable in relation to an old infarct.

Ischaemic lesions may be experimentally produced in boundary zone areas of the monkey

brain following profound hypotension (Brierley and Excell, 1966) and similar ischaemic lesions have been described in man following precipitate and sustained hypotension (Adams *et al.*, 1966). These lesions have been found in the cerebral hemispheres and in the superior and posterior inferior cerebellar arterial territories boundary zones where more than three-quarters of the focal cortical infarcts in the present series were situated. The cerebellar arteries communicate freely over the surface of the cerebellum (Vander Eecken, 1959) and the occurrence of ischaemic necrosis in these boundary zones seems to be the result of a general decrease in blood flow within this normally efficient anastomotic network. Although the multiple stenoses both in the feeding and component vessels of the cerebellar network could be expected to have reduced perfusion in the boundary zones and contributed to infarction in a majority of the cases in the present study, it appears that vascular occlusion by embolism or thrombosis has been of little significance in precipitating the perfusion failure. It seems that a general decrease in blood flow can be regarded as the likely factor precipitating focal cerebellar infarction in the present cases.

Summary

The nature and site of more than 80 cerebellar infarcts and the associated vascular lesions found at necropsy in 21 elderly patients with a clinical history suggestive of ischaemc brain disease are described.

There were 2 massive recent infarcts associated with basilar thrombosis and 2 old focal infarcts associated with intra-cerebellar haemorrhage. There were 7 recent focal cortical infarcts, 6 solitary focal cortical infarcts, 9 focal medullary infarcts in 5 cases and 63 old focal cortical infarcts in 13 cases. In 12 of these 19 cases there was an old occlusion or severe stenosis in the vertebral-basilar-cerebellar artery system. The focal infarcts were predominantly in boundary zones of the territories of the superior cerebellar and posterior inferior cerebellar arteries. Conclusive evidence of embolism was lacking and a general decrease in blood flow in the cerebellar anastomotic network is sugested as the likely factor precipitating focal infarction.

Acknowledgments

The initial part of this study was carried out wth the help of Drs. L. M. Dugdale and G. D. Calvert in the Department of Pathology at the University of Otago, Dunedin, aided by a grant from the New Zealand Medical Research Council. The technical assistance and photographic work of Mr. S. W. Mathers of this Department in Hobart is gratefully acknowledged.

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NEUROPATHY ASSOCIATED WITH LYMPHOMA

J. C. WALSH*

INTRODUCTION

Neuropathy is a rarely reported complication of the lymphomas. Williams *et al.* (1959) reported a retrospective study of 3,725 subjects with lymphoma which included only 3 cases of a symmetrical peripheral neuropathy; in addition, several isolated case reports have appeared (Hutchinson *et al.*, 1958; Simpson, 1962). Root infiltration (Allison and Gordon, 1955) or multiple microemboli (Barron, Rowland and Zimmerman, 1960) have been demonstrated in some cases, but in others (Blanchard, 1962) no aetiological factor has been demonstrated. There has been no previous study on a group of randomly selected subjects with lymphoma to determine the incidence of neuropathy of lesser severity.

The aim of this study has been to determine whether abnormalities of nerve conduction are present in clinically normal subjects with lymphoma, and to establish the nature of the pathological changes that may occur.

MATERIALS AND METHODS

Subjects

Seventy-nine subjects with malignant lymphoma were examined.

To exclude other causes of neuropathy a glucose tolerance test, urinary porphyrin assay, blood urea, marrow biopsy and measurements of serum folate and B_{12} levels were performed on those subjects with evidence of involvement of the peripheral nervous system.

Nerve Conduction Studies

Electrophysiological studies were performed on 62 subjects with malignant lymphoma and on 35 control subjects matched for age. There is a gradual loss of myelinated fibres with ageing (O'Sullivan and Swallow, 1968; Ochoa and Mair, 1969b) resulting in impairment of conduction. For this reason the subjects have been separated into two groups on the basis of age (Table 1).

Motor conduction velocities were determined in the median, ulnar and lateral popliteal nerves. Sensory action potentials were recorded from the median and ulnar nerves, and mixed motor and sensory action potentials were recorded from the ulnar nerve at the elbow and from the lateral popliteal nerve at the knee. The techniques used are standard in this laboratory (Walsh and McLeod, 1970).

The skin temperature of the arm and leg was measured with a thermistor and ranged from 31°C to 35°C.

Histological Methods

Sural nerve biopsy was performed on 5 subjects with lymphoma who had abnormalities of nerve conduction.

Light Microscopy: The nerves were divided into three pieces about 1 cm long and attached to card. One piece was stained for myelin with Flemming's solution (Walsh and McLeod, 1970) and photomicrographs of transverse sections of selected fascicles were printed on photographic paper at an enlargement of $\times$ 1,000. The external diameters of all myelinated fibres not undergoing active degeneration were measured and histograms of fibre size were constructed (Swallow, 1966; Fullerton and O'Sullivan, 1968). A second piece of nerve was fixed in picric acid (Holmes, 1943), embedded in paraffin wax and 5μ sections stained with haematoxylin and eosin, and with Congo Red. A third piece of nerve was fixed in 10% formal saline, and subsequently stained in 1% osmic acid and single fibres isolated by teasing (Thomas, 1955). Measurements were made of the length and diameter of each internode and

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TABLE

Motor and Sensory Conduction
Group A: Subj

	Motor Conduction						
	Median			*Ulnar*			
	Latency (m sec)	*Amplitude* (mV)	*Velocity* (M/sec)	*Latency* (m sec)	*Amplitude* (mV)	*Velocity* (M/sec)	*Latency* (m sec)
CONTROLS (15) Age 31·7±10·2 (18–48)	*3·3±0·4 2·8–4·3	7·2±1·8 3·6–10·4	60·4±4·9 50–68	2·6±0·4 2·1–3·3	8·0±2·0 3·7–10·8	56·3±4·9 50–69	4·4±0·7 3·8–6·0
LYMPHOMA (20) Age 34·3±12·2 (14–50)	3·6±0·9 2·6–5·8	8·6±2·9 3·6–16·0	53·3±12·3 42–65	2·9±0·7 2·0–4·2	8·1±1·9 4·7–12·2	55·5±7·9 47–76	5·6±1·6 3·8–8·6
SIGNIFICANCE	ns	ns	P < 0·05	ns	ns	ns	P < 0·05

Group B: Subject

CONTROLS (20) Age 63·4±9·7 (50–80)	3·8±0·8 2·6–5·1	6·6±2·7 2·7–13·4	52·2±4·1 43–59	2·9±0·7 2·2–4·9	8·7±1·8 5·0–12·0	53·2±2·5 42–61	5·2±1·3 3·3–7·7
LYMPHOMA (42) Age 64·6±9·4 (51–83)	3·8±0·8 2·4–5·3	5·9±2·6 1·8–12·9	50·3±4·6 42–64	3·0±0·5 2·1–4·3	7·9±2·4 3·7–13·5	51·6±5·2 36–64	5·7±1·3 3·2–8·9
SIGNIFICANCE	ns	ns	ns	ns	ns	ns	ns

* Mean ± S.D.
Range

the results plotted in the manner described by Fullerton, Gilliatt, Lascelles and Morgan-Hughes (1965).

Electron Microscopy: Pieces of nerve 5 mm in length from 4 subjects with lymphoma and from a control subject were fixed in iced 2% osmic acid on 0·1 M Cacodylate buffer (pH 7·3) for 3 hours. The specimens were then dehydrated, counterstained wth 1% phosphotungstic acid in absolute alcohol and embedded in Araldite. Thin sections were stained on copper grids with uranyl acetate and lead citrate and examined in a Philips EM200 electron microscope. Unmyelinated fibres were counted and their diameters measured on prints at a final enlargement of about × 6,000, and histograms of fibre density constructed in the manner described by Ochoa and Mair (1969a).

RESULTS

Clinical Studies

Seventy-nine subjects with malignant lymphoma were examined. Seventeen were excluded from the study because there was a history of alcoholism, diabetes mellitus, dietary privation or of therapy with neurotoxic drugs. Among the 62 subjects selected for electrophysiological studies there were 5 subjects with clinical evidence of mild peripheral neuropathy. Forty-one subjects had no systemic symptoms, the remainder having varying amounts of disability. One subject was found to have high concentrations of uroporphyrins and coproporphyrins in the urine.

Electrophysiological studies were performed on 35 control subjects with no historical or clinical evidence of neurological disease.

Nerve Conduction Studies

The subjects were divided into two groups on the basis of age (Table 1). Among the 35 control subjects there were 4 who had an asymptomatic localized lesion of the median, ulnar or lateral popliteal nerve and one who had generalized impairment of motor and sensory conduction; these subjects were retained

dies in 62 Subjects with Lymphoma
Younger than 50 Years

		Sensory Action Potentials				*Mixed Action Potentials*			
teral Popliteal		*Median*		*Ulnar*		*Ulnar*		*Lateral Popliteal*	
Amplitude (mV)	*Velocity* (M/sec)	*Latency* (m sec)	*Amplitude* (μV)	*Latency* (m/sec)	*Amplitude* (μV)	*Latency* (m sec)	*Amplitude* (μV)	*Latency* (m sec)	*Amplitude* (μV)
5·0±2·2 2·0–9·7	49·6±3·9 41–56	2·7±0·3 2·2–3·4	22·4±8·2 6–40	2·4±0·3 2·0–3·1	17·1±7·2 6–36	5·0±0·5 4·1–5·8	57·4±16·7 35–88	6·2±0·8 5·1–7·2	6·7±3·3 3–13
3·6±1·8 0·6–6·9	44·4±5·1 34–55	3·4±0·7 2·6–5·0	11·7±3·3 7–20	3·2±0·7 2·6–4·8	7·1±2·8 2–12	5·6±0·8 4·2–7·3	29·2±14·6 6–62	6·8±0·1 5·6–8·9	3·5±1·6 0–7
P < 0·05	P < 0·01	P < 0·01	P < 0·001	P < 0·001	P < 0·001	P < 0·05	P < 0·001	P < 0·05	P < 0·01
Older than 50 Years									
3·0±1·7).6–6·9	44·1±4·9 33–55	3·4±0·6 2·8–5·0	11·5±3·9 4–20	2·9±0·4 2·3–3·8	8·7±3·6 0–15	5·6±0·6 4·0–6·7	31·0±12·3 9–67	6·6±0·9 5·4–8·6	3·2±3·3 0–15
2·0±1·1 0–4·3	40·2±6·4 28–50	3·6±0·6 2·8–5·0	9·0±5·2 0–30	3·2±0·6 2·1–4·8	5·8±3·3 0–34	6·0±0·8 4·1–8·0	21·3±10·2 7–51	6·7±0·8 5·3–9·0	3·5±0·9 2·3–4·9
P < 0·01	P < 0·05	ns	P < 0·05	P < 0·05	P < 0·01	P < 0·05	P < 0·01	ns	P < 0·01

in the control group to allow a less biased comparison with lymphoma group.

The results of motor and sensory conduction in both age groups have been compared, and the significance of the difference determined by the student *t* test (Table 1). It may be seen that the motor latency tends to be increased in the median, ulnar and lateral popliteal nerves but the difference becomes significant only in the lateral popliteal nerve in younger subjects. There is a similar trend with the motor conduction velocity measurements, the difference becoming significant in the median nerve in young subjects, and in the lateral popliteal nerve in both age groups.

By contrast there is a significant increase in latency and a significant reduction in amplitude of the sensory action potentials recorded from the median and ulnar nerves, and of the mixed nerve action potentials recorded from the ulnar and lateral popliteal nerves of both age groups. It may be seen that the difference is more significant in the younger age group (Table 1, Fig. 1). The relationship of age to impairment of conduction is illustrated in Fig. 2.

The results of electrophysiological studies on each subject have been compared with the normal range. The abnormalities found in individual subjects are given in Table 2. It can be seen that in 22 subjects there was a generalized impairment of conduction. Five of these had clinical evidence of a mild neuropathy but 17 had no clinical evidence of a peripheral neuropathy. Eleven subjects had evidence of a localized lesion of the median, ulnar or lateral popliteal nerve, which did not exceed the incidence of isolated lesions in control subjects.

Sural Nerve Biopsy

Sural nerve biopsy was performed on 5 subjects with lymphoma who had electrophysiological evidence of impaired conduction. Relevant clinical and laboratory details are summarized in Table 3. One subject (Case 1) had clinical evidence of mild peripheral neuropathy. In 2 subjects (Cases 3 and 5) the total

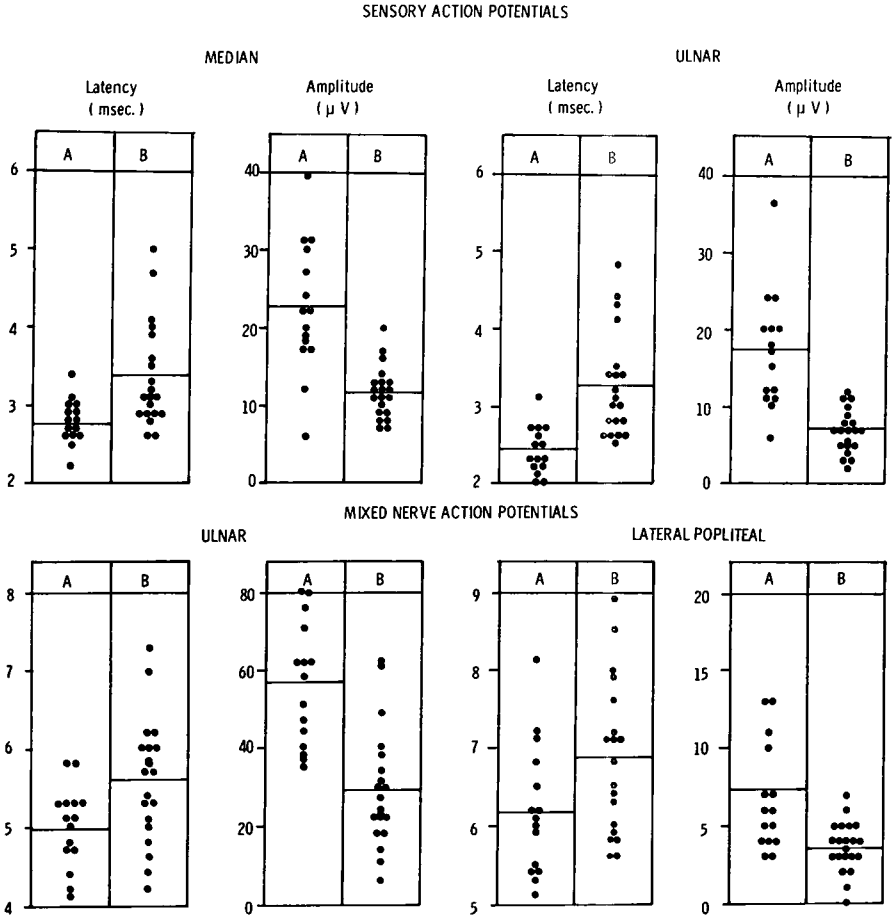

FIG. 1—The results of sensory conduction studies in control subjects (Column A) and in lymphoma subjects (Column B) less than 50 years of age. The mean for the group is indicated by a horizontal line.

TABLE 2

Peripheral Nerve Involvement in 62 Subjects with Malignant Lymphoma

Diagnosis	*Number Studied*	*Mild Clinical Neuropathy*	*Generalized Impairment of Nerve Conduction*	*Localized Nerve Lesions*		
				Median	*Ulnar*	*Lateral Popliteal*
Hodgkins disease	20	1	4			2
Chronic lymphatic leukemia	18	1	8	1	3	1
Lymphosarcoma	9	1	5			
Reticulum cell sarcoma	10	2	5		1	2
Giant follicular lymphoma	5			1		
	62	5	22	2	4	5

density of myelinated fibres was within the normal range and in 3 there was a reduction in the number of fibres of all diameters. There was no evidence of arteritis, amyloid or cellular infiltrate in any of the nerves examined.

Abnormalities were found in all nerves on examining teased fibre preparations. In 2 subjects (Cases 2 and 5) the dominant abnormality was segmental demyelination (Fig. 3a); only an occasional fibre was seen undergoing axonal degeneration; wide variations in internodal length were common on individual fibres. The distribution of myelinated fibres in Case 2 is illustrated in Fig. 4.

In one subject active axonal degeneration was present in some fibres while others appeared normal. There was little variation in internodal length on individual fibres (Fig. 3b). In 2 subjects over the age of 70 there were changes due to both axonal degeneration and segmental loss of myelin.

Electron microscopy was performed on a control nerve and on the nerves from 4 subjects with lymphoma. The density and distribution of unmyelinated fibres in the control subject were within the range reported by Ochoa and Mair (1969a) in a study of 5 normal subjects. The density and distribution of unmyelinated fibres in the 4 subjects with lymphoma were within the normal range. The fibre distribution in Case 2 is illustrated in Fig. 5.

Discussion

In 1948, Denny-Brown reported 2 cases of carcinoma of the lung who had a senory neuropathy. Since that time there has been increasing awareness of the involvement of the peripheral nervous system in carcinoma, and the high incidence of sub-clinical involvement of the peripheral nervous system in this condition has been established (Moody, 1965; Hildebrand and Cöers, 1967; Trojaborg *et al.*, 1969). Peripheral neuropathy is a rarely reported complication of lymphoma and there has been no detailed study performed on a group of subjects with lymphoma to determine the incidence of peripheral nerve involvement. Electrophysiological studies were performed on a group of

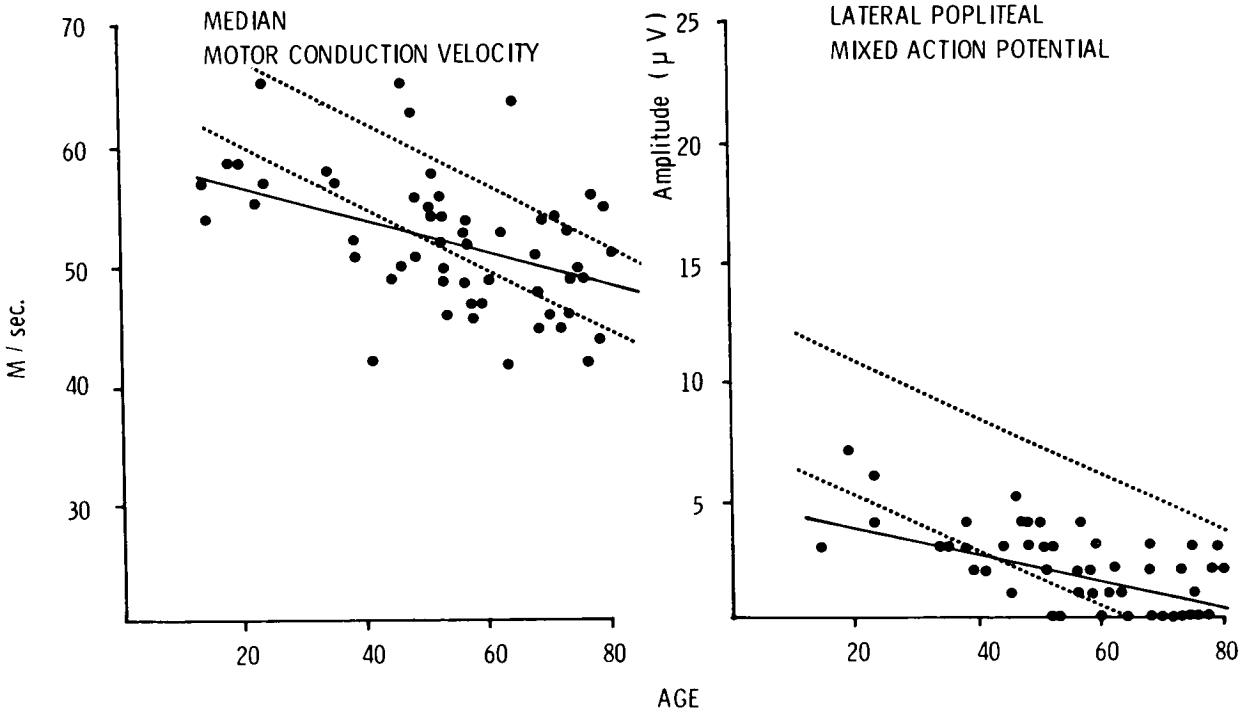

FIG. 2—The relationship of age to impairment of conduction. The results of two electrophysiological measurements in subjects with lymphoma are plotted against age. The calculated regression line is indicated by a solid line and the normal range by dotted lines.

TABLE 3

Clinical and Laboratory Data on 5 Subjects with Malignant Lymphoma

	Age	*Sex*	*Diagnosis*	*Relevant Clinical and Laboratory Findings*	*Density of Myelinated Fibres (thousands/ sq.mm.)*	*Area (sq.mm.)*	*Histology*
Case 1	76	M	Chronic lymphatic leukemia	2-point discrimination impaired on feet; ankle jerks reduced.	2·40	0·27	Active axonal degeneration and segmental demyelination.
2	60	M	Reticulum cell sarcoma	Syncope due to autonomic neuropathy. No clinical evidence of peripheral neuropathy.	1·80	0·26	Segmental demyelination.
3	42	M	Reticulum cell sarcoma	Gross wasting. No clinical evidence of neuropathy. High level of urinary porphyrins.	6·07	0·23	Active axonal degeneration.
4	75	M	Hodgkin's disease	Extra dural mass at T8 level removed. No clinical evidence of neuropathy at time of study but ankle and knee jerks disappeared 3 months later.	1·55	0·59	Segmental demyelination and occasional axonal degeneration.
5	40	M	Lympho-sarcoma	No clinical evidence of neuropathy.	5·51	0·09	Segmental demyelination and moderate axonal degeneration.

62 subjects with lymphoma of varying degrees of severity. The presence of a peripheral neuropathy was confirmed in 5 subjects with clinical evidence of a mild neuropathy, and demonstrated in 17 subjects who were clinically normal. Abnormalities were most common in subjects with lymphosarcoma and reticulum cell sarcoma.

Impairment of sensory conduction was more significant than impairment of motor conduction, because this is a more sensitive method of evaluation. It is of considerable interest that the impairment of conduction is more marked in the younger subjects with lymphoma. This may indicate a lowered resistance of subjects with malignancy to the factors which produce age changes in the peripheral nervous system, such as repeated minor trauma.

Sural nerve biopsy was performed on 5 of the 22 subjects with impaired conduction. The sural nerve biopsy from Cases 2 and 5 both had a marked reduction in myelinated fibre density, fibres of all diameters being affected. On examination of teased fibres, segmental demyelination was found in numerous fibres examined. The finding of segmental demyelination indicates a local metabolic or toxic disorder of the Schwann cell. There was no evidence of arteritis, amyloid or cellular infiltration in the nerves examined. If the peripheral neuropathy in these 2 subjects was the result of proximal microemboli or of infiltrative or toxic lesions of the dorsal root ganglia the biopsy would be expected to show the changes of axonal degeneration. A relative nutritional deficiency has been suggested as a factor in the production of malignant neuropathy, but the dominant pathological change of nutritional neuropathy is axonal degeneration (Pekelharing and Winkler, 1893; Dyck *et al.*, 1968; Walsh and McLeod, 1970). Fibres undergoing axonal degeneration were rarely seen in the nerves from these two patients.

By contrast, the nerve from Case 3 had no gross reduction of myelinated fibres in transverse section but examination of teased fibres revealed a mixture of normal fibres, and of fibres undergoing active axonal degeneration. Electrophysiological studies indicated that there was a diffuse impairment of both motor and

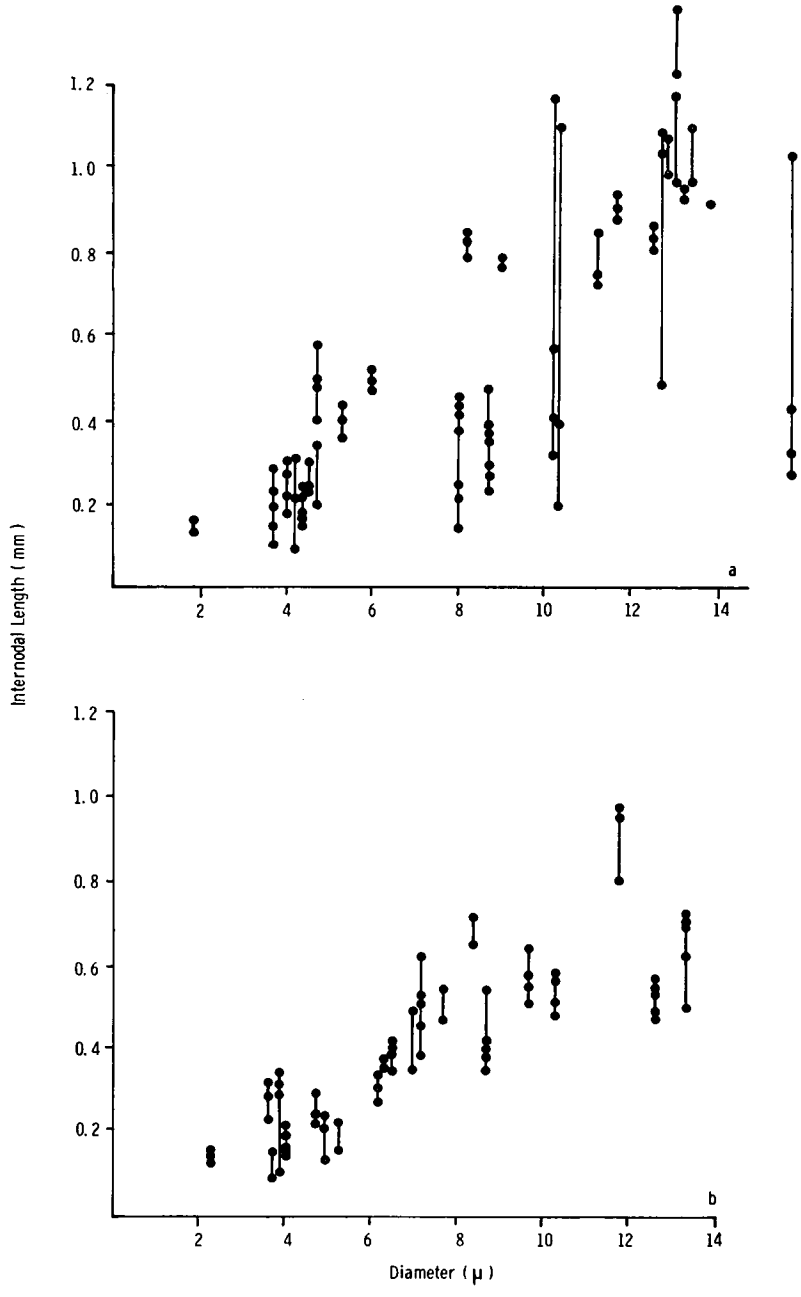

FIG. 3a, b—The distribution of internodal length of single fibres teased from the sural nerves of two subjects with lymphoma. The internodal lengths of individual fibres are plotted against the diameter of the widest internode and joined by a vertical line.

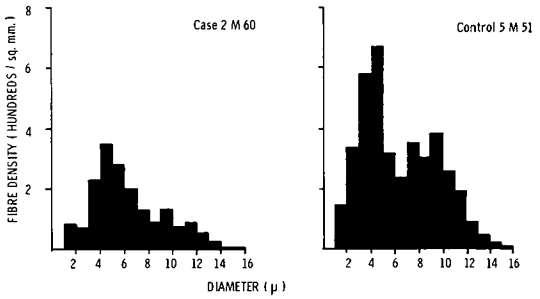

FIG. 4—Histogram of the distribution of myelinated fibres from the sural nerve of a control subject, and from a subject with lymphoma.

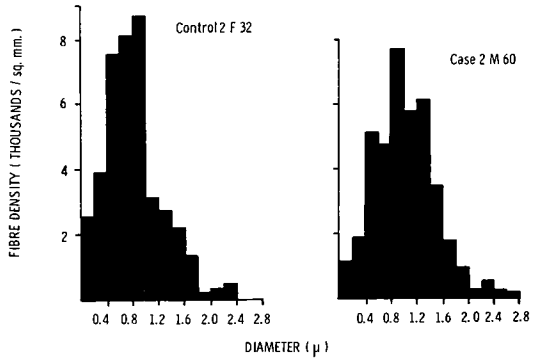

FIG. 5—Histogram of the distribution of unmyelinated fibres from the sural nerve of a control subject, and from a subject with lymphoma.

sensory conduction. This subject was grossly wasted and although a toxic or nutritional aetiology could be suggested, the possibility of multiple microemboli proximally, or widespread root involvement, cannot be excluded. It is of interest that the urine of this subject contained large amounts of uroporphyrins and coproporphyrins. There is no established association between the neuropathy of malignancy and excessive porphyrin production by the tumour but this possible association warrants further attention.

In the 2 subjects (Cases 1 and 4) over the age of 70 there was a significant reduction in fibre density, and examination of teased fibres established that both axonal degeneration and segmental demyelination were present. The significance of these findings in subjects over the age of 70 is difficult to assess as both changes are found in the aged peripheral nervous system (Lascelles and Thomas, 1966).

Electron microscopy was performed on the sural nerve of 4 subjects with lymphoma and on a control subject. There was no significant loss of unmyelinated fibres in the lymphoma subjects.

Electrophysiological evidence of impaired conduction has been demonstrated in 17 of 57 subjects with lymphoma who had no clinical evidence of peripheral neuropathy, and confirmed in 5 subjects with clinical evidence of a mild peripheral neuropathy. The incidence of sub-clinical neuropathy in the lymphoma group was 30%, which may be compared with the incidence of 33% of sub-clinical affection demonstrated by electromyography in a group of patients with carcinoma of the lung (Trojaborg *et al.*, 1969). More than one type of pathological lesion may be responsible for neuropathy in the malignant lymphomas. The presence of axonal degeneration may be a reflection of nutritional deficiency, or of toxic, embolic or infiltrative lesions proximally. The presence of segmental demyelination in some cases indicates that a local disorder of the Schwann cell may also occur in the malignant lymphomas. Acceleration of ageing changes in the peripheral nervous system may be a contributing factor.

It is considered that the subjects studied form a representative group, as less than one-third of the group were suffering from significant systemic symptoms. The stage of the disease was not related to the presence of neurological involvement. It is concluded that sub-clinical involvement of the peripheral nervous system is common in malignant lymphoma.

SUMMARY

Electrophysiological studies have been performed on 62 subjects with malignant lymphoma and compared with 35 control subjects. There is a high incidence of sub-clinical involvement of the peripheral nervous system. Detailed histological studies were performed on a sural nerve biopsy from 5 subjects with lymphoma. More than one factor contributes to impaired conduction. In some nerves axonal degeneration is the dominant feature, and in others there is segmental demyelination. The unmyelinated fibres do not appear to be involved.

ACKNOWLEDGMENTS

This work was supported by grants from the

New South Wales State Cancer Council, and from the Postgraduate Medical Foundation, University of Sydney. Valuable technical assistance was given by Miss Julia Baverstock. The advice and criticism of Associate Professor J. G. McLeod is gratefully acknowledged.

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ULTRASTRUCTURAL CHANGES IN THE PERIPHERAL NERVES IN EXPERIMENTAL DYING-BACK POLYNEUROPATHIES

JOHN W. PRINEAS*

When a peripheral nerve fibre is cut at one point, both the axon and myelin sheath distal to the site of injury degenerate. Nerve fibres showing a similar Wallerian-like pattern of degeneration on light microscopy occur at the distal extremities of long peripheral nerves in certain symmetrical polyneuropathies. Why nerves degenerate in this way is not understood, although it has been suggested that distal axonal degeneration in these "dying-back" polyneuropathies may be due to a diminished output from the neuronal perikaryon of essential axonal metabolites. Recent ultrastructural and biochemical studies indicate that more than one subcellular mechanism can lead to this type of neuronal degeneration.

In experimental tri-ortho-cresyl phosphate (TOCP) neuropathy in chickens and cats (Bischoff, 1967; Prineas, 1969a), the earliest subcellar change observed in the peripheral nerves is an accumulation of membrane-bound irregular vesicles within the axoplasm of affected nerves (Figs. 1 and 2). This change occurs a week or more before the onset of clinical signs and several weeks before changes are visible light microscopically. At the time of onset of clinical signs, large osmiophilic concentrically lamellated bodies appear within terminal axoplasmic expansions at neuromuscular junctions. Axonal changes show a marked distal-proximal gradient and rarely extend as high as the spinal nerve roots. Perikarya of affected neurons show a mild increase in neurofilaments and smooth endoplasmic reticulum. *Boutons terminaux* and small axons within the central nervous system show a sequence of changes identical to that seen at the periphery.

In experimental acrylamide polyneuropathy in the cat, the light microscopical changes in the peripheral nerves are similar to those seen in TOCP polyneuropathy. The early fine-structural changes occurring within affected axons in these two dying-back polyneuropathies are, however, quite different. In acrylamide polyneuropathy (Prineas, 1969b), the earliest change seen is a marked accumulation of

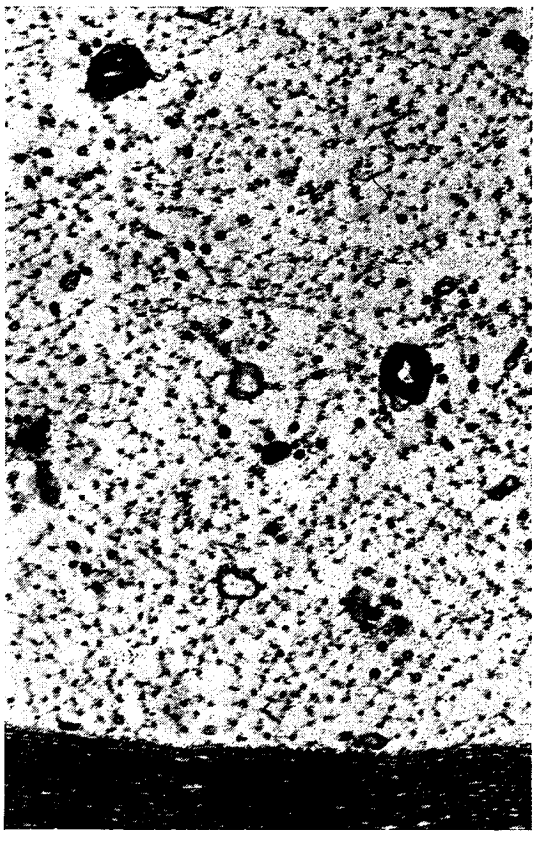

FIG. 1—Normal peripheral axon showing neurofilaments, neurotubules, mitochondria and sparse endoplasmic reticulum. × 50,000.

* Sydney.

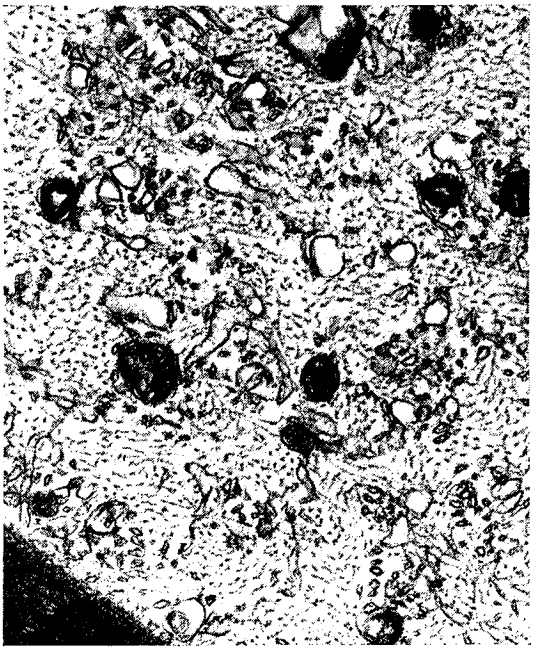

FIG. 2—TOCP polyneuropathy. The axoplasm contains a system of abnormal membrane-bound spaces. × 42,000.

neurofilaments in distal axons (Fig. 3) and in terminal axoplasmic expansions. A similar increase in axonal neurofilaments and degenerative changes in axonal neurofilaments and in axonal mitochondria are seen later along the whole length of peripheral nerves and may be conspicuous in the spinal nerve roots, especially the posterior roots. In the central nervous system, small axons and *boutons terminaux* in the gracile tract and nucleus also show an early accumulation of neurofilaments.

It is difficult to induce a polyneuropathy in experimental animals by dietary means but this has been achieved by several groups of investigators using thiamine deficient diets over long periods and the changes found on light microscopy have been those of a dying-back polyneuropathy. In rats maintained on a thiamine deficient diet, fine-structural changes have been observed in distal intramuscular nerves which differ from those seen in pair-fed controls (Prineas, 1970) or those produced by simple starvation (Collins *et al.*, 1964). The earliest changes seen include the appearance of flattened membrane-bound sacs within the axoplasm (Fig. 4) and a decrease in the number of neurotubules present. Similar axoplasmic changes have been described in the central nervous system in thiamine deficient rats (Tellez and Terry, 1968).

The fact that the early fine-structural changes within axons in these three experimental dying-back polyneuropathies differ suggests that different subcellular mechanisms are responsible for distal axonal degeneration in these polyneuropathies. In TOCP polyneuropathy, degenerate organelles continue to accumulate in terminal axoplasmic expansions for a period following the appearance of changes in axons, which suggests that the bulk flow of axoplasm in this polyneuropathy may not be affected during the early stages of degeneration. In acrylamide polyneuropathy, the axonal fine-structural changes resemble those present in nerve fibres above points of partial constriction (Weiss, 1967), and it is possible that, in this instance, bulk flow of axoplasm may be impaired from an early stage in the development of the neuropathy. Pleasure *et al.* (1969) measured the rate of flow of isotopically

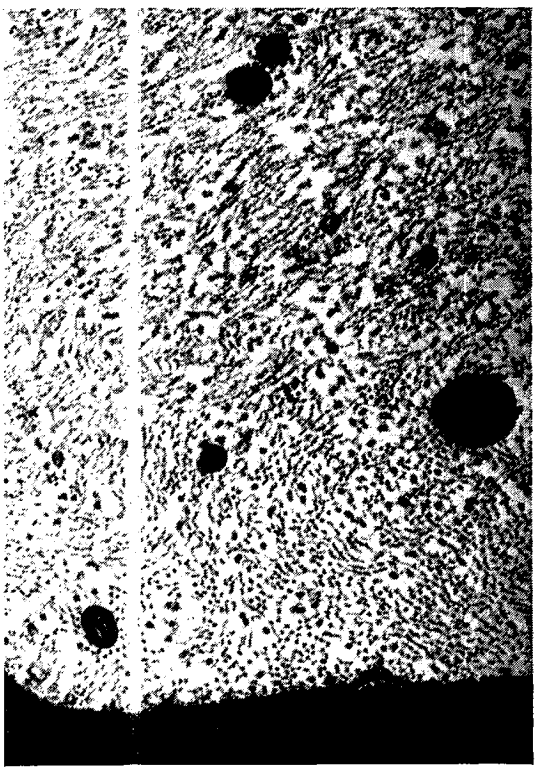

FIG. 3—Acrylamide polyneuropathy. Neurofilaments are present in excessive numbers and the mitochondria are enlarged. × 33,000.

labelled protein in the spinal nerve roots in cats in these two experimental polyneuropathies and observed that in TOCP polyneuropathy, protein moved at a normal rate whereas in acrylamide polyneuropathy, the flow was markedly reduced. In thiamine deficiency polyneuropathy, the early loss of neurotubules observed suggests that fast axoplasmic transport, in which it is thought neurotubules play a special role, may be specifically impaired.

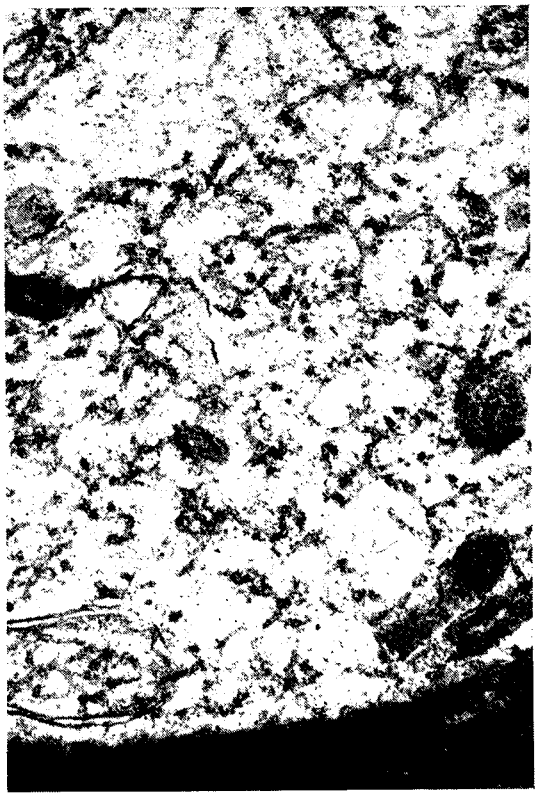

FIG. 4—Thiamine deficiency polyneuropathy. The axoplasm contains a system of flattened membrane bound sacs. Neurofilaments and neurotubules are depleted. × 45,000.

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ONION BULB FORMATIONS IN CHRONIC POLYNEUROPATHIES

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Introduction

Onion bulb formation is the name given to the concentric arrays of endoneurial elements around nerve fibres which may be seen in transverse sections of peripheral nerves in certain types of neuropathy. Classically, onion bulb formations are associated with the recessively inherited hypertrophic neuropathy of Déjèrine and Sottas, but it is now recognized that they may be seen in the peripheral nerves in other conditions, including sporadic cases of hypertrophic interstitial polyneuropathy (Thomas and Lascelles, 1967), Refsum's syndrome (Reese and Bareta, 1950), Charcot-Marie-Tooth disease (Dyck, 1966) and diabetic neuropathy (Thomas and Lascelles, 1966).

Recent electronmicroscope studies have clearly established that the onion bulbs are composed of concentric laminae of Schwann cells with intervening layers of collagen fibrils (Dyck, 1966; Thomas and Lascelles, 1967; Webster, Schröder, Asbury and Adams, 1967). However, several views have been expressed concerning their mechanism of production and the hypotheses advanced fall into three major groups (Ballin and Thomas, 1968):

(1) a primary proliferation of Schwann cells with secondary damage to nerve fibres (Garcin, Lapresle, Fardeau and de Recondo, 1966),
(2) repeated axonal degeneration and regeneration (Webster *et al.*, 1967), and
(3) segmental demyelination and remyelination (Thomas and Lascelles, 1967; Dyck, 1969).

There is little evidence to support the first of these hypotheses, and the main argument has been whether onion bulb formations are associated with segmental demyelination or axonal degeneration. In order to investigate the matter further, we have examined sural nerve biopsies from patients with subacute or chronic neuropathy; the presence of onion bulbs recognized by light and electron microscopy has been related to the pathological process of degeneration seen in teased single nerve fibres, and to the results of nerve conduction studies.

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Methods

Motor conduction studies were performed on the median, ulnar and lateral popliteal nerves; sensory action potentials were recorded in the median and ulnar nerves, and mixed nerve action potentials were recorded in the ulnar and lateral popliteal nerves (Walsh and McLeod, 1970).

Sural nerve biopsies were performed at the level of the lateral malleolus. Portion of the nerve was fixed in Flemming's solution and transverse sections were stained with Kultschitzky's haematoxylin and counter-stained with Van Gieson. Another portion of the nerve was fixed in 10% formaldehyde, stained in 1% osmic acid and single nerve fibres teased out and measured (Walsh and McLeod, 1970).

Material for electron microscopy was fixed in 3% glutaraldehyde in a 0·1 M cacodylate buffer for 3 hours followed by 2% osmium tetroxide for 1 hour. Some material was obtained from paraffin blocks and subsequently fixed as above. After fixation, the nerve was embedded in araldite and examined in a Siemens 1A or a Philips 200 Electron Microscope after staining with uranyl acetate and lead citrate.

Results

Sural nerve biopsies from 27 patients with chronic or subacute neuropathies were ex-

amined. Of these, 14 were found to have onion bulb formations by light or electron microscopy or both, and 13 had no evidence of onion bulb formation. For the purposes of analysis, they were divided into 3 groups.

Group A: Nerves in which gross onion bulbs were conspicuous on light microscopy

Six cases fall into the group. The results are summarized in Table 1.

(a) *Light Microscopy*

The occurrence of inappropriately thin or absent myelin sheaths in relation to the axon diameter was regarded as evidence of primary demyelination (Dyck, 1966). Such evidence was found in all 6 cases (Fig. 1).

(b) *Teased Fibre Measurements*

Fibres were teased from only 3 of the 6 sural nerves. In one case no myelinated fibres were found, but in the other 2 cases there was unequivocal evidence of segmental demyelination (Fig. 2).

FIG. 1—An epon section showing large onion bulbs around demyelinated axons. × 1,400.

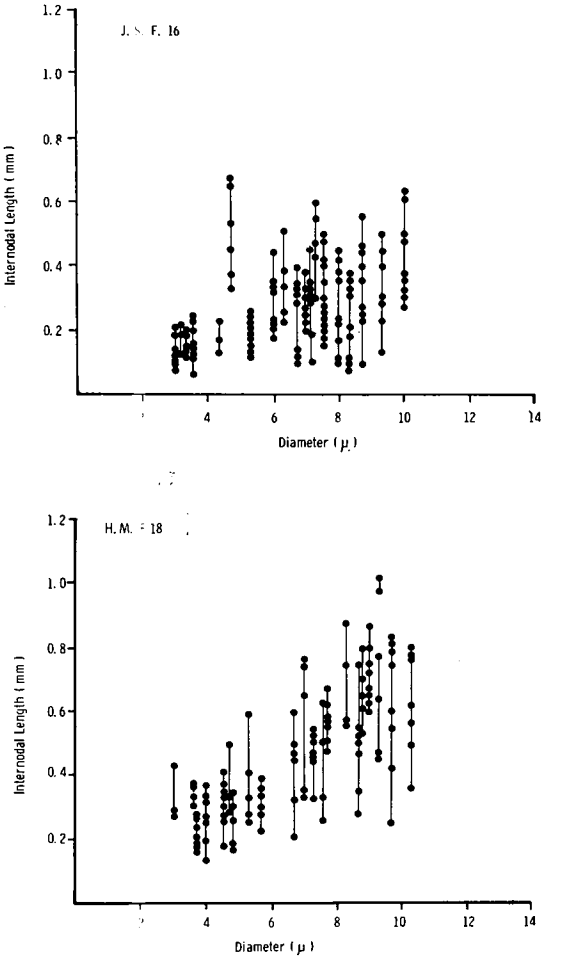

FIG. 2—Relationship between internodal length and fibre diameter in teased single nerve fibres from two patients with chronic polyneuropathy. Onion bulb formations were seen on electron microscopy.

(c) *Electron Microscopy*

The occurrence of thin or absent myelin sheaths in respect to the axon diameter was regarded as evidence of demyelination, and was found in all 4 nerves examined electronmicroscopically (Fig. 3).

(d) *Nerve Conduction Studies*

Nerve conduction studies were performed in 4 subjects. In 2 cases, the neuropathy was so gross that no action potentials could be recorded. In 2 cases, there was gross slowing of conduction. The degree of slowing of conduction in the upper

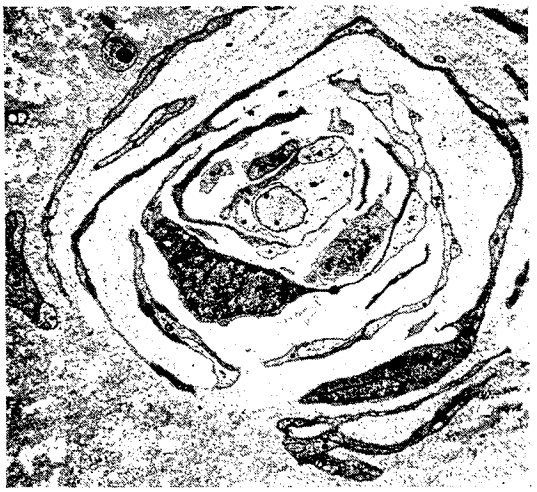

FIG. 3—An electron micrograph of an onion bulb showing a central demyelinated axon and concentric lamellae composed of Schwann cell processes and processes of unidentified mononuclear cells. × 4,000.

limbs has been arbitrarily defined as follows:

mild	40-50 M/sec.
moderate	30-40 M/sec.
gross	less than 30 M/sec.

Group B: Nerves in which onion bulb formations were inconspicuous on light microscopy, but were apparent on electron microscopy

Eight cases fell into this group. The results are summarized in Table 2.

(a) *Light Microscopy*

There was evidence of primary demyelination in 3 cases.

(b) *Teased Fibre Measurements*

Single fibres were teased from all the nerves, and in all cases there was definite histological evidence of segmental demyelination.

(c) *Electron Microscopy*

All nerves were examined by electron microscopy and all showed evidence of primary demyelination (Fig. 4).

(d) *Nerve Conduction Studies*

Gross slowing of conduction was found in 4 of the 8 subjects.

Group C: Nerves in which no onion bulbs were found on light or electron microscopy

There were 13 cases in this group. The results are summarized in Table 3.

(a) *Light Microscopy*

There was no evidence of primary demyelination.

(b) *Teased Fibre Measurements*

Single fibres were teased from 10 of the 13 nerves. In 1 case no myelinated fibres remained. In 2 cases there was conspicuous segmental demyelination, and in the remaining 7 cases axonal degeneration was the predominant pathological abnormality.

(c) *Electron Microscopy*

All the nerves were examined by electron microscopy. There was no evidence of primary demyelination.

(d) *Nerve Conduction Studies*

In 1 subject there was gross slowing of conduction. In the remaining subjects, mild degrees of slowing of conduction occurred.

DISCUSSION

The present study indicates that there is a very high degree of correlation between onion bulb formation and segmental demyelination. In fact, there was evidence of segmental de-

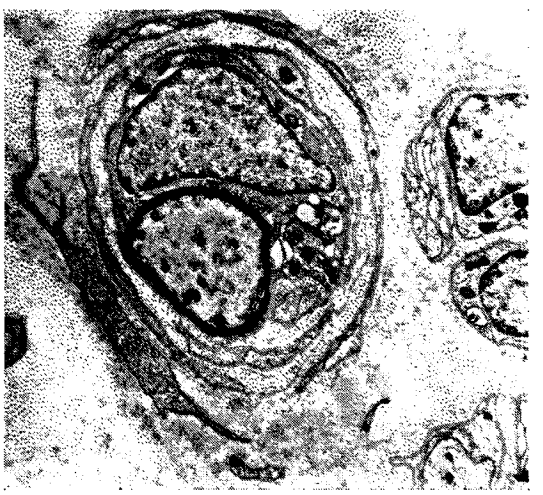

FIG. 4—An electron micrograph of a small onion bulb containing a remyelinating axon. × 5,400.

myelination in all 14 cases in which onion bulbs were visible on light or electron microscopy. These findings support the hypothesis expressed by other workers (Thomas and Lascelles, 1967; Dyck, 1969) that onion bulbs develop in response to segmental demyelination and remyelination. However, their formation is not an invariable consequence of the pathological process, since no onion bulb formations were seen on electron microscopy in 2 patients with

TABLE 1

A. Onion Bulbs on Light Microscopy

Patient	*Sex*	*Age*	*Pathological Evidence of Demyelination*			*Impairment of Conduction*
			L.M.	*Teasing*	*E.M.*	
M.R.	F	13	+	No fibres	+	Unrecordable
J.S.	F	15	+	+	+	Gross
S.O.	M	6	+	+	+	Unrecordable
A.F.	F	55	+	N.E.	N.E.	N.E.
D.L.	M	29	+	N.E.	+	Gross
M.P.	F	16	+	N.E.	N.E.	N.E.

TABLE 2

B. Onion Bulbs on Electron Microscopy Only

Patient	*Sex*	*Age*	*Pathological Evidence of Demyelination*			*Impairment of Conduction*
			L.M.	*Teasing*	*E.M.*	
L.P.	F	26	−	+	+	Gross
P.E.	M	9	−	+	+	Gross
M.W.	F	41	−	+	+	Gross
W.C.	M	60	−	+	+	Mild
R.S.	M	18	−	+	±	Gross
M.S.	M	14	+	+	+	Mild
H.M.	F	18	+	+	+	Moderate
A.C.	F	18	+	+	+	Mild

TABLE 3

C. No Onion Bulbs on Light or Electron Microscopy

Patient	*Sex*	*Age*	*Pathological Evidence of Demyelination*			*Impairment of Conduction*
			L.M.	*Teasing*	*E.M.*	
R.A.	M	40	−	No fibres	−	Mild
C.P.	M	47	−	−	−	Mild
E.R.	F	64	−	+	−	Gross
S.M.	M	38	−	+	−	Mild
B.O.	M	43	−	N.E.	−	Mild
C.F.	M	63	−	N.E.	−	Mild
E.R.	F	64	−	N.E.	−	Mild
M.C.	F	18	−	−	−	Mild
A.J.	M	41	−	−	−	Mild
J.P.	M	45	−	−	−	Mild
S.B.	M	50	−	−	−	Mild
W.C.	M	56	−	−	−	Mild
D.T.	M	59	−	−	−	Mild

chronic neuropathies in whom segmental demyelination was found in the teased fibres. The formation of onion bulbs in response to repeated episodes of axonal degeneration and regeneration must be unusual, if it occurs at all, since it was not seen in any of the 11 cases of chronic neuropathy in which axonal degeneration was the primary pathological feature.

Onion bulb formations may be of different types. Gross onion bulbs, which are readily visible on light microscopy, may be associated with a central myelinated axon, or with one in which there has been no attempt at remyelination. In some nerves, there was considerable variation in the thickness of the myelin sheath at the centre of the onion bulb and in these cases there was electron microscopical evidence that cellular structures other than the Schwann cell contributed to the concentric lamellae. In other nerves in which the myelin sheath appeared to be of normal thickness, the surrounding lamellae were composed of only Schwann cell elements (Fig 5).

In all subjects in whom gross slowing of conduction occurred in the peripheral nerves there was also pathological evidence of segmental demyelination (Gilliatt, 1966). However, the converse was not necessarily true and in some cases segmental demyelination was associated with only mild or moderate degrees of slowing of conduction. It seems probable that the degree of slowing of conduction depends upon the extent of demyelination; gross slowing of conduction will be seen only when the vast majority of fibres in the peripheral nerve are affected by the pathological process.

FIG. 5—An electron micrograph of a fully myelinated axon surrounded by concentrically arranged Schwann cell processes. × 5,000.

SUMMARY

Electrophysiological studies and sural nerve biopsies were performed on 27 patients with chronic and subacute polyneuropathies. The presence of onion bulb formations on examination by light and electron microscopes of the sural nerves was associated in all cases with segmental demyelination in teased single fibres, and in the majority of cases with gross slowing of nerve conduction.

ACKNOWLEDGMENTS

The skilled technical assistance of Miss Julia Baverstock with the histological preparations is gratefully acknowledged. The work was supported by grants from the Postgraduate Medical Foundation, University of Sydney, and the Wellcome Foundation.

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AN OBJECTIVE ASSESSMENT OF A GAMMA AMINOBUTYRIC ACID DERIVATIVE IN THE CONTROL OF SPASTICITY

DAVID BURKE*, J. D. GILLIES† and JAMES W. LANCE

INTRODUCTION

Spasticity has been defined as a state of hyperactive stretch reflexes (Pedersen, 1969). The measurement of spasticity therefore involves an assessment of the stretch reflex, and its management will require agents that dampen down the pathological increase in reflex activity. Until now, diazepam has been the most effective drug in the treatment of spasticity, but it is not effective in some patients, and in others tolerance may develop following prolonged use. Drowsiness is a common side-effect, which may be transient with low dosages of diazepam, but may be incapacitating with high doses (30-60 mg/day).

Recently attention has focused on derivatives of Gamma aminobutyric acid, because of the central inhibitory effects of the parent substance. One such derivative is Beta (4-chlorophenyl) gamma-aminobutyric acid, which is known as CIBA 34,647-Ba.

We have recently conducted a preliminary trial of this agent in human spasticity, using an objective measurement of spasticity based on the amount of electromyographic activity (EMG) produced by a given velocity of stretch.

PATIENTS AND METHODS

Design of the Trial

A double-blind trial was performed in six patients, all quadriplegic following trauma to the cervical spinal cord. Two patients had clinically complete cord lesions. All had established spasticity, the injury having occurred within the preceding twelve months in five patients, and four years previously in the sixth.

The patients were examined before commencement of the trial when they were all on diazepam, 15-30 mg daily. Placebo or active drug was then administered in incremental dosage for a period of two weeks, at which time the patients were re-examined, prior to changing to the alternative drug. CIBA 34,647-Ba was started at a dose of 5 mg three times daily, and this was increased by 15 mg daily every three days to a maximum of 60 mg daily. Placebo tablets were administered in an identical manner. In one patient, the dose of 34,647-Ba was increased to 75 mg per day after the conclusion of the trial.

Clinical assessment was performed at regular intervals. Blood pressure and pulse rate were recorded twice daily, urinalysis was performed daily and microurine weekly. Blood count, ESR, blood urea, serum electrolytes and liver function tests were performed when on diazepam, and then each two weeks. An electroencephalogram was also performed at these times.

Electromyographic Testing

The electromyographic testing was performed on three occasions: before the start of the trial while on diazepam, and after each two-week period. The patients were tested when lying prone, and the quadriceps muscle was stretched by passive flexion of the knee. All stretching movements commenced with the knee fully extended.

The EMG of the quadriceps was recorded by surface electrodes, and integrated (time constant 0·2 sec.). The same electrode placement

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was used for each assessment. Knee joint angle was measured by a goniometer, and its output was differentiated (time constant 5 msec.) to produce a voltage proportional to angular velocity. The direct EMG, integrated EMG, joint angle and angular velocity were recorded simultaneously on a four-channel Offner dynograph. Direct EMG and joint angle were also monitored on an oscilloscope.

For each stretching movement, the peak integrated EMG was plotted against the velocity of stretch. The calculated line of best fit was then determined by regression analysis of those points for which EMG was present. The intercept of the line of best fit on the velocity axis was taken as the threshold velocity of the stretch reflex. The EMG:velocity relationship of any patient was then expressed in terms of the threshold velocity and the slope of the line of best fit.

A reduction in degree of spasticity was apparent when the amount of EMG at any given velocity of stretch was reduced. This was seen as a change in the threshold velocity and the slope of the line of best fit. The percentage improvement was determined as the percentage change in EMG produced at the velocity 200°/sec. This velocity was chosen as a point of comparison because it is a reasonable upper limit to the rate of physiological movement.

Results

1. Assessment of Therapy

In all six patients, for any given velocity of stretch less EMG was produced when on 34,647-Ba than when on placebo. The amount of EMG produced at 200°/sec. was decreased to a mean of 37·5% (range 0-67%) of the level obtained when on placebo. The decrease in spasticity, which was apparent both clinically and electromyographically, resulted from an increase in the threshold velocity and a decrease in slope of the EMG:velocity relationship (Fig. 1).

In three patients, the threshold velocity was greater when on 34,647-Ba than when on placebo; in one of these three no stretch reflex could be obtained when the patient was receiving 34,647-Ba. In two patients, spasticity was so marked when on placebo that even the slowest stretching movements resulted in a stretch reflex. When receiving 34,647-Ba,

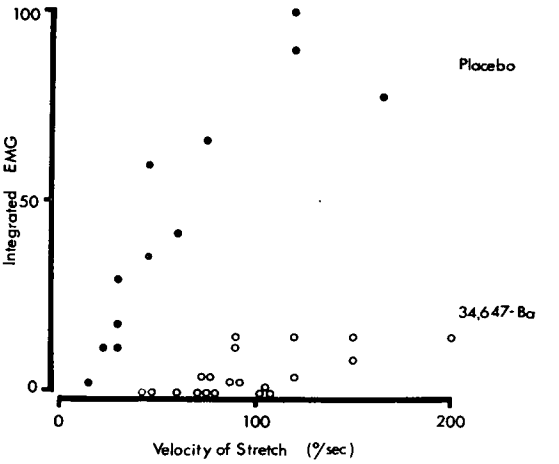

FIG. 1—The effect of 34,647-Ba on the EMG: velocity relationship. At any given velocity of stretch, less EMG is produced when the patient is receiving the active drug. The threshold velocity has increased from 25°/sec. to 75°/sec. and the slope of the EMG:velocity relationship has decreased.

velocities of 23°/sec. and 104°/sec. were required to produce a stretch reflex. In the first of these two patients, the electromyographic improvement was not apparent clinically, and the dose of 34,647-Ba was later increased to 75 mg daily. At this dosage a further increase in threshold velocity occurred, to 145°/sec., and clinical improvement was readily detected (Fig. 2).

In the sixth patient, spasticity was intense when receiving placebo, but only slightly less marked when on the active drug. There was no change in the threshold velocity since slow movements still produced a stretch reflex. However, the slope of the EMG:velocity relationship decreased, so that at all velocities of stretch, less EMG was produced when taking 34,647-Ba (Fig. 3). In this patient, the stretch reflex at 200°/sec. was decreased to only 67% of its former level, and clinically only a slight decrease in the degree of spasticity was noted. This patient had been equally resistant to diazepam.

An uncontrolled comparison of the results obtained when on diazepam with those of 34,647-Ba was also made, but only limited conclusions can be drawn from such a comparison. Spasticity was less marked in four patients when on 34,647-Ba than when on diazepam, and in one patient there appeared

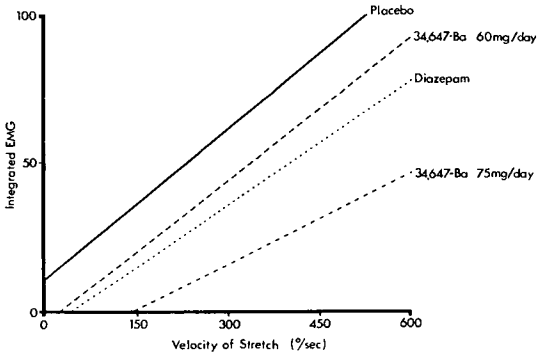

FIG. 2—Dose-related response to 34,647-Ba in an intensely spastic patient. The EMG:velocity relationships are represented by the calculated lines of best fit. When on placebo, even slow stretching movements evoked a reflex response. The threshold velocity increased to 23°/sec. when on 34,647-Ba 60mg daily, and to 145°/sec. when on 75 mg daily. With diazepam, the threshold velocity was 41°/sec.

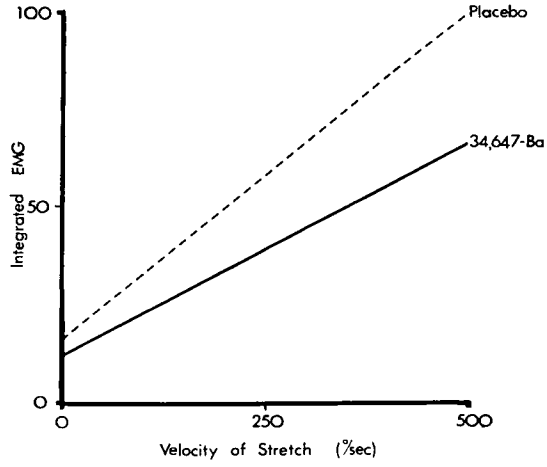

FIG. 3—Resistant spasticity. No change in the threshold velocity was produced by 34,647-Ba. With both placebo and active drug a stretch reflex was produced even by slow stretching movements. However, the intensity of spasticity was slightly less on 34,647-Ba, and the amount of reflex EMG at any given velocity was reduced.

to be no difference between the preparations. In the sixth patient (Fig. 2), when 34,647-Ba was given in a dose of 60 mg daily, diazepam appeared to be slightly superior (threshold velocity 41°/sec. on diazepam, 23°/sec. on 34,647-Ba). When the dose of 34,647-Ba was increased to 75 mg daily, it was clearly the more effective (threshold velocity 145°/sec.).

2. Side-effects

No serious side-effects were noted. Five patients on the active drug and two on placebo suffered transient attacks of nausea, with vertigo and sweating, but these cleared spontaneously. Easy fatiguability and muscle weakness occurred for one day in two patients, and one patient became depressed transiently, all when on 34,647-Ba.

No renal, hepatic or haemopoietic abnormality was found, and no change in blood pressure was recorded.

The EEG's of two patients taking placebo were found to be abnormal. One was overtly epileptic, and a similar but less severe pattern was found when this patient was taking 34,647-Ba. In the second patient, minor abnormalities were found on photic stimulation and on overbreathing, but the EEG taken when on 34,647-Ba was within normal limits.

DISCUSSION

The electromyographic method of assessment described in this paper is based on the linear relationship between the spastic stretch reflex and the velocity of stretch (Burke, Gillies and Lance, 1970). A reduction in spasticity produced by the active drug resulted in a smaller EMG response at any given velocity of stretch. This was associated with an increase in the threshold velocity and a decrease in slope of the EMG:velocity relationship. CIBA 34,647-Ba therefore appears to affect both the threshold and the sensitivity of the stretch reflex.

34,647-Ba was effective in all patients even though the improvement in one patient was slight and resulted in little therapeutic benefit. The results are in accord with those of other clinical trials, which also concluded that this drug is effective and clinically useful (Bergamini, Riccio and Bergamasco, 1966; Birkmayer, Danielczyk and Weiler, 1967; Castaneda, 1967; Morales, 1967; Jerusalem, 1968).

The absence of side-effects in this trial is notable. In other trials, excessive salivation, thirst, sedation, confusional states, euphoria and hypotension have been recorded. The relative freedom from side-effects in this trial may result from the slowly incrementing dosage, which was restricted to 60 mg/day. However, in one patient a higher dose was necessary to

achieve a satisfactory therapeutic response, and this dose was well tolerated.

The efficacy of 34,647-Ba does not appear to depend on the integrity of supraspinal structures, since it was equally effective in patients with complete or incomplete spinal cord lesions. It presumably has a site of action in the spinal cord.

Summary

An electromyographic method of assessing changes in spasticity has been used to evaluate a Gamma aminobutyric acid derivative, CIBA 34,647-Ba, in human spasticity. This drug has been found to be an effective agent, with minimal toxicity.

Acknowledgments

The authors would like to thank Dr. R. F. Jones and Dr. J. E. Marosszeky, who managed all clinical aspects of this trial, and CIBA Co. Pty. Ltd. for supplies of active drug and placebo.

Generous financial support has been received from Mr. and Mrs. Edwin Street, the Rockdale Lions Club, and CIBA Co. Pty. Ltd.

This research programme is supported by grants from the National Health and Medical Research Council of Australia and the Adolph Basser Trust.

Illustrations were prepared by the Department of Medical Illustrations, University of New South Wales.

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AN ELECTROMYOGRAPHIC ANALYSIS OF THE CLASP-KNIFE PHENOMENON

DAVID BURKE*, J. D. GILLIES† and JAMES W. LANCE

INTRODUCTION

The clasp-knife phenomenon has long been recognized as one of the characteristic features of spasticity. The inhibition of the stretch reflex which produces this phenomenon has been attributed to the action of Golgi tendon organs and their Ib afferent fibres (Jansen, 1962; Pedersen, 1969). However, recent work on these receptors has demonstrated a low threshold to active contraction with a relatively high threshold to passive stretch (Jansen and Rudjord, 1964; Houk and Henneman, 1967). Sustained inhibition of the stretch reflex therefore cannot be adequately explained on the basis of activation of tendon organs, since there is little stimulus to the tendon organ in the absence of muscle contraction.

In addition, Denny-Brown (1964) has pointed out that in the lower limbs, the clasp-knife pattern of hypertonicity is present only in extensor muscles such as the quadriceps, even though tone may be equally increased in flexor muscles.

Because of these discrepancies, we have undertaken the present study of the stretch reflexes of the quadriceps and hamstring muscles of spastic man.

SUBJECTS AND METHODS

Twenty-five spastic subjects were studied. The quadriceps stretch reflex was examined in 20 patients, chosen because of prominent clasp-knife hypertonicity, and the hamstrings stretch reflex was examined in 15 patients. Eight patients suffered from multiple sclerosis. Eleven were paraplegic or quadriplegic resulting from spinal cord trauma (8 patients), spinal osteomyelitis (1 patient), spinal metastases (1 patient) and spinal cord angioma (1 patient). Two patients suffered from familial spastic paraplegia, three from cerebral vascular lesions and one from a glial cyst of the premotor cortex.

The patients were tested when lying prone, the quadriceps and hamstrings muscles being stretched by passive movements of the knee joint. The electromyogram (EMG) of the muscle being studied was recorded by surface electrodes 10 cm apart, and was integrated (time constant 0·2 sec.). The direct EMG was monitored on an oscilloscope to detect artefact. The knee joint angle was measured by a goniometer, whose output was differentiated (time constant 5 msec.) to produce a voltage proportional to angular velocity. The joint angle was also monitored on an oscilloscope so that any given joint position could be accurately reproduced. The direct EMG, integrated EMG, joint angle and angular velocity were recorded on a four-channel Offner Dynograph.

Both linear and sinusoidal stretching movements were used. The amplitude of the stretching movement and the starting position were varied, and a range of velocities was used. The height of the integrated EMG was used as a measure of the stretch reflex produced by the stretching movement.

RESULTS

The spastic stretch reflex

In both quadriceps and hamstrings muscles the stretch reflex fatigued on repeated testing. Usually a stable base-line was then obtained, and recordings were taken. Occasionally it was

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difficult to reach such a stable level because spasms frequently interrupted testing, and spasticity was enhanced following spasm. This was most marked in spinal injury patients.

The stretch reflex of the quadriceps was tested in patients in whom clasp-knife hypertonicity was prominent. The resistance to stretch developed early in the stretching movement and then subsided, so that with further flexion of the knee no resistance was encountered. In some patients, this was so pronounced that with the knee at 90° flexion the stretch reflex was completely suppressed and the knee jerk appeared to be pendular.

By contrast, in the hamstrings the resistance to passive stretching appeared maximal as the muscle became fully stretched with the knee joint extended. Consequently the clasp-knife phenomenon was not encountered in the hamstrings.

The effect of velocity

In both quadriceps and hamstrings, there was usually a threshold velocity, below which no stretch reflex was produced. In some patients, however, spasticity was so intense that even slow stretching movements produced EMG.

In both muscles, the stretch reflex then increased as the velocity of stretch increased (Fig. 1). In the quadriceps, this relationship appeared linear throughout the range of velocities used. For most patients, a similar linear relationship was present in the hamstrings (Fig. 2), but in seven patients a disproportionately greater reflex response was produced with velocities of stretch which exceeded the rate of physiological movement (300°/sec.).

The effect of starting length

With the quadriceps, when the stretching movement started from a more stretched position, a similar linear relationship between EMG and velocity was obtained, but the stretch reflex was smaller for any given velocity. There was an increase in threshold velocity and a decrease in the slope of the EMG:velocity relationship (Fig. 3).

With the hamstrings, no significant change was produced by starting the stretching movement from a more stretched position, and the EMG:velocity relationships at each starting position were superimposable (Fig. 4).

When the stretching movement was divided into three sequential steps, it was found in the quadriceps that the stretch reflex was maximal

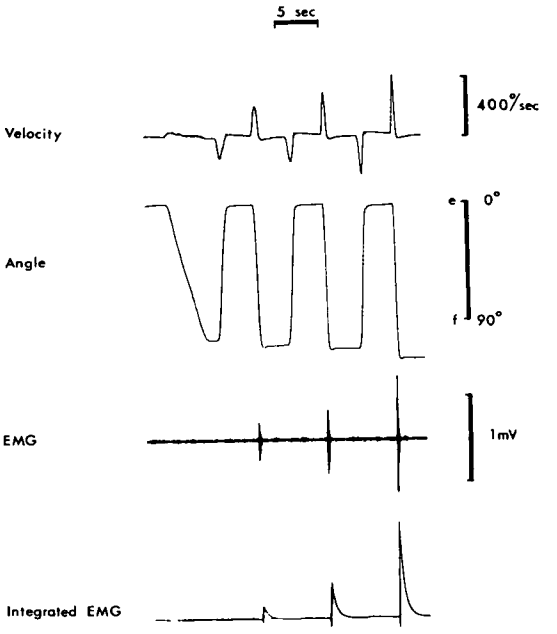

FIG. 1—Velocity dependence. As the velocity of stretch is increased the stretch reflex increases as seen by the increasing EMG response. This trace is from the quadriceps and stretching is a downward deflection of the angle record.

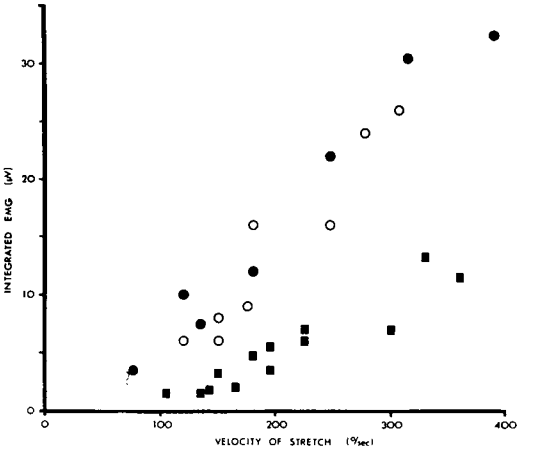

FIG. 2—Linearity of the EMG:velocity relationship. The relationships obtained in the hamstrings muscles of three patients are illustrated, each by a separate symbol. As the velocity of stretch increases the reflex EMG increases so that the relationships obtained appear linear.

in the first step, and that little EMG was produced by subsequent stretching (Fig. 5). In the hamstrings, however, the stretch reflex occurred in the last step which approached the fully extended position of the knee joint (Fig. 6).

These findings were confirmed with sinusoidal stretching. In the quadriceps, the EMG was maximal when the limb was oscillating about the muscle's least stretched position (Fig. 7), while in the hamstrings the EMG activity occurred around its most stretched position (Fig. 8).

To determine whether the changes in the stretch reflexes produced at different positions of the knee joint arose as a result of muscle stretch or as a result of stimulation of joint afferents, an ischaemic block of knee joint afferents was produced by a sphygmomanometer cuff above the knee. The cuff pressure was maintained for 30-45 minutes until joint proprioception was abolished. Although this resulted in a decrease in spasticity, the quadriceps stretch reflex was still inhibited by increasing muscle stretch so that the clasp-knife phenomenon persisted.

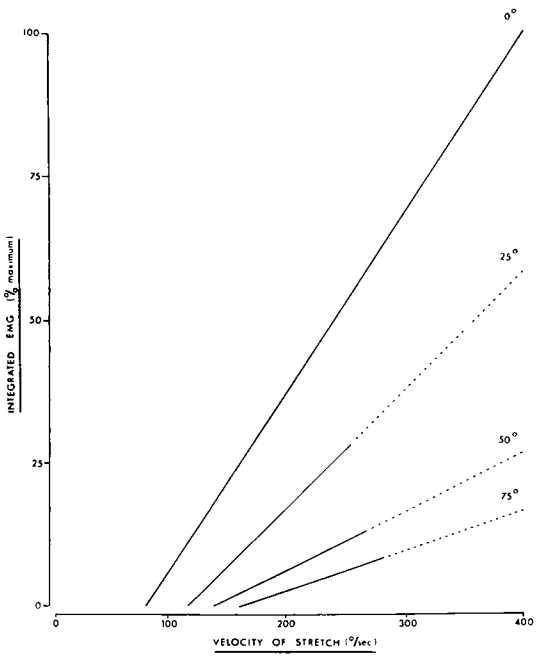

FIG. 3—The effect of change in starting position on the quadriceps stretch reflex. The relationship between reflex EMG and velocity of stretch has been determined at four different starting positions, and each relationship is represented by the calculated line of best fit. As the knee is flexed, the threshold velocity of the stretch reflex increases and the slope of the relationship decreases, so that for any given velocity of stretch less EMG is produced.

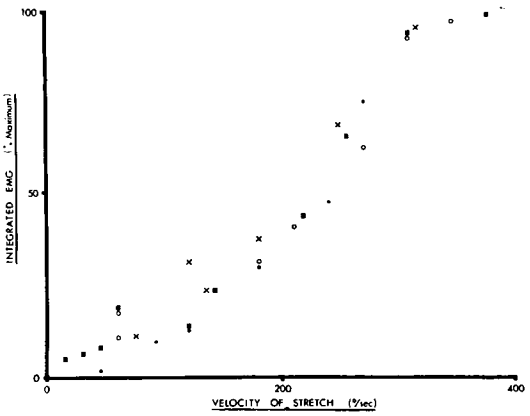

FIG. 4—The effect of change in starting position on the hamstrings stretch reflex. Four different starting positions are represented, each by a different symbol. The relationships are superimposable, and no significant difference in the stretch reflex has been produced by altering the starting position of the stretching movement.

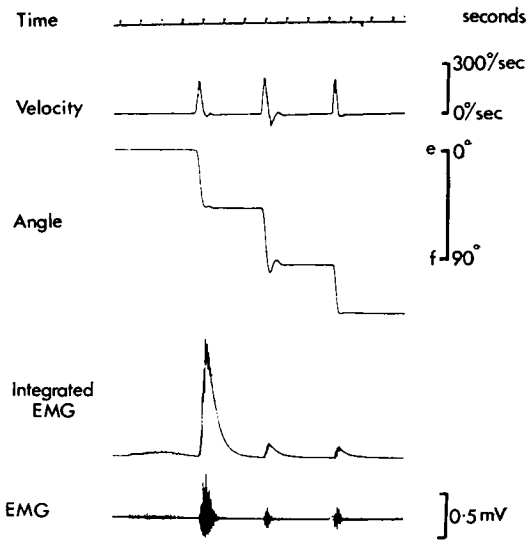

FIG. 5—Stepwise stretching of the quadriceps muscle. The stretching movement has been divided into three sequential steps, of approximately equal amplitude and at the same velocity. The stretch reflex is maximal in the first step and subsequent stretching produces only a small reflex response. Stretching is a downward deflection of the angle trace.

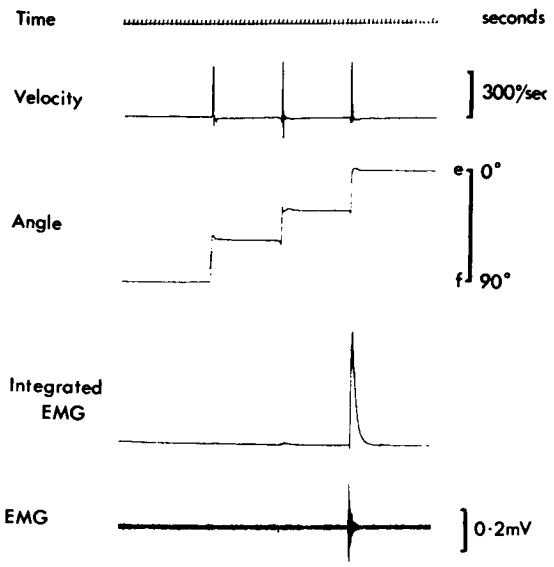

FIG. 6—Stepwise stretching of the hamstrings muscles. In three sequential steps, the stretch reflex occurs during the final step, and little EMG is produced by the earlier stretching movements. Stretching is an upward deflection of the angle trace.

DISCUSSION

In the spastic patient, increased muscle tone is only appreciated clinically when the muscle is stretched sufficiently rapidly. This suggests that the stretch reflex depends on the velocity of stretch, and that a threshold velocity must be exceeded before a stretch reflex is produced. Our results confirm these clinical impressions for both the extensor and flexor muscles of the knee joint, but in addition it has been shown that the relationship between the velocity of stretch and the resulting stretch reflex is linear. In the hamstrings muscles of some patients, a non-linearity began to appear at faster velocities of stretch, but these velocities greatly exceeded the rate of physiological movement. The velocity dependence presumably arises from activation of the primary endings of the muscle spindles, which are sensitive to dynamic stretch (B. H. C. Matthews, 1933; P. B. C. Matthews, 1963).

The effect of increasing muscle stretch on the stretch reflex differs in the two muscles. In the quadriceps, this effect is inhibitory, so that as the muscle is stretched, the stretch reflex diminishes and the examiner appreciates a diminishing resistance (the "clasp-knife phenomenon"). In the hamstrings, however, increased muscle stretch results in an increased stretch reflex, which becomes maximal as the muscle is fully stretched, so that the clasp-knife phenomenon does not occur.

Ischaemic block of knee joint receptors did not abolish the inhibitory effect of muscle stretch on the quadriceps stretch reflex, and presumably these receptors play little part in this reflex pattern. This suggestion is confirmed by Skoglund (1956) who showed that artificial and physiological stimulation of knee joint afferents had little effect on segmental stretch reflexes in the cat. It would therefore appear that the effect of muscle stretch on the quadriceps and hamstrings stretch reflexes arise in the muscles themselves, due to increasing length.

The Golgi tendon organ cannot be responsible since it is sensitive more to muscle contraction than to changes in muscle length, and in the absence of muscle contraction, passive stretching produces only slight increase in tendon organ firing rate (Jansen and Rudjord, 1964; Houk and Henneman, 1967). Moreover, tendon organs from flexor muscles appear to have similar autogenic inhibitory effects as do those of extensor muscles (Bianconi, Granit and Reis, 1964) so that activation of tendon organs cannot explain the differences between the stretch reflexes of the quadriceps and the hamstrings muscles.

Both the primary and secondary spindle endings are sensitive to changes in length, but only the secondary ending produces reflex effects that fit this flexion reflex afferent pattern. Activation of extensor secondary spindle endings in the spinal preparation inhibits extensor reflexes, while similar activation of flexor secondary endings facilitates flexor reflexes (Eccles and Lundberg, 1959a; Laporte and Bessou, 1959; Hunt and Perl, 1960).

It is suggested that the clasp-knife phenomenon arises due to inhibition of the quadriceps stretch reflex by activation of the secondary spindle endings of the quadriceps muscle. Since the secondary endings of the hamstrings muscles facilitate the hamstrings stretch reflex the clasp-knife phenomenon is not found in these muscles. In normal and in the decerebrate state transmission from these flexion reflex afferents is tonically inhibited by a bulbo-spinal pathway (Eccles and Lundberg, 1959b; Holmqvist and

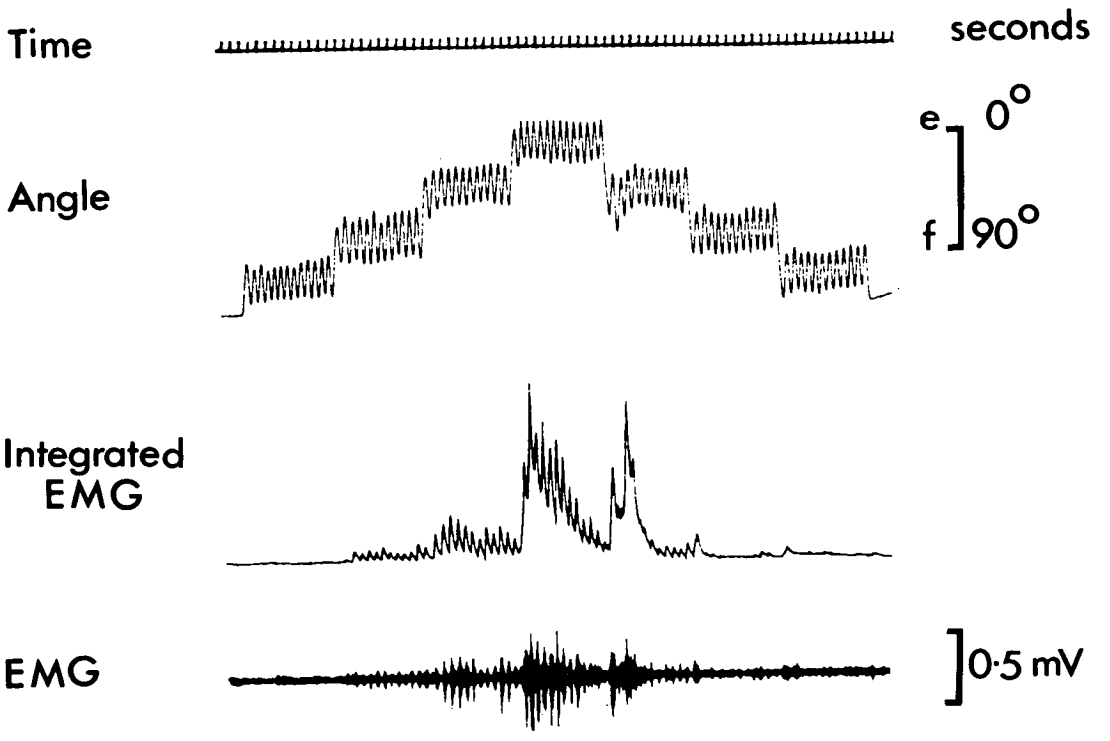

FIG. 7—Sinusoidal stretching of the quadriceps muscle. The quadriceps is being stretched at about 1 c.p.s. through an amplitude of approximately 30°. The stretch reflex becomes maximal as centre of oscillation approaches the least stretched position.

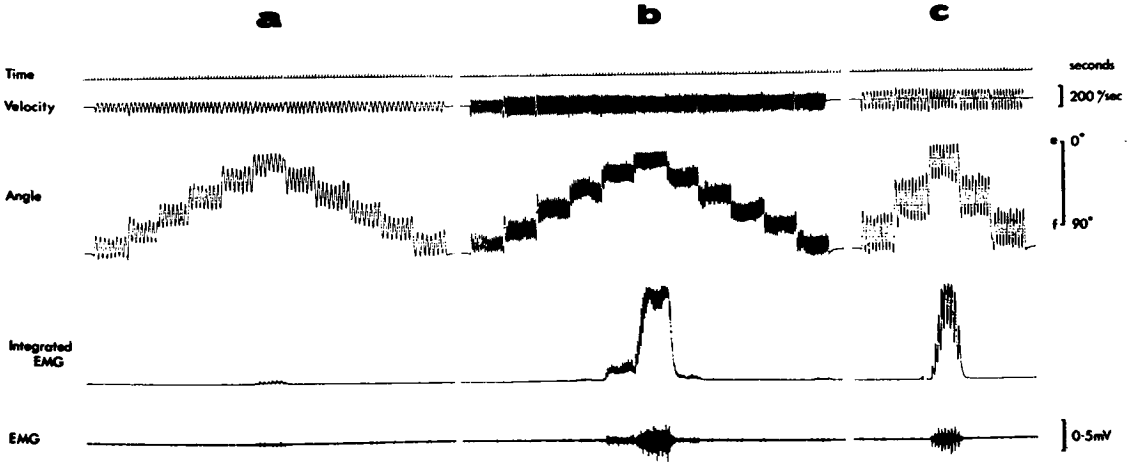

FIG. 8—Sinusoidal stretching of the hamstrings muscles. The stretch reflex becomes maximal as the centre of oscillation approaches the most stretched position of the hamstrings.
a and b show that the reflex response is velocity dependent.
b and c show that a change in amplitude of the stretching movement has little effect on the reflex response provided that the velocity of stretch remains constant.

Lundberg, 1959, 1961) so that in the decerebrate cat inhibition of the quadriceps stretch reflex with increasing muscle length is not apparent, and a clasp-knife phenomenon is not prominent. Spinalization disinhibits the afferent pathway from the secondary spindle ending, so that the characteristic features of spasticity become apparent.

Summary

In the quadriceps and hamstrings muscles of spastic man, the stretch reflex increased as the velocity of stretch increased, presumably due to the dynamic sensitivity of the primary spindle endings.

In the quadriceps, the stretch reflex decreased as the muscle was stretched while in the hamstrings the stretch reflex increased with increasing stretch. As a result, the clasp-knife phenomenon was present in the quadriceps but not in the hamstrings.

This pattern of response is consistent with activation of the secondary spindle endings, rather than activation of Golgi tendon organs.

Acknowledgments

The authors wish to thank the National Health and Medical Research Council of Australia and the Adolph Basser Trust for their continuing support of this project. Financial assistance from Mr. and Mrs. Edwin Street, The Rockdale Lions Club and Ciba Co. Pty. Ltd. is gratefully acknowledged. Mr. P. D. Neilson, Mr. K. Norcross and Mr. N. Skuse have assisted with technical problems. Illustrations were prepared by the Department of Medical Illustration, University of New South Wales.

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Discussion

Associate Professor J. G. McLeod: What is the experimental evidence that the Golgi tendon organs are not playing a significant role in the clasp-knife phenomenon?

Answer: We have performed no experiments to eliminate the tendon organ. Our results, however, are not consistent with the known properties of tendon organs.

Associate Professor J. W. Lance: The action of tendon organs is the same in both muscles so that the different effects of muscle stretch in the muscles cannot be due to tendon organs.

Associate Professor J. G. McLeod: Could the facilitation in the hamstrings caused by stretch be due to a relative depression of fusimotor activity of muscle spindles in the flexor muscles?

Answer: The relative depression of fusimotor activity in flexor muscles is actually a feature of

the decerebrate state not the spastic state. In the acute spinal cat, the dynamic fusimotor activity is released in both flexor and extensor muscles while there is little evidence of static fusimotor activity in either muscle. It is possible that stretch of the hamstrings does not become significant mechanically until later in the stretching movement, but, nevertheless, the facilitatory effect of length is a graded phenomenon even if only over a limited range.

Mr. Mawson: What steps were taken to control other reflexes such as Philipson's reflex? What was the position of the patient and the leg being tested?

Answer: The patients were tested when clinically stable. Most were on continuous catheter drainage of the bladder so that bladder distension did not occur, and we usually did not test patients with bed sores so that flexion reflex afferent stimulation did not vary from this source. The patients all lay prone with their legs extended. The position of the leg not being examined did not vary, so Philipson's reflex was not a factor.

THE SUPRASPINAL CONTROL OF THE TONIC VIBRATION REFLEX

J. D. GILLIES, D. J. BURKE and J. W. LANCE*

INTRODUCTION

In most normal and pathological conditions, control of tone and posture is mediated through the stretch reflex. As the stretch reflex in normal subjects is small and of brief duration, most studies of the stretch reflex have been made on the abnormal exaggerated stretch reflexes of spasticity and Parkinsonian rigidity in man, or the stretch reflex of the decerebrate cat.

When vibration is applied to a muscle, it activates the primary spindle endings of that muscle to fire in a manner similar to normal stretch. The reflex contraction produced in the muscle vibrated by this activation of muscle afferents therefore simulates the normal stretch reflex.

This vibration-induced reflex, present in normal man (Lance, 1965; de Gail, Lance and Neilson, 1966; Hagbarth and Eklund, 1966), and the decerebrate cat (Matthews, 1966), has been termed the "Tonic Vibration Reflex" (TVR) by Hagbarth.

Spinalization markedly reduces or abolishes the TVR in man, indicating supraspinal facilitation of the reflex. As the TVR is present in postcollicular decerebrate cats, but is abolished by spinalization at the C_1 level, brain stem structures must facilitate the reflex.

The experiments outlined below localize the descending motor pathways in the spinal cord which facilitate the reflex, and identify the brain stem structures from which they arise.

MATERIALS AND METHODS

Experiments were carried out in 18 cats. Six animals were anaesthetized by intraperitoneal sodium pentobarbitone; the remaining 12 were initially anaesthetized with ethyl chloride and ether, and both carotid arteries ligated. Of this group 7 were decerebrated by precollicular section, and the remaining 5 were given small intravenous doses of pentobarbitone to maintain anaesthesia after ether was discontinued.

The spinal cord was approached either dorsally, by laminectomy from the fourth to the eighth thoracic vertebra, or ventrally through the body of the fourth cervical vertebra. The brain stem was displayed by posterior fossa craniotomy and cerebellectomy. The hind limb was denervated except for the triceps surae. The tendo Achillis was detached from the calcaneum and tied to a hook extending from an isometric strain gauge. The strain gauge was bolted to a Ling Altec type 201 shaker which could be driven at frequencies up to 500 c.p.s. by a DC power amplifier. Both hind limbs of the cat were prepared so that the ipsilateral and contralateral effects of unilateral lesions of the cord and brain stem could be recorded. Tension and EMG of both triceps surae were simultaneously recorded on a four-channel pen recorder, and the tension and EMG of the muscle being vibrated was also observed on an oscilloscope.

The muscle was stretched by a tension of 150-220 gm and this tension was relaxed at intervals during the experiment. Vibration at 300 c.p.s. and an amplitude of 150μ was applied to the tendon for periods of ten seconds and the reflex tension and EMG recorded. Almost all the primary spindle endings in the muscle will fire at the frequency of the vibrator, independent of gamma efferent activity when the above tension, amplitude and frequency of vibration are employed (Brown, Engberg and Matthews, 1967).

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Lesions were made in the spinal cord with a dural hook, and the cerebellum and brain stem nuclei were ablated by suction. All lesions were confirmed histologically by serial sections.

RESULTS

Section of a dorsal quadrant of the spinal cord had no effect on the tonic vibration reflex (TVR) of the triceps surae. Provided that the cat remained in good condition, extension of the lesion to involve the entire dorsal half of the cord also produced no change in the reflex. However, when a small lesion was made in the cervical cord, involving only the ventral funiculus on one side, the TVR was reduced to 40% of its previous level on the side of the lesion, with no change in the TVR of the contralateral side (Fig. 1). When the lesion was extended to involve both ventral funiculi, the TVR was reduced in both hind limbs. One constant observation was that, after the lesion in the ventral quadrant, the tonic plateau level of tension of the TVR was depressed significantly more than the initial phasic spike. When the EMG and tension at the commencement of vibration were recorded on 35 mm film at high speed, this initial spike of tension was seen to correspond to the first burst of EMG activity induced by the vibrator movement.

No change in the TVR was noted when the cerebellum was removed. However, when the lateral vestibular nucleus (LVN), at the base of the cerebellar peduncle, was ablated, the TVR was reduced to 20-40% of its control level (Fig. 2). Again the tonic portion of the TVR was more affected than the initial phasic spike. Ablation of both LVN resulted in bilateral reduction but not complete suppression of the TVR. As the terminals of the eighth nerve are partly destroyed when the LVN is ablated, this nerve was cut prior to the ablation in 6 preparations. Section of the nerve had little effect on the TVR.

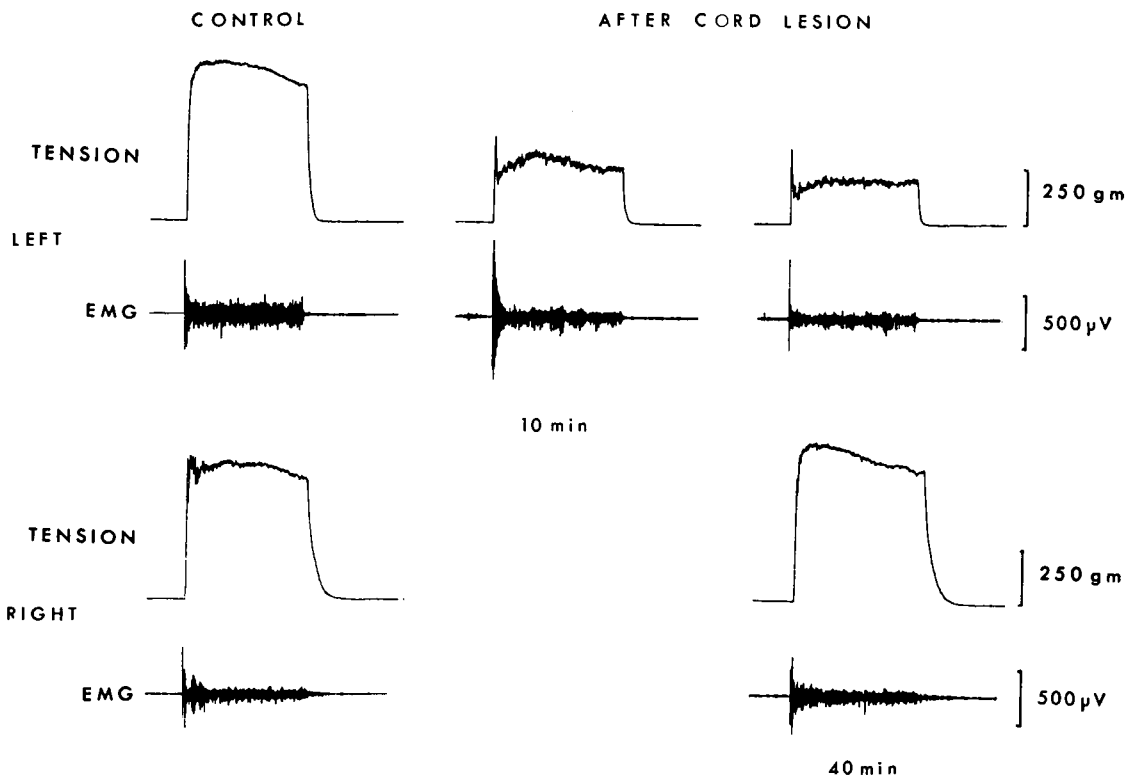

FIG. 1—The TVR of the left side is depressed by a lesion involving the left ventral quadrant of the cord. On the contralateral side there is no change in the TVR. The increase in depression over 40 min. is due to the cessation of injury induced activity at the site of the lesion. A large phasic spike remains after the lesion.

To exclude the possibility that destruction of the LVN had reduced the TVR by damaging the reticular formation medial to the lesion, a stimulating electrode was placed in the brain stem just caudal to the lesion, in the path of the efferent fibres from the nucleus. Stimulation in this region did not produce any EMG activity or tension in the hind limb, but when vibration was applied, a normal TVR was recorded (Fig. 3).

Discussion

Section of the ventral quadrant of the spinal cord markedly reduced the ipsilateral TVR. The descending motor pathways from the brain

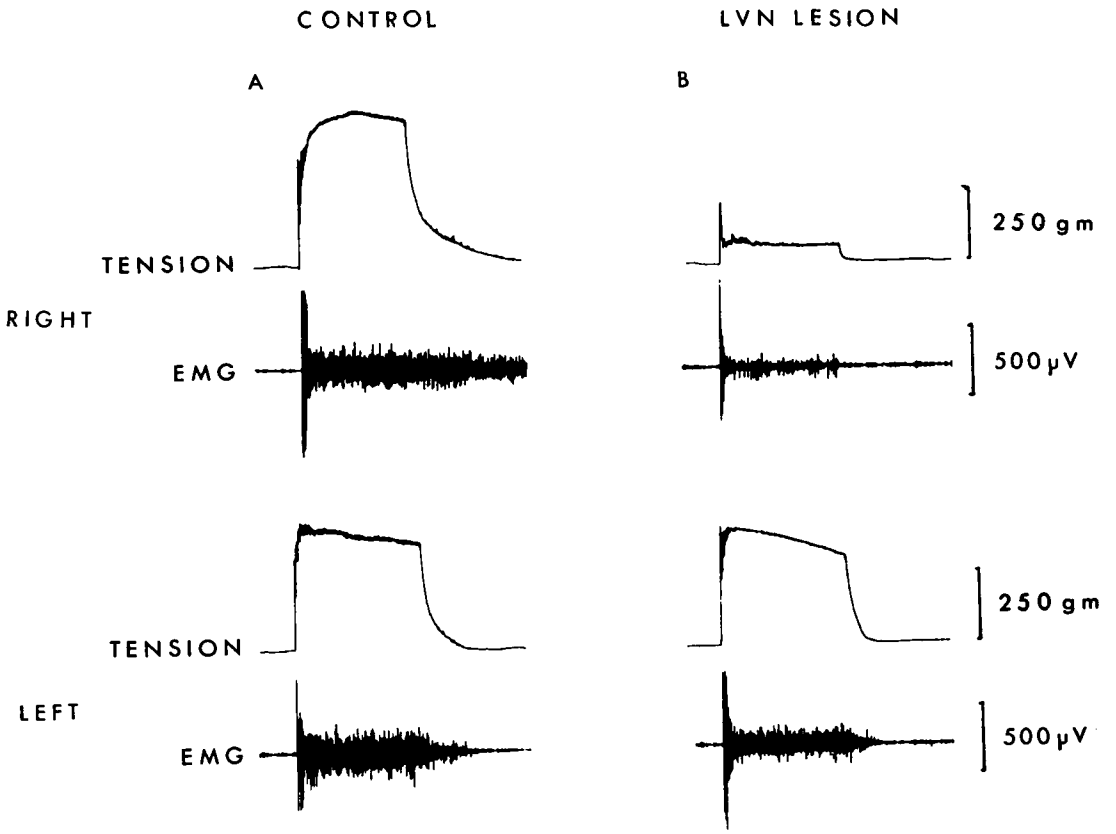

Fig. 2—The ablation of the right lateral vestibular nucleus markedly reduces the TVR on the right side, with no effect on the contralateral side. The lesion also leaves a large phasic spike.

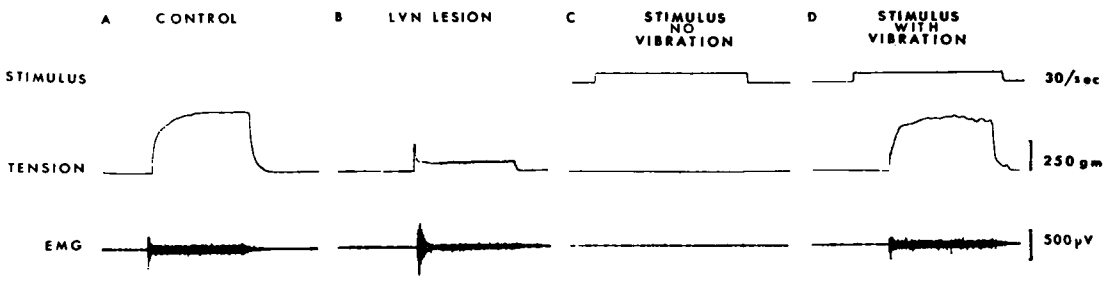

Fig. 3—The control TVR (A) has been suppressed by ablation of the lateral vestibular nucleus (B). At C the efferent fibres from the LVN are stimulated with no effect. When vibration is applied during stimulation, the TVR is returned to its control level (D).

stem which facilitate hind limb extensor motoneurones are the pontine reticulospinal tract, and the lateral vestibulospinal tract, arising from the nucleus pontalis caudalis, and the lateral vestibular nucleus respectively (Nyberg-Hansen, 1966). As both these tracts run in the ventral funiculus, and both are ipsilateral, severance of one or both could account for the reduction in the TVR.

The lateral vestibular nucleus, through its projection in the ventral quadrant of the cord, appears to tonically facilitate the TVR. This facilitation appears to arise from tonic activity in the nucleus itself, rather than afferent activity from the vestibular endorgans, as the section of the eighth nerve had little effect on the TVR. The failure of bilateral LVN ablation to completely abolish the TVR, as does spinalization, suggests that some other brain stem structures, possibly the pontine reticular formation, also facilitates the TVR. As the TVR is completely abolished by spinalization, but not entirely suppressed by removal of both lateral vestibular nuclei, other brain stem structures, for example the pontine reticular formation, presumably have some facilitatory effect on the TVR.

The initial rise in tension produced at the commencement of vibration is due to the initial burst of EMG. By examining the delay from the commencement of vibration to this spike, Matthews (1966) concluded that it was a monosynaptic reflex produced by the first burst of vibration induced afferent activity. This portion of the reflex was far less affected by the lesions than the tonic plateau level of tension. The depression of tonic reflexes, with the maintenance of brisk phasic reflexes, is a common clinical observation. This differential effect on tonic and phasic reflexes has been observed after anteriolateral cordotomy (Sweet and White, 1957).

Summary

The brain stem structures which facilitate the tonic vibration reflex (TVR) have been localized by the use of small discrete lesions in the spinal cord and brain stem. The maintenance of a normal TVR depends on the integrity of the ventral quadrant of the cord, and the lateral vestibular nucleus. Reasons are given for suggesting that the normal TVR is maintained by tonic activity of the lateral vestibular nucleus which is independent of vestibular afferent activity and projects into the ventral quadrant of the cord. Lesions of both the ventral quadrant and the lateral vestibular nucleus depress the tonic component of the reflex more than the phasic.

Acknowledgments

This work was supported by a grant from the National Health and Medical Research Council.

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DIFFERENT CEREBRAL SCAN CHARACTERISTICS OF DIFFERENT CEREBRAL PATHOLOGIES: DOUBLE ISOTOPE SCANNING

J. BARRIE MORLEY and R. G. SEPHTON

INTRODUCTION

At the last meeting of this Association, preliminary experience was presented of attempts to categorize the differing scanning characteristics of different cerebral pathologies. In that report, the approach was based on a comparison of scans with Tc-pertechnetate, performed early (5-10 minutes), and late (1 hour), after injection of the isotope. Also were described two patients in whom Technetium (99m Tc) and Indium (113m In) were employed; reasons were given by which the cerebral lesion's 99m Tc scan could be equated with the combined characteristics of vascularity and tissue permeability of the lesion, whereas the features of the 113m In scan could be taken to represent vascularity properties alone. By assuming that, for different types of cerebral lesions, there would be differing relative properties of tissue vascularity and tissue permeability, then the comparison of these two scans might afford means of leading to a diagnosis of the type of pathology, as well as its presence.

Since then, the isotope 113m In has become readily available in this country, and 51 patients now have been examined by this double isotope technique. However, of these there remain several in whom the final pathological diagnosis is unknown or uncertain, and there are others in whom the anatomical site of the lesion was not such as to permit confident comparison of the two scans. Accordingly, this report is confined to the experience of 26 patients, in whom all of the features, clinical, pathological, and radio-isotopic, are unequivocal.

MATERIALS AND METHODS

Patients were selected for double isotope scanning in one of two ways: either on clinical grounds, where there seemed good reason to believe that the scan would be abnormal, or already the patients had had performed a routine 99m Tc scan which was clearly abnormal. As mentioned above, only those patients were included in whom the scan abnormality was completely definite, and situated in an area devoid of interpretative confusion.

After preliminary oral potassium perchorate (400 mg), 15-18 mCi 99m Tc-pertechnetate was injected intravenously. Immediately thereafter, a similar intravenous injection was administered to the patient, 7-12 mCi 113m In in "blood pool" preparation (Sephton, Morley, Steven, Andrews, and Cornell, 1969). The patient immediately was scanned ("early" 99m Tc), in the most appropriate view for the presumed lesion, this scan taking some 15 minutes. Immediately, without moving the patient, the scanning equipment was reset for the 113m In scan, which then proceeded without delay. Before returning to the "late" 99m Tc scan (about 1 hour, post injection), usually the other lateral view, plus an anterior or posterior view for 99m Tc were performed to complete the routine scanning examination. The "late" 99m Tc scan concluded the procedure, in the same view as for the "early" 99m Tc and 113m In scans.

The equipment comprised a standard Picker Magna-Scanner and Collimator (Cat. No. 2111), using a 5-inch crystal.

The appearances of each of the scans 113m In, "early" 99m Tc, and "late" 99m Tc then were compared. Unfortunately, to date it has not been possible to finalize the "subtraction" system as outlined in the previous report, and to date these comparisons have had to be performed by numerical counting of the respective ratios of uptake in each of the three scans for that cerebral lesion. For each scan this ratio

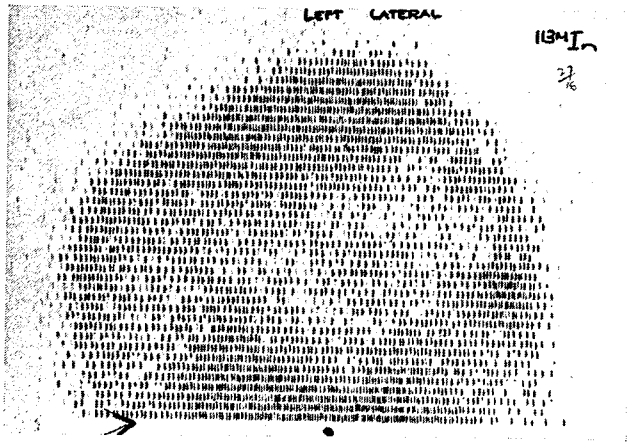

FIG. 1—Left lateral scan of parietal angioma: 113m In (Indium) scan.

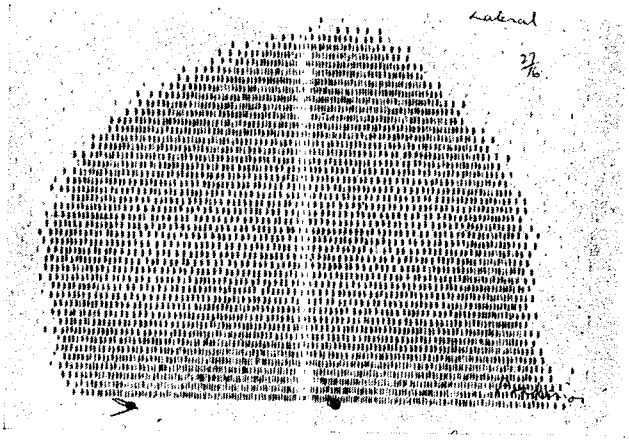

FIG. 2—Left lateral scan of parietal angioma: "Early" 99m Tc (Technetium) scan.

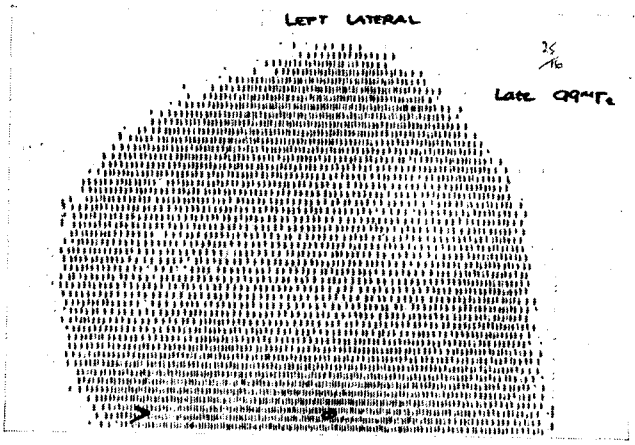

FIG. 3—Left lateral scan of parietal angioma: "Late" 99m Tc (Technetium) scan.

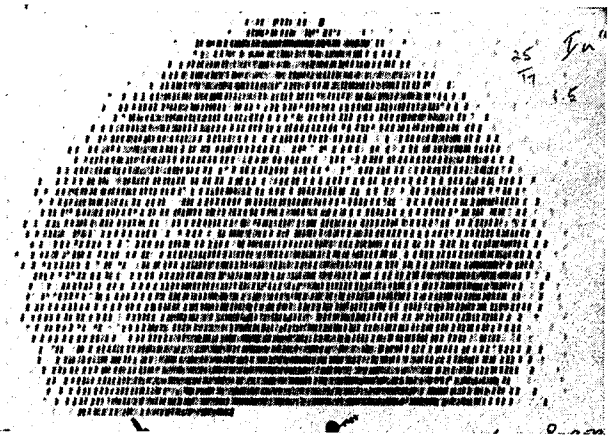

FIG. 4—Left temporal glioma, left lateral view: 113m In (Indium) scan.

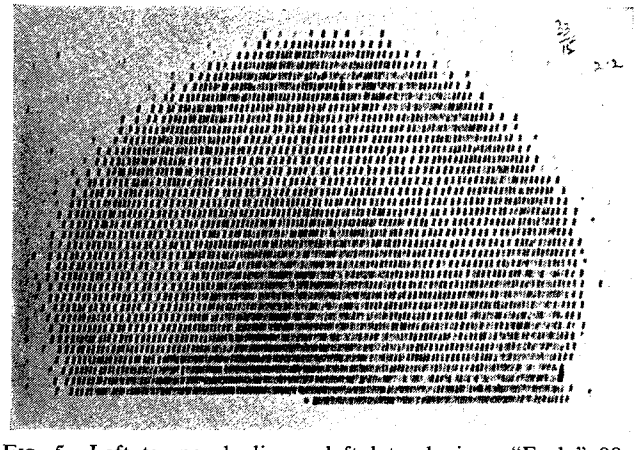

FIG. 5—Left temporal glioma, left lateral view: "Early" 99m Tc (Technetium) scan.

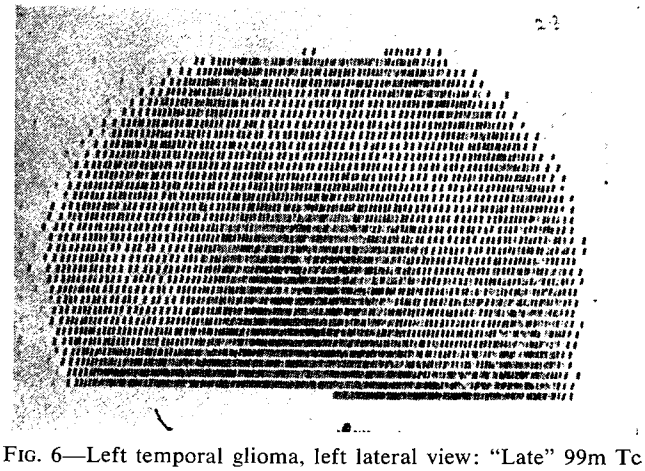

FIG. 6—Left temporal glioma, left lateral view: "Late" 99m Tc (Technetium) scan.

was calculated by counting from the "print out" of the scan, over an area of 1 cm^2, of the uptake over the abnormal area, and of the uptake of an unequivocally normal area, again 1 cm^2. In each of the three scans, as closely as was possible, the same respective normal and abnormal areas were counted.

RESULTS

It seemed logical first to compare the proportions of "vascularity" and "permeability" of each scan, i.e. to compare the Indium with the "late" Technetium scan. In those scans in which the Indium and Technetium ratios were the same (degree of precision calculated at $\pm 0 \cdot 1$), the assumption was that the abnormal uptake of the scan was due to vascularity factors, and that there was virtually no contribution from increased tissue permeability. Scans showing this appearance were classified as "impermeable (vascular)". These scans were further subdivided according to whether the vascularity was "marked" (uptake ratio greater than 1·4), or "mild" (uptake ratio less than or equal to 1·4). The results are tabulated in Table 1, from which it can be seen that the three angiomas and two meningiomas in this study came into this "impermeable" or "vascular" category; moreover, but for one angioma, all of these lesions were of "marked vascularity" character.

Where the Indium uptake (or ratio) was less than that seen on the "late Technetium" scan (or ratio), the lesion was classified "permeable". Additional subdivision was attempted by distinguishing those lesions in which the "permeability" was "slow", as manifest by the "early Technetium" scan showing a lesser uptake than the "late Technetium" scan; a lesion was designated "rapidly permeable" if the "early" and "late" Technetium scan showed ratios of equivalent intensity. Reference to Table 1 indicates that the 9 gliomas and glioblastomas all had characteristics of "rapid permeability" and it was of interest that this was irrespective of the histological degree of malignancy in each case. The majority of patients with cerebral infarctions, and with secondary cerebral tumours, had brain scans of "slow permeability" in character.

DISCUSSION

By drawing this simple classification, it can be seen that it is possible to make a systematic distinction between various cerebral pathologies that may be demonstrable on the brain scan. As in the previous report to this Association, these results are viewed with cautious optimism. Although the number of patients admissable for this presentation is, as yet, too small to warrant firm conclusions, it is believed that a significant trend has been demonstrated.

Brief comments might be made regarding both the cerebral infarctions and the secondary cerebral tumours. It may be anticipated that both of these types of lesions each could be represented in more than one of the semi-arbitrary categories as adopted in this study. Thus, it is conceivable that in the earlier stages of a cerebral insult, there may be significant representation of both "permeability" and "vascularity" factors, and later as the infarction undergoes repair, and resolution, the "per-

TABLE 1

Tabulation of Results

IMPERMEABLE (or vascular) (i.e. 113m In ≈ late 99m Tc)		*PERMEABLE* (i.e. 113m In < late 99m Tc)	
1. *Mild vascularity:* (i.e. 113m In ratio ⩽ 1·4)		1. *Slow permeability:* (i.e. early 99m Tc < late 99m Tc)	
Secondary (lung)	1	INFARCTIONS	3
Infarct	1	SECONDARIES	3
Intracerebral clot	1		
ANGIOMA	1	2. *Rapid permeability:* (i.e. early 99m Tc = late 99m Tc)	
2. *Marked vascularity:* (i.e.113m In ratio > 1·4)		GLIOMAS	6
		GLIOBLASTOMAS	3
ANGIOMAS	2	Subacute extradural haematomas	2
MENINGIOMAS	2	Infarct	1

meability" facet could become a good deal less evident. In other words, the categorization of the scan abnormality may depend on the stage of the illness in which the patient is scanned. With cerebral secondary tumours, it is possible that lesions of differing primary origin may have differing contributions of "vascularity" and "permeability".

Finally, it is also envisaged that an additional diagnostic parameter might be available, by studying the ultimate or maximum permeability eventually attained within the lesion, as well as the rate at which the "permeability" contribution develops. This additional characteristic could be examined quite simply, by repeating the salient scan views several hours after injection.

Figures 1-6 consist of illustrative scans.

Summary

Experience is presented with double isotope cerebral scanning, of various proven cerebral lesions, in 26 patients. 113m In and 99m Tc are used in preparations to represent "vascularity" and "vascularity plus permeability" characteristics respectively of the lesion. By adopting a simple theoretical classification, it appears that a fairly systematic method of distinction between the various cerebral pathologies is possible.

Acknowledgments

Mrs. D. Morley, and the Staff of the Radio Isotopes Department, are thanked for their technical assistance. Colleagues of the Honorary Medical Staff at Prince Henry's Hospital kindly referred patients for examination.

Discussion

Dr. B. Turner: Does Dr. Morley have any information as to whether the "permeability" represents extra-cellular or intra-cellular distribution, or both?

Dr. Morley: I cannot provide definitive information on this point. We believe that we have a working model by which to examine this, by employing colloidal gold in radio isotopic form. We have preliminary results which suggest that glial tissue semi-specifically "takes up" this isotopic preparation, and any resulting brain scan would provide an "intra-cellular" representation.

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