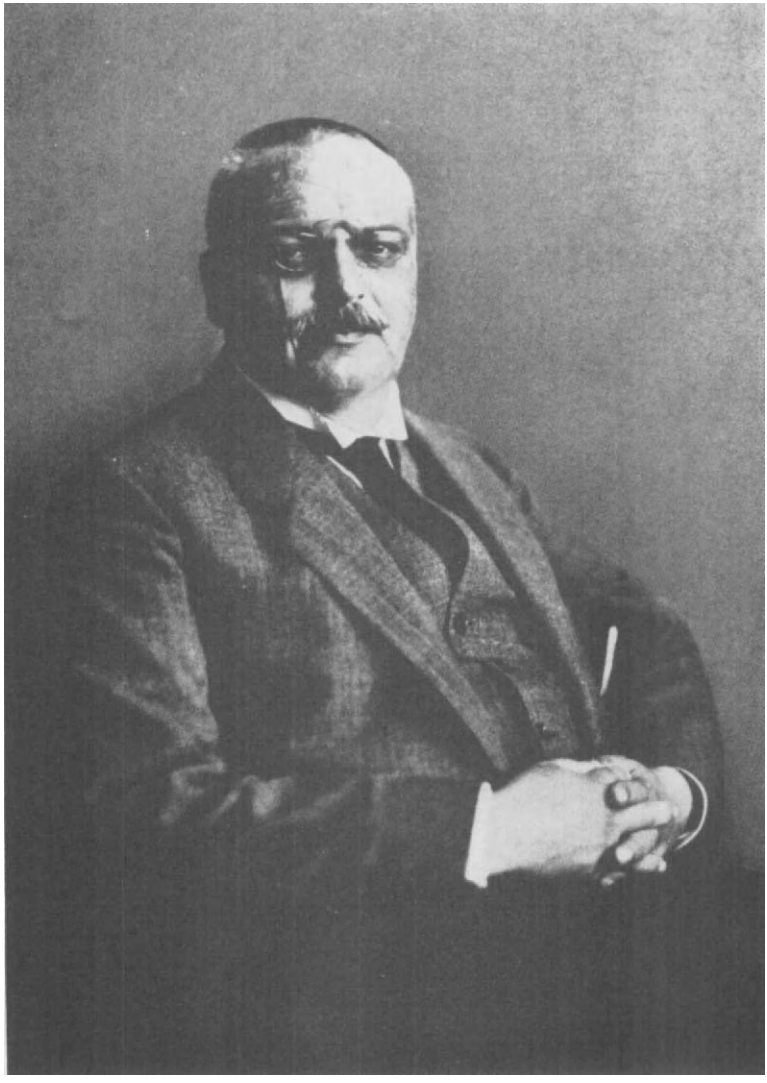
ALZHEIMER'S DISEASE



Alois Alzheimer, 1864–1915

ALZHEIMER'S DISEASE AND RELATED CONDITIONS

A Ciba Foundation Symposium

Edited by
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and
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J. & A. CHURCHILL
104 GLOUCESTER PLACE, LONDON
1970

First published 1970

With 95 illustrations

Standard Book Number 7000 1484 5

Printed in Great Britain

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Membership

Symposium on Alzheimer's Disease and Related Conditions held 11th–13th November, 1969

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The Ciba Foundation

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The Foundation's house at 41 Portland Place, London, has become well known to workers in many fields of science. Every year the Foundation organizes six to ten three-day symposia and three to four shorter study groups, all of which are published in book form. Many other scientific meetings are held, organized either by the Foundation or by other groups in need of a meeting place. Accommodation is also provided for scientists visiting London, whether or not they are attending a meeting in the house.

The Foundation's many activities are controlled by a small group of distinguished trustees. Within the general framework of biological science, interpreted in its broadest sense, these activities are well summed up by the motto of the Ciba Foundation: *Consocient Gentes*—let the peoples come together.

Preface

THIS symposium, held at the suggestion of Professor W. H. McMenemey, brought together neurologists, pathologists, psychiatrists, biophysicists and others. In spite of illness, Professor McMenemey gave much time and thought both to the organization of the meeting and later to the preparation of the material for publication. The Foundation is deeply grateful to him for his initiative, continuing interest, advice and help.

We are also much indebted to Professor M. Roth for taking the chair at a meeting where such different disciplines were represented. His sympathetic direction of the proceedings enabled clinicians and laboratory scientists to communicate effectively and to appreciate each other's work.

Finally, the editors wish to thank all the participants, who by their work before, during and after the meeting have made this book a record which we hope will be of wide interest.

* * *

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ALZHEIMER'S DISEASE AND RELATED CONDITIONS
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CHAIRMAN'S OPENING REMARKS

PROFESSOR M. ROTH

I should like to welcome you all to this meeting and to express both my pleasure and my misgivings in having been asked to act as your Chairman. Research in this as in every other field of neuropsychiatry has become a highly professional, specialized activity and the task of a Chairman is a difficult one. In this context I recall with a certain sympathy the comment of Dr Johnson when he was listening with evident apathy to the performance of a certain violin player. His host tried to encourage him and said: "Dr Johnson, you know this piece is very difficult." "Difficult do you call it, Sir? I wish it were impossible." However, the interest of the subject and the work of the international group of scientists invited by the Ciba Foundation more than compensates for the challenge.

I should like to make certain general points about the groups of disorders we shall be discussing, in an attempt to construct a conceptual framework for our discussions here. There is evidence to suggest that Alzheimer's disease is partly determined by heredity. However, the genetic factor in question does not appear to be of the classical, major, all-or-none Mendelian kind. We are in that very difficult ill-charted territory of genes with additive effects. The second point follows from the first and is a paradoxical one. If we deal with additive genes, we should normally expect the disease in question to have the features of a quantitative variant of the norm. Alzheimer's disease does not conform to this expectation, the clinical findings being in far better accord with an all-or-none phenomenon. Yet the pathological changes have been demonstrated to occur in a less pronounced form in normal elderly subjects, as Gellerstedt (1933) and others have shown. The third point again follows naturally and relates to the specificity of the pathological changes. Are they the common end-result of a number of distinct forms of neuronal degeneration and destruction, or is it possible that the modern ultrastructural and histochemical techniques that have taught us so much in recent years would reveal a

diversity beneath the apparent uniformity of the light microscope appearances in Alzheimer's disease, normal senescence, senile dementia, amyotrophic lateral sclerosis and other disorders? It would, I believe, be generally agreed that these changes are in some measure to be found in a variety of disorders.

Fourthly, the phenomenon appears to be linked in some as yet undisclosed manner, as are so many of the problems of modern medicine—arterial hypertension, cancer, cerebrovascular and cardiovascular disease—with the degenerative processes of middle and late life. What is this relationship? Are we dealing with a caricature of the ageing process, or are the clinical and morphological similarities to Alzheimer's disease deceptive and misleading? One finding which enhances the general biological interest of the subject is the markedly increased mortality in Alzheimer's disease and related disorders.

Fifthly, this is a disease in which the primary, most conspicuous and specific changes are in the brain and the brain has a fixed population of post-mitotic cells. Such organs are particularly prone to exhibit adverse changes in the course of senescence. Sixthly, memory disturbance is invariable and generally among the earliest manifestations of the condition. In this memory disturbance we are looking at failure of one of the basic processes of the cerebrum—its capacity for forming a short-term or a lasting record of experience. How fundamental is this change and what is its pathological correlate? Is there a generalized change at synapses or some specific localized lesion in the hippocampus or limbic system which has aroused such wide-ranging neurobiological interest in recent years?

We embark on this symposium as a collection of experts with different special interests. At the end of Ciba Foundation symposia, as far as my experience goes, the participants form a more closely aggregated group and have often achieved a useful consensus about a number of problems. I hope that we shall succeed in sustaining the Foundation's splendid record. Lewis Carroll may have dimly foreseen a predicament similar to our own when he wrote, in *Through the Looking Glass*, "All the time the guard was looking at her, first through a telescope, then through a microscope, and then through an opera glass. At last he said, 'You are travelling the wrong way!'." We have been set the task of evaluating what has been achieved in Alzheimer's disease with the aid of different methods of investigation applied at different organizational levels. We may be fortunate enough to achieve fresh insights. But a wide range of long-established and novel approaches

are represented here and some of us may have to arrive at the more modest, though not less useful, conclusion that we are travelling the wrong way.

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ALZHEIMER'S DISEASE AND RELATED CONDITIONS
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ALOIS ALZHEIMER AND HIS DISEASE

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THE trail we are to follow during these three valuable days was laid in Tübingen in the first week of November 1906, at a meeting of the South West German Society of Alienists. Alois Alzheimer recounted his clinical and neuropathological findings in a 51-year-old woman whose symptoms began with loss of memory and disorientation; later came depression and hallucinations, and in under five years profound dementia and death. The brain was found to be atrophied and the cerebral cortex contained miliary lesions (*Herdchen*), presumably of the type which Blocq and Marinesco described in 1892 and which were at that time being studied by Fischer. But Alzheimer also noted, thanks to the use of Bielschowsky's method of silver impregnation, a curious clumping and distortion of the cortical neurofibrils which we now associate with his name.

His presentation was noted by title only in the *Neurologische Zentralblatt* in 1906 and the report, without illustrations, is to be found in the *Allgemeine Zeitschrift für Psychiatrie* in the following year. There was no discussion after his presentation. He seems to have been impressed not only with the severity of the pathological process and especially the changes in the neurons but also with the early age of onset of a clinical condition which in an older person he would have regarded as senile dementia.

In 1908 Bonfiglio described a neurosyphilitic male with the disease but Perusini's four cases published in 1910 were more straightforward. He believed the condition to be independent of senility and therefore a disease *sui generis*. This was also the view of Kraepelin who in the same year proposed that it be named after his observant neuropathologist, Alzheimer. Italian writers have understandably called it the Alzheimer-Perusini disease.

Alzheimer was 42 years old when he read his historic paper and he could boast of having received part of his training in Würzburg under von Kölliker, the histologist and one-time colleague of Virchow. His fruitful association with Franz Nissl, a brilliant technician and his senior by four years, began in 1889 in Frankfurt-am-Main and lasted for six years. Later

their times in Kraepelin's department in Heidelberg overlapped for a year until Alzheimer accompanied Kraepelin to Munich in 1903. As a man of private means, Alzheimer could afford to combine his histological studies with clinical work, an association of pursuits which although unpopular in some quarters today continues to produce important results and deserves every encouragement.

It is fitting that in our tribute to the memory of Alzheimer we should feel it necessary to review both the clinical and pathological aspects of the disease, and I would ask those who decry the use of eponymic terms in medicine what alternative name workers in the second decade of this century could have used? We must ask ourselves whether we are ready to change this designation to another which succinctly conveys our present-day notions on its aetiology and pathogenesis.

An eponym in medicine raises the question of how far we are justified in departing from the criteria originally laid down by the person or persons who first described the malady. Many believe that when a disease has a firm basis in pathology, pathological rather than clinical findings constitute the more important criterion for acceptance of diagnosis. In Alzheimer's disease, therefore, the association of more or less generalized cortical atrophy with the presence of many argyrophilic plaques and neurons afflicted with changes in the neurofibrils ("tangles") constitutes the essence of the pathology. Granulovacuolar degeneration of the pyramidal cells of the hippocampus is clearly acceptable but it was only described in 1911 by Simchowicz and the same can be said of two further landmarks in our knowledge of the disease, namely, the recognition of amyloid by Divry (1934), of which we shall hear more later, and the interesting electron microscopic features of this disease. Pathological findings unknown to Alzheimer and Perusini can surely be accepted provided they are constant and the case histories are typical.

The symptomatology in Alzheimer's disease has also been widened since the year 1910 and these additions are presumably acceptable provided the pathological findings are typical. The disease has been found in persons, particularly those with heredofamilial histories, who have not reached an age when they could be regarded as in any way presenile. The age range therefore has been widened.

But the so-called "senile plaques", one of the two hallmarks of Alzheimer's disease, are to be found in a variety of other conditions, as well as in the elderly, and the same is true of that other hallmark, the curious neurofibrillary change: only in well-established cases of Alzheimer's disease, however, does one find them so constantly together and in such

numbers. Some may say "what about in senile dementia?" and the answer to this could be "if this brain came from a person aged 86, then pathologically speaking she was a senile case of Alzheimer's disease", which is of course the reverse of what Alzheimer apparently thought in 1906 in his presenile case of presumed senile dementia! Fischer (1907) would have called these late examples of Alzheimer's pathological syndrome "presbyophrenic dementia", and there is perhaps a case for resuscitating this term as a pathological diagnosis if plaques and (especially) altered neurons are more scantily and locally distributed in the brains of many elderly dements—and they are practically all women—than in a classical instance of Alzheimer's disease. There are cases too of elderly dements whose brains lack the classical pathological features of Alzheimer's disease although there is a conspicuous increase in the lipochrome content of the surviving cortical neurons.

Dr Wolstenholme has entitled this symposium "Alzheimer's disease and related conditions". He knew well, I think, that the confines of an eponymic disease are liable to be indefinite. I am sure it would help if we could pronounce on the legitimate boundaries of Alzheimer's disease. There have been those who argue that there are differences in symptomatology between it and "senile dementia" which cannot be accounted for by diminished ability on the part of an old person to react to a cerebral disorder with its train of disturbing symptoms. If therefore we are prepared to accept or reject a demarcation between Alzheimer's disease and presbyophrenic dementia—let us call it—or Fischer's disease, it would be useful. The paper by Dr Sourander which follows will, I am sure, help us to make up our minds because the author comes from a country noted for its intensive genetic and clinical studies as well as for careful neuropathology.

What other "related conditions", then, are relevant to our symposium? Pick's disease springs to mind, not because it can be difficult to distinguish on clinical grounds, but because of the occasional reports of classical histological features of Alzheimer's disease confined to the atrophied parts of the brain, the curious association of the two diseases as reported by Berlin (1949) and, as we shall hear today, the greater involvement of one lobe or side in some cases of Alzheimer's disease, just as in Pick's disease and in Lissauer's dementia. Finally that orphan of neuropathology—or perhaps family of orphans—Creutzfeldt-Jakob disease, is unlikely to be far from our thoughts.

The pathologist and the electron microscopist—in spite of the undoubted value of biopsy and the advances in cytochemistry—see only the debris of the battlefield and it is left to the clinician, the neurophysiologist and the

chemist to deduce what actually happens in the struggle between the healthy brain and the disturbance which affects it. As neurons seem to be selectively and extensively picked out in Alzheimer's disease can a dormant virus or slow virus be considered in part or wholly responsible? Can geneticists explain why one uniovular twin in Edinburgh died after 19 years with this form of dementia while her sister in Australia was in good health four years after her death? It is worth remembering that the afflicted twin experienced a period of febrile delirium during the 1918–19 epidemic of influenza but she also had acne rosacea, for which reason Davidson and Robertson (1955) suggested that somatic mutation might account for the double discordance.

The term "abiotrophy" was coined by Gowers (1902), although the idea of localized diminished vitality was advanced by Aristotle. Is the word still respectable or, in the wider group of diseases under discussion—occasionally heredofamilial though usually sporadic—shall we soon be able to implicate a particular gene or group of genes as being wholly or partly responsible?

The Ciba Foundation has to its credit a series of five colloquia on the subject of ageing, and the spirited discussion which followed Dr Kallmann's paper (1957) on twin data was mostly concerned with the relationship of the presenile dementias to ageing. That debate could well be regarded as the prelude to this symposium, but would we be justified in attributing Alzheimer's disease to ageing any more than to, say, diabetes mellitus or cancer? The Alzheimer cell change has been found in many conditions unrelated to senility. However we shall know more about this when we have heard of the recent work on the spatial arrangement and chemistry of neurofilaments and neurotubules and on the pathology of synapses: by then we may be nearer to understanding the basic lesions of Alzheimer's disease and important aspects of protein metabolism in the brain.

Fortunately, in this gathering we have many types of specialists who can help to solve our problems and we are of varied ages: those recently qualified are proficient in the use of new techniques and see the problems with sharp eyes and fresh minds; the older of us have the benefit of experience and longer memories which should serve us well, provided that our strata glomerulosa are functioning properly and are not already encumbered by bloated neurofibrils.

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ALZHEIMER'S DISEASE AND RELATED CONDITIONS
G. E. W. WOLSTENHOLME & MAEVE O'CONNOR

THE CONCEPT OF ALZHEIMER'S DISEASE AND ITS CLINICAL IMPLICATIONS*

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"Nomina si nescis, perit et cognitio rerum" (Linnaeus, in *Philosophia Botanica*, 1751)

IN order to determine the place of Alzheimer's disease in the spectrum of neuropsychiatric disorders the clinical and morphological characteristics of the condition must be described, and the borderlines between this disease and related conditions must be defined by using suitable structural and functional parameters.

GENERAL FEATURES OF ALZHEIMER'S DISEASE

Light microscopic and clinical characteristics

The disease first described by Alzheimer (1907, 1911), for which Kraepelin (1910) coined the eponym Alzheimer's disease, is a primary degenerative polioencephalopathy of unknown aetiology and pathogenesis. Morphologically it appears as cortical atrophy with many neurofibrillary tangles and so-called "senile" plaques, best shown in light microscopy by silver stains, birefringence after Congo red staining (Divry, 1934) and fluorescence after treatment with thioflavine (Schwartz, 1965). These changes occur in the isocortex and allocortex of both cerebral hemispheres and are sometimes seen also in the subcortical grey matter and occasionally in the brain stem. Neuronal loss, accumulation of lipofuscin and astrogliosis are subsidiary features without relevance for the differential diagnosis.

The clinical symptoms and course of Alzheimer's disease have been regarded by many investigators as fairly well defined (Rothschild and Kasanin, 1936; Sjögren, 1950, 1952, 1956; Delay, Brion and Garcia Badarocco, 1955). In our material (68 cases) the onset was between 45 and

* The investigation was supported by grants from the Swedish Medical Research Council, Project 21X-618-01, A-B, and from the United Life Group Insurance Co., Stockholm.

59 years of age in 50 per cent, between 60 and 64 years in 30 per cent and between 65 and 69 years in 20 per cent.

The course is progressive, without remissions. Sjögren (1950, 1951, 1952) distinguished three stages in the development of the disease. The initial stage, lasting two to four years, is characterized by mnestic disturbances, spatial disorientation and in most cases a pronounced lack of spontaneity. In the second stage progressive organic dementia and focal symptoms appear, particularly agnosia, aphasia and apraxia. Certain motor disturbances of a hypertonic-akinetic character are also frequently observed. The terminal stage is characterized by complete dementia, often accompanied by cerebral seizures and in most cases by a Klüver-Bucy-like syndrome (described separately later on). In a previous investigation (Sjögren, Sjögren and Lindgren, 1952) many of the symptoms in Alzheimer's disease were attributed to damage to the frontal and parietal lobes. In this presentation the importance of temporal lobe damage for the symptomatology will be particularly stressed.

Sjögren (1956) reported observations on a number of patients showing a symptomatology which in some respects resembled Alzheimer's disease, but which on the other hand was not typical of this disease. The characteristic features were: (1) much later onset than in Alzheimer's disease proper; (2) none of the lack of spontaneity characteristic of Alzheimer's presenile dementia; (3) absence of extrapyramidal features; (4) absence of cerebral seizures; (5) progressive dementia with agnosia, aphasia and apraxia as in Alzheimer's disease in younger individuals. The degree and distribution of cerebral atrophy was the same as in Alzheimer's disease but plaques and particularly fibrillary tangles were much less frequent than in classical Alzheimer cases.

This condition, which was called "atrophia senilis cerebri", differed markedly from cases of dementia senilis simplex and presbyophrenia. In the latter conditions focal neurological symptoms were absent, brain atrophy was rather slight and tangles in the isocortex were rare. There is a close clinical and morphological resemblance between "atrophia senilis cerebri" and the "démence sénile alzheimerisée" of the Geneva school of psychiatry (cf. Arab, 1954). The cases covered by the eponym possibly represent late manifestations of Alzheimer's disease. At least some of the so-called "juvenile" cases reported in the literature may represent early manifestations, although the clinical deviations from the classical type of Alzheimer's disease appear even more marked in these cases than in senile "alzheimerized" dementia.

A considerable number of familial cases of Alzheimer's disease have been

reported (Lowenberg and Waggoner, 1934; McMenemey *et al.*, 1939; Essen-Möller, 1946; Lauter, 1961; Bucci, 1963; Nahman and Rabinowicz, 1963; Heston and Loventhal, 1966). Some of these were cases with an early onset (van Bogaert, Maere and de Smedt, 1940). In the familial cases the genetic transmission has usually been described as autosomal and dominant. For the sporadic cases T. Sjögren assumed there was a cumulative polygenic action (Larsson, Sjögren and Jacobson, 1963).

Histochemical, ultrastructural and biochemical findings

Numerous papers have been written on the histochemistry of "senile" plaques and Alzheimer's neurofibrillary tangles. The main interest has been focused on the amyloid-like properties of a substance accumulating in the core of the plaques, in the tangles and in the walls of cortical vessels (cf. Hechst, 1929; Divry, 1934, 1935, 1939; Scholz, 1938; Missmahl and Hartwig, 1954; Margolis, 1959; Diezel and Vogel, 1965). In biopsy material Friede (1962, 1965) demonstrated increased enzyme activity in cortical zones corresponding to the early stage of plaque formation. The oxidative enzymes were increased at the periphery of the plaques, whereas acid phosphatase activity was increased throughout the fully developed plaques, particularly in the central deposits of granular material and in invading microglia. The marked increase in oxidative enzymes was interpreted as indicating that plaque formation may be induced by activation of metabolic processes in the neuropil. The considerable size and central degeneration of mature plaques were explained on the basis of inadequate capillarization.

Electron microscopic studies by Terry (1963) on cortical biopsies in Alzheimer's disease showed a neuropil structurally normal save for moderate gliosis. The ultrastructural localization of the acid phosphatase activity within the plaques in Alzheimer's disease was studied by Suzuki and Terry (1967). Dense bodies, probably of lysosomal character, were seen in distended axons and dendrites within the plaques. By analogy with changes occurring in early Wallerian degeneration, Suzuki and Terry hypothesized that formation of the plaques was "secondary to a proximal degeneration in neuronal perikaryon, possibly related to the neurofibrillary tangle of Alzheimer". Gonatas, Anderson and Evangelista (1967) identified some of the neuronal processes within the plaques as being presynaptic terminals. According to Terry (1968), the ultrastructural studies of brain biopsies in Alzheimer's disease suggest that "the clinical symptoms may be due to intraneuronal neurofibrillary lesions and to loss of cortical neurons".

For the highly characteristic neurofibrillary tangles originating in the cytoplasm of neurons the eponym Alzheimer's neuronopathy may be suggested. It has been known since the time of Cajal that changes resembling Alzheimer's neuronopathy may occur spontaneously, e.g. during hibernation, or may be induced by various methods, e.g. cold combined with inanition (cf. Jackson, 1925) or dehydration (Alexander, 1934; Stern and Elliott, 1949). Recently aluminium phosphate injected intracranially into rabbits has provided an interesting experimental model for both the acute and the chronic stages of Alzheimer's neuronopathy (Klatzo, Wiśniewski and Streicher, 1965; Terry and Peña, 1965; Terry, 1968; Wiśniewski, 1968). The use of quantitative cytochemical and histochemical techniques with this model indicated that the synthesis of proteins of a neurofibrillary type increased in the neurons showing tangles (Embree, Hamberger and Sjöstrand, 1967). This observation is of considerable interest since the electron microscopic studies by Kidd (1964, 1965) on cortical biopsies in Alzheimer's disease have shown that the filaments in this disease are not identical with normally occurring smooth neuronal filaments but appear to have periodically arranged expansions suggestive of the formation of an abnormal protein. Further discussion of the experimental models falls outside the scope of this presentation.

Since plaques, tangles and amyloid-like changes of the cortical vessels (congophilic angiopathy) are not specific for Alzheimer's disease, their histochemical characterization does not provide a reliable basis for delineation of the concept of Alzheimer's disease.

Only a few biochemical studies have been made in Alzheimer's disease. The content of non-haemin iron in various cortical fields in this disease and in normal senile involution showed (Hallgren and Sourander, 1960) no increase corresponding to the histologically detectable cortical iron deposits which Goodman (1953) assumed to be due to a primary disturbance of iron metabolism in Alzheimer's disease. The cortical accumulation of stainable iron, particularly in glial cells, may be secondary to a redistribution of iron caused by altered binding of the iron in the severely degenerated cortical tissue. Studies by Svennerholm on brain lipids from ten individuals in our series of Alzheimer cases did not provide evidence of any primary disturbance of lipid metabolism, and the normal figures for non-lipid hexosamine made a primary disturbance of the glycosaminoglycan metabolism less likely (Sjögren, Sourander and Svennerholm, 1966). Suzuki, Katzman and Korey (1965) found an increase in total acid polysaccharides in the cerebral cortex and this was assumed to reflect the presence of amyloid in the core of the plaques.

In recent years disturbances in monoamine metabolism, particularly in Parkinson's disease, have received much attention. Low values for homovanillic acid (HVA), the *o*-methylated acid metabolite of dopamine, were shown in autopsy specimens from the neostriatum in individuals with senile dementia (Gottfries, Gottfries and Roos, 1969) and Alzheimer's disease (Roos, personal communication). It was suggested that these conditions may be associated with a disturbance of monoamine metabolism which is reflected in a reduction of HVA in the neostriatum. A statistically significant relation was found between a low HVA concentration and a high degree of dementia, but since the clinical diagnosis of Alzheimer's disease seems not to have been neuropathologically verified no definite conclusion can yet be drawn about the monoamine metabolism in this malady.

COMPARATIVE STUDIES ON ALZHEIMER'S DISEASE AND RELATED CONDITIONS

The results presented here are essentially based on a joint clinical (Sjögren) and morphological (Sourander) investigation. All cases derive from the same source: Clinic I, Women's Department, Lillhagen Mental Hospital.

Brain atrophy

The weight of the brain was determined in 400 cases (Sjögren, 1965). The age at death of 318 patients is shown in Fig. 1. Fig. 2 shows the marked differences in distribution of brain weights in the three main diagnostic groups, i.e. the "nuclear" group consisting of patients afflicted with progressive organic presenile or senile dementia, the cerebrovascular group, and patients with other psychiatric disorders principally of the geriatric functional type. This third group includes disorders of mainly psychogenic origin, such as paraphrenic, melancholic and psychoneurotic states, without obvious structural changes in the brain. Brain atrophy is most pronounced in the nuclear group and Fig. 3 shows the distribution of brain weights and the degree of atrophy in this group. The reduction in brain weight is much more pronounced in most cases of Alzheimer's disease than in senile dementia (dementia senilis simplex and presbyophrenia). In the clinically somewhat different but morphologically related cases of Alzheimer-like senile dementia, the severity of brain atrophy is comparable to that seen in Alzheimer's disease, and its location is the same. In both conditions there is marked atrophy of the temporal-limbic structures.

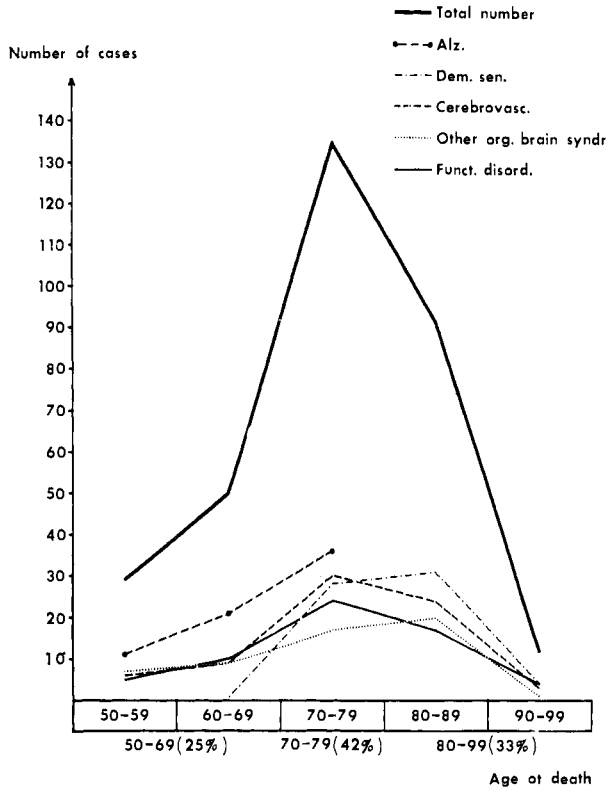

FIG. 1. Age at death of 318 patients.

Argyrophilic plaques and neurofibrillary tangles

The presence and intensity of plaques and tangles were studied in 318 cases. The clinical classification of the material is presented in Table I. It is well known that plaques and tangles are not specific for Alzheimer's disease but may occur in many different disorders. Gellerstedt (1933) in a meticulous study showed that they may also appear in so-called normal brains of mentally and neurologically presumably healthy individuals over 65 years of age. Since no detailed neuropsychiatric and psychological examinations had been performed on this material, the significance of these high figures for the so-called senile changes obtained in some cases is questionable. Corsellis (1962) showed that plaques and tangles were absent in the brains of most psychiatric patients belonging to the cerebrovascular group and the functional division. We carried out the same type of semiquantitative screening on our material and, as Fig. 4 shows, the two sets of results agree remarkably well.

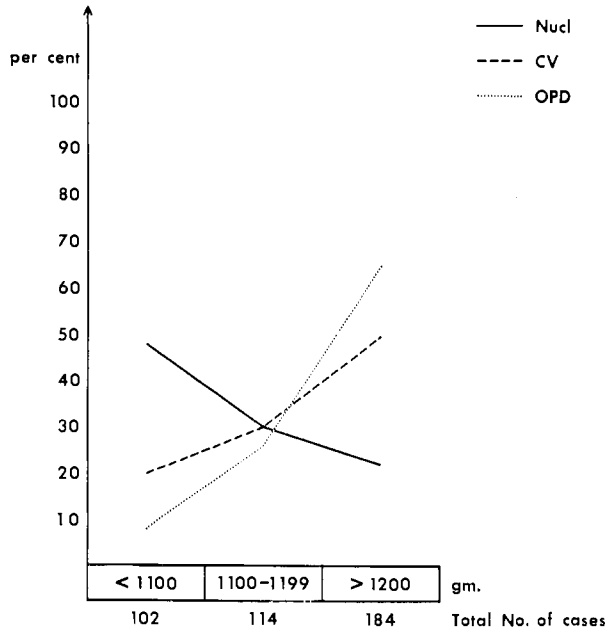

FIG. 2. Brain weight in 400 cases.
Nucl: "nuclear" group
CV: cerebrovascular group
OPD: group with other psychiatric disorders

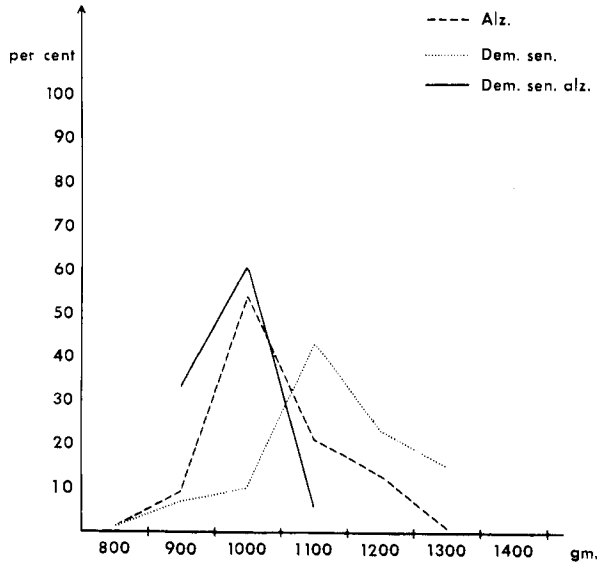

FIG. 3. Brain weight in "nuclear" group (Alzheimer's disease and senile dementia).

TABLE I

CLASSIFICATION OF THE LILLHAGEN MATERIAL (318 CLINICALLY AND NEUROPATHOLOGICALLY EXAMINED CASES)

	No. of cases
I. Presenile-senile nuclear group	132
(a) Alzheimer's disease	68
(b) Dementia senilis	64
Dementia senilis alzheimerisata	20
Dementia senilis simplex and presbyophrenia	44
II. Cerebrovascular group	72
III. Other organic encephalopathies	54
IV. Functional disorders	60

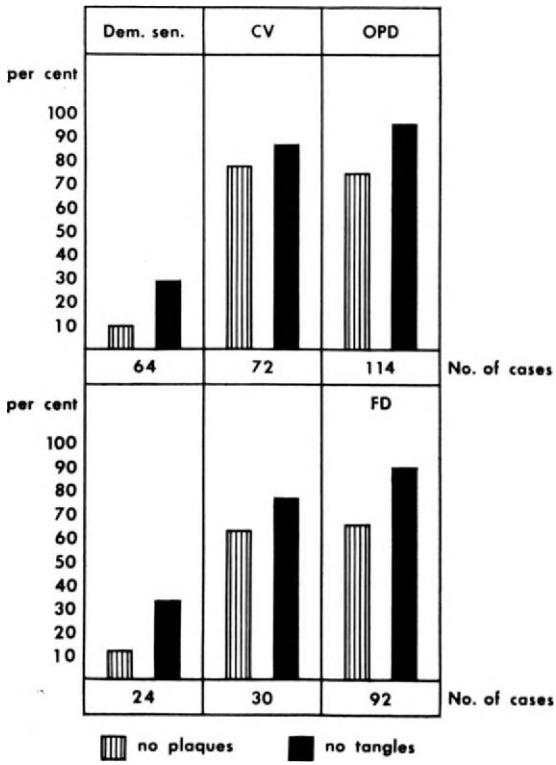

FIG. 4. Absence of plaques and tangles in psychiatric diseases of later life.

Above: Lillhagen cases.
Below: From Corsellis (1962)
CV: cerebrovascular group
OPD: other psychiatric disorders
FD: functional division

In senile dementia (dementia senilis simplex and presbyophrenia) which is clinically characterized by progressive mental deterioration without focal neurological symptoms, neurofibrillary tangles occur in both phylogenetically old and new regions of the temporal lobe. Fig. 5 shows the frequency and distribution of tangles in Alzheimer's disease and in senile dementia in the hippocampus, amygdala and temporal neocortex in corresponding age groups (age at death). In both diseases the tangles are

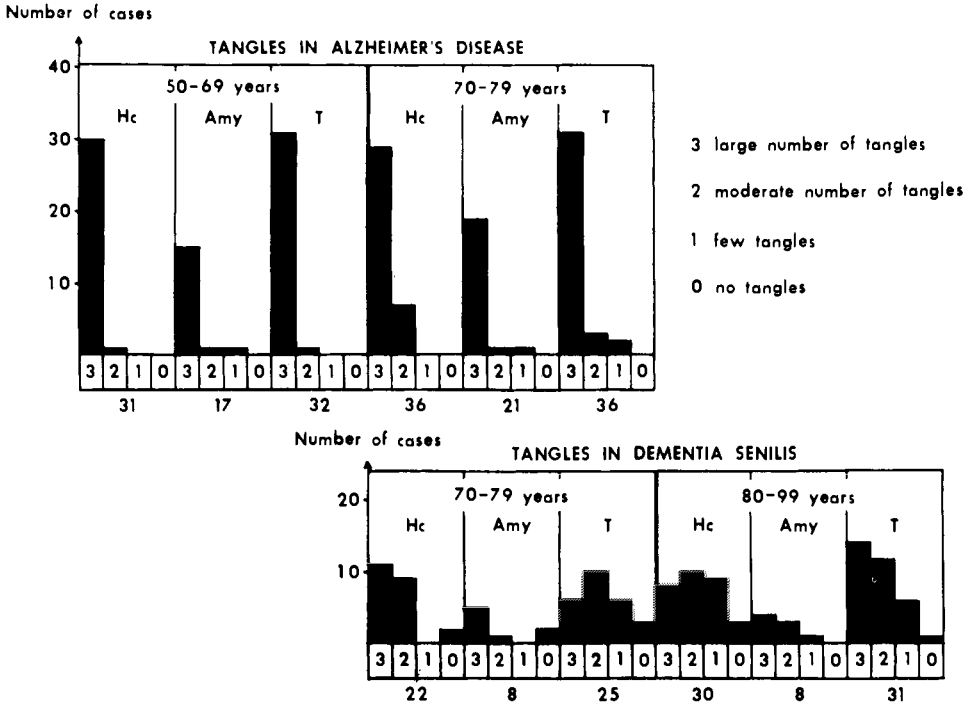

FIG. 5. Numbers of tangles in Alzheimer's disease and in senile dementia.
HC: hippocampus
Amy: amygdala
T: temporal neocortex

present in all three regions, constantly in cases of Alzheimer's disease and with few exceptions in cases of senile dementia.

There is, however, a marked difference in the degree of involvement not only of temporal structures but also of extratemporal neocortical fields. Fig. 6 shows the results of a semiquantitative comparison of the occurrence of tangles in five different brain regions in Alzheimer's disease and senile dementia. Regions showing only a few fibrillary tangles (+ instead of + + and + + +) were excluded from the comparison. Young and old

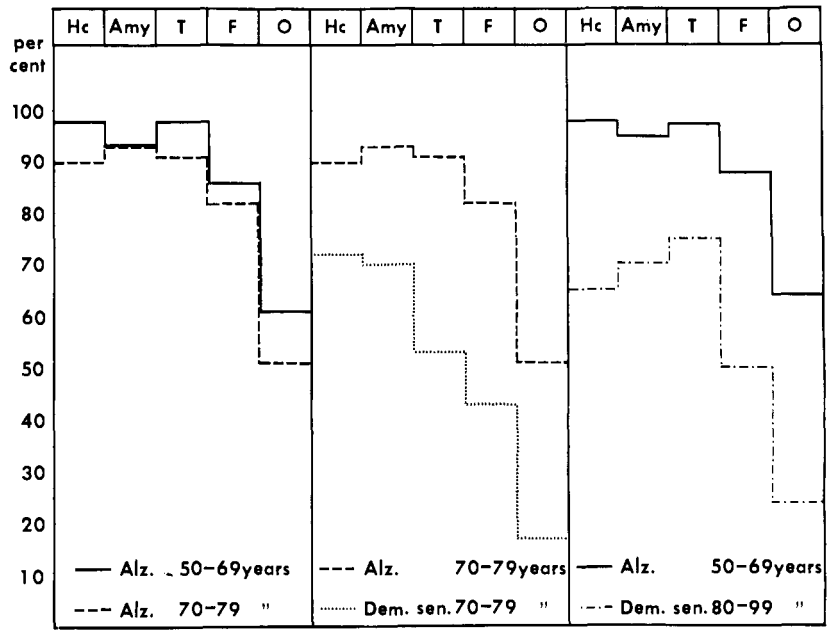

FIG. 6. Comparison of Alzheimer's disease and senile dementia: cases with numerous neurofibrillary tangles
HC: hippocampus
Amy: amygdala
T: temporal neocortex
F: frontal cortex
O: occipital cortex

cases of Alzheimer's disease showed similar numbers of tangles in the various brain areas examined, in striking contrast to the differences seen between cases of Alzheimer's disease and senile dementia. The marked differences in the occurrence of tangles between the younger Alzheimer cases and the very old senile dementia cases is particularly interesting, since it clearly shows that advanced age of the patients is not the crucial factor determining the severity of the lesions. Since Alzheimer's neurofibrillary tangles do not occur in the isocortex during normal senile involution (Gellerstedt, 1933), and since even old cases of senile dementia do not show the same degree of severity of the lesions, we may conclude that Alzheimer's disease is probably not a presenile form of senile dementia as is frequently claimed. This statement by no means excludes the possibility of common pathogenic factors for both diseases.

Vascular lesions

The occurrence and significance of atherosclerosis of basal brain arteries

and of cerebral infarctions in Alzheimer's disease and in senile dementia have been discussed in several papers (Mutrux, 1953; Arab, 1954; Rothschild, 1956; Sjögren, Sourander and Svennerholm, 1966; Jamada and Mehraein, 1968*b*). Sjögren, Sourander and Svennerholm (1966) showed that in presenile and senile dementia the basal arteries of the brain are generally only slightly or not at all affected by atherosclerosis and the incidence of cerebral infarctions is very low. In both respects the group with progressive dementia resembles the group with mainly functional disorders. Jamada and Mehraein (1968*b*) concluded from their investigation of the occurrence of atherosclerosis in basal brain arteries that a negative correlation was indicated between the presenile (Alzheimer's disease) and atherosclerotic processes. The absence of atherosclerosis does not exclude the possibility that changes in small cortical vessels, e.g. capillary fibrosis (Gellerstedt, 1933) and senile vascular deformities of the type recently described by Hassler (1965, 1967), may be present, the functional significance of which is so far unknown. The role played by marked congophilic angiopathy in some cases must also be further investigated.

Cerebral circulation

Recently multiple simultaneous measurements of regional blood flow using the ^{133}Xe intracarotid injection method developed by Lassen and co-workers (1963) and Lassen and Ingvar (1963) have been performed in clinically diagnosed cases of presenile and senile dementia (Ingvar *et al.*, 1968; Obrist *et al.*, 1970). Focal reductions were consistently found in the frontotemporal regions of the senile cases, but had a more variable localization in the presenile. Post-mortem examination of one of the patients, who died at the age of 66 years after four years of mental illness characterized by progressive dementia and marked impairment of verbal communication, provided histopathological evidence of Alzheimer's disease. This patient had less grey matter than normal in all regions but a normal blood flow for grey matter in most regions. Future research with this technique will show whether and to what extent the regional blood flow is reduced in the severely atrophic temporal and frontal lobes in Alzheimer's disease.

INVOLVEMENT OF THE TEMPORAL LOBES

Klüver-Bucy syndrome

In the 1940s, at an early stage of the Lillhagen investigation, Sjögren was already paying attention to the association of a dementia-amnestic syndrome with severe damage to the hippocampus and entorhinal cortex,

characterized by numerous plaques and fibrillary tangles. Neuropathological post-mortem examinations were initiated, the first 96 being carried out by the late Professor Nils Gellerstedt, whose classical study (1933) of cerebral changes during normal senile involution also included detailed observations on the hippocampal formation. Additional impetus for further clinical and pathological studies came from observations by Grünthal (1947) and Glees and Griffith (1952) on dementia and memory disturbances in man after bilateral destruction of the hippocampus, and from the famous paper by Papez (1937) on "A proposed mechanism of emotion", suggesting a closed circuit of interconnected structures, including the hippocampus, for elaboration of "the functions of central emotion". The strongest argument for combined clinical and histopathological studies on the temporal lobes and especially their limbic structures came from the detailed analysis by Klüver and Bucy (1937, 1938, 1939) and Klüver (1951, 1952) of a striking syndrome occurring in monkeys after bilateral temporal lobectomy including the amygdala, uncus, hippocampus and most of the temporal neocortex. Essentially the same syndrome in monkeys had already been observed in 1888 by Brown and Schäfer. The Klüver-Bucy syndrome, which is said to represent the most striking behaviour change ever produced by a brain operation in an animal, consists of the following six symptoms (Klüver and Bucy, 1937): (1) Visual agnosia, i.e. inability to recognize animate and inanimate objects, including non-recognition of simian faces, on the basis of visual criteria alone. (2) Hyperorality, i.e. an extremely strong tendency to examine and touch all objects with the mouth. (3) "Hypermetamorphosis" (in the sense of Wernicke). The animal appears strongly stimulus-bound, not only taking notice of and attending to visual stimuli but also "trying, as if acting under some compulsion, to contact and touch every object in sight". (4) Loss or diminution of emotions. (5) Hypersexuality. (6) Profound changes in dietary habits ("bulimia").

A more or less complete Klüver-Bucy syndrome has been repeatedly described in man after surgical removal of the same temporal-limbic structures as in the operated monkeys (Liddell and Northfield, 1954; Terzian and Dalle Ore, 1955).

From 1940 to 1964 systematic clinical tests were performed on neuropsychiatric patients at Lillhagen Hospital, Clinic I, to find out whether and to what extent symptoms of the Klüver-Bucy syndrome occurred. Particular attention was paid to Alzheimer's disease and related conditions. Fig. 7 shows the frequency of marked symptoms of the Klüver-Bucy syndrome in 60 cases of Alzheimer's disease. Visual agnosia was often noticed as the

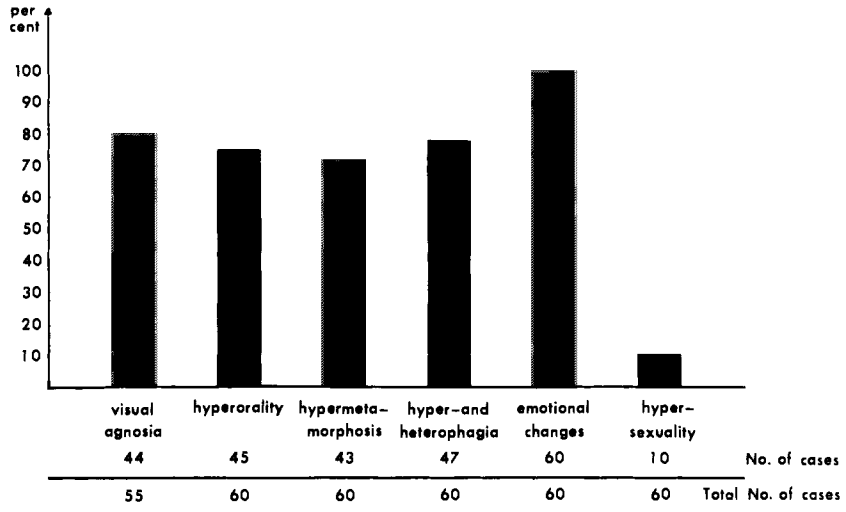

FIG. 7. Frequency of symptoms of the Klüver-Bucy syndrome in Alzheimer's disease.

first focal neurological symptom in the early stage of the disease, while hyperorality, hypermetamorphosis and hyperphagia and heterophagia were late phenomena. Visual agnosia or "psychic blindness" was particularly manifested as prosopagnosia, i.e. non-recognition of the face of relatives or the patient's own face (mirror sign). The hyperorality and hypermetamorphosis were essentially of the same type as in operated monkeys. The changes in dietary habits consisted of a strong inclination to eat voraciously—not only food but all sorts of objects such as gauze bandages, flowers, and match boxes. Emotional changes appeared as extreme dullness and apathetic behaviour corresponding to the "tameness" in monkeys after removal of both temporal lobes. Hypersexuality was infrequent but if present it consisted of increased autosexual, heterosexual, and homosexual activity, sometimes of a violent type. In most cases all the symptoms save the last one were present, constituting an almost fully developed Klüver-Bucy syndrome comparable to that seen in bilaterally lobectomized monkeys.

Morphologically the temporal lobes of all our patients showing a pronounced Klüver-Bucy syndrome were severely affected. Macroscopically a more or less marked atrophy of the temporal-limbic structures, i.e. the amygdaloid complex, hippocampus and temporal neocortex, particularly of the anterior pole and of the inferior and basal parts, was seen. As shown in Fig. 8, in most cases examined histologically there was an

abundance of argyrophilic plaques and fibrillary tangles in the hippocampus and amygdaloid complex as well as in the temporal neocortex. The frontal cortex and temporal neocortex appeared somewhat less affected.

Pilleri (1961*a*) examined various forms of presenile-senile dementia clinically and neuropathologically with regard to the occurrence of oral motor patterns and temporal lobe involvement. In six cases of Alzheimer's disease, showing various oral behaviour patterns, the temporal lobes or parts of

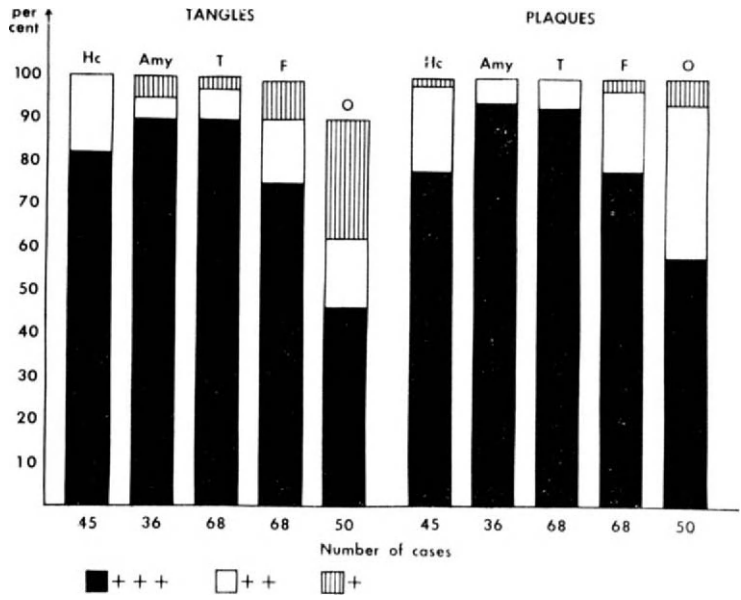

FIG. 8. Percentage of tangles compared with that of plaques in five brain regions in cases with pronounced Klüver-Bucy syndrome.

them were severely affected. According to Pilleri (1961*b*) the full syndrome of Klüver-Bucy, being specific for primates, is not likely to appear in man although isolated symptoms are often observed. However, he has recently (1966) reported an additional case of Alzheimer's disease which, like most of our Alzheimer cases, showed all the symptoms of the syndrome except abnormal sexual behaviour. Anatomically there was, as in our cases, severe damage to both neocortical and limbic parts of the temporal lobes. The same distribution of lesions has also been described in a juvenile case of Alzheimer's disease associated with a Klüver-Bucy syndrome (Jelgersma, 1964).

The Klüver-Bucy syndrome seems to develop in man only when there is also severe damage in extratemporal cortical regions (Pilleri, 1966). This

hypothesis gains support from a recently reported case with extensive vascular destruction of the temporal lobes and emotional changes but no other symptoms of the syndrome (Pilleri, 1967). In this case there were only minor changes in the extratemporal parts of the brain.

Although the spontaneously appearing Klüver-Bucy syndrome in man is not confined to Alzheimer's disease but may also occur in other conditions, e.g. in Pick's disease and certain cases of cerebral arteriosclerosis or brain tumours involving mainly the temporal lobes, in most cases it forms an essential part of the clinical symptomatology of this disease, as clearly shown in the Lillhagen material. Since the temporal lobes and their limbic structures are always severely affected in patients revealing the syndrome, its presence has a local diagnostic value.

The interpretation of the relationship, if any, between the various symptoms of the Klüver-Bucy syndrome is still much debated. Klüver (1958, 1965) tried to find a common denominator for the apparently very different behavioural changes and assumed this to be an interference with the normal fundamental capacity of the brain to deal with "inconstancies and fluctuations". According to Klüver (1958) the temporal-limbic system with its varied functions ("poikilofunctions") is "poised between the isofunctions of the cortex guaranteeing an approximate constancy of the external environment and the isofunctions of the diencephalon guaranteeing an approximate constancy of the internal environment". The pathology of poikilofunctions is considered to involve essentially a loss of the modulatory, inhibitory and regulatory functions of the limbic system.

According to Akert and co-workers (1961) the early literature on the localization of the individual symptoms of the Klüver-Bucy syndrome seemed to indicate that elements concerned with drives and emotion, such as hyperorality, tameness and hypersexuality, could be referred to damage to the rostral half of the pyriform lobe, including the amygdala and rostral parts of the entorhinal area. Visual agnosia seemed to be related to the lateral and ventral temporal cortex. However, further experimentation (Akert *et al.*, 1961) led to the conclusion that a Klüver-Bucy syndrome can also be elicited by lesions of certain areas of the temporal neocortex, particularly the anterior temporal pole, connected by intratemporal association systems with the medial ("rhinencephalic") temporal structures. In Alzheimer's disease both the phylogenetically old and young parts of both temporal lobes are heavily affected. This may be the reason for the persistence of the syndrome, since in animals where only one of these parts has been damaged the symptoms may gradually disappear (Brown and Schäfer,

1888; Akert *et al.*, 1961). Recent studies by Delgado (1967) on monkeys stimulated by remote radio control indicate that the "limbic system" may be more involved in planning, i.e. organization of the temporo-spatial sequence of behaviour, than in behavioural performance.

Herrick (1956) presented the view that the richly developed neuropil, constituting the greater part of the cortex in man, forms the substrate material for the integrative functions of the brain, and that these functions are not firmly bound to definite localized arrangements of nerve cells and fibres. They are dynamic field functions, "the nature and limits of which are determined by patterns of interaction of the energies released and not by any arrangements in space and stable structures". Walshe (1964) claimed that the cortical neuropil representing the integrative apparatus of the brain, in contrast to the projecting systems, has no known somatotopic arrangement. However, he admitted that the equipotentiality of the integrative apparatus is only relative and that there must be some measure of functional differentiation.

The ultrastructural and histochemical studies previously quoted indicate that the essential structural manifestations of Alzheimer's disease, i.e. the argyrophilic plaques and the neurofibrillary tangles, may reflect metabolic disturbances in neurons and the neuropil. The macroscopic and light microscopic investigations show that in most cases of this disease the severest lesions are in the temporal lobes. The Klüver-Bucy syndrome may thus be said to reflect profound disturbances in the most severely affected parts of the brain in Alzheimer's disease, namely the integrative apparatus, including neuropil and neurons, of the temporal lobes.

Hippocampal lesions and the mnestic syndrome

The significance of temporal lobe damage for the mnestic disturbances in the initial stage of Alzheimer's disease is difficult to evaluate.

Penfield and Milner (1958) suggested that the hippocampus in man plays an important role in memory functions and stated that bilateral lesions in this structure are followed by an unexpected loss of recent memory. Gol and co-workers (1963) studied the effects of highly selective removal of the hippocampus in the monkey, baboon and cat. They concluded that while hippocampal function is important for facilitating affective behaviour, its significance in the learning processes is confined to modifying influences. According to Adey (1963), it may be concluded that the hippocampus participates in learning processes by establishing functional patterns with subcortical structures. It may be regarded as related to transmission and

transaction and not to storage of information. The severe disturbances of memory in Alzheimer's disease may at least partly be due to the profound bilateral changes in the hippocampus.

Cerebral seizures

The syndrome "cerebral seizures with onset late in life" is dealt with surprisingly briefly in the medical literature. In a study of the occurrence of cerebral seizures in elderly patients with various neuropsychiatric disorders, Sjögren (1964) found a high frequency of "epilepsia tardiva" in Alzheimer's disease, probably attributable to the organic brain lesions.

In the present series of 68 histopathologically verified cases of Alzheimer's disease, epilepsy was recorded in 75 per cent. In 44 per cent of cases with seizures these were recorded as *grand mal* and in 64 per cent as minor spells seen as characteristic "drop fits—hypokinetic fits" (Sjögren, 1952) and masticatory seizures. Epileptic seizures of both types were more common in Alzheimer's disease than in senile dementia or cerebrovascular diseases, as shown in Fig. 9 (204 patients).

In most cases (60 per cent) *grand mal* occurred during the last six months

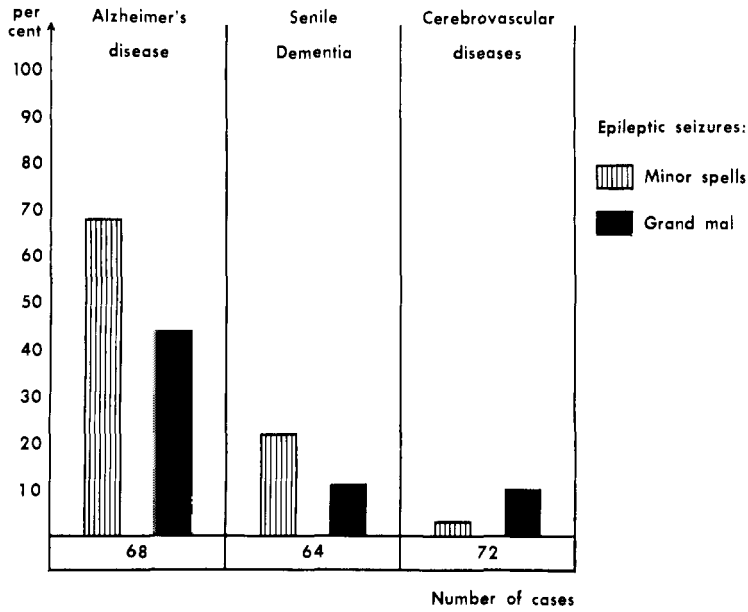

FIG. 9. Percentage of minor epileptic seizures and of *grand mal* in 204 patients with Alzheimer's disease, senile dementia and cerebrovascular disease.

of life while minor spells frequently appeared comparatively early in the course of the disease (in 70 per cent one to three years and in 12 per cent four to nine years before death). In 45 per cent of the cases with epilepsy both types of seizure were observed.

Masticatory seizures were recorded in 45 per cent of the patients with epilepsy and were manifested as rhythmic chewing, smacking, tasting and lip-licking, accompanied by loss of consciousness. These phenomena are of particular interest in regard to temporal lobe damage. Magnus, Penfield and Jasper (1952), discussing stimulation results and seizure findings, suggested the amygdala as the subcortical structure most likely to be involved in masticatory hyperactivity. Our studies, like the quantitative estimations of Jamada and Mehraein (1968*a*, *b*), have demonstrated that the amygdala is markedly affected in Alzheimer's disease.

EXTRACEREBRAL PATHOLOGY

The study of extracerebral pathogenic factors in Alzheimer's disease has been much neglected, particularly as regards the endocrine functions. Schnitzler (1911) described a case of dysthyroidism with an abundance of neurofibrillary tangles. Schob and Güntz (1932) reported a case of Alzheimer's disease associated with cachexia hypophyseopriva (Simmonds' disease). Constantinidis, Garrone and Wildi (1962) studied the urinary excretion of steroids in Alzheimer's disease and other psychiatric conditions of later life. They found a decreased proportion of 17-ketosteroids to corticoids in Alzheimer's disease.

Extreme and rapid decrease in body weight, apparently unrelated to inadequate feeding and to cancer, was a common finding in the terminal stage of Alzheimer's disease in our material. Morphological changes in the hypothalamic nuclei in Alzheimer's disease have been described by Morel and Wildi (1950). In our material the limbic structures which are regarded as related to hypothalamic and endocrine functions were severely affected. A careful search for signs and symptoms of possible endocrine dysfunction is therefore necessary. Almost 30 years ago McMenemey (1940) stressed the need for a more complete histological examination of other organs of the body in Alzheimer's disease. However, this appeal has not yet produced an adequate response.

CONCLUDING REMARKS

In closing we would like to state that from the pathological point of view the delineation of Alzheimer's disease is a problem which cannot be solved

by methods in current use. However, before new techniques can be usefully applied, the clinical material must be classified according to known

TABLE II

ALZHEIMER'S DISEASE AND RELATED CONDITIONS

I. *Primary degenerative polioencephalopathy of Alzheimer type*
   (1) Alzheimer's disease proper (presenile and delayed)
   (2) Alzheimer-like senile dementia
   (3) Alzheimer-like early adult ("juvenile") dementia

II. *Secondary Alzheimer's disease*
   Down's syndrome and possibly other oligophrenic conditions
   Post-traumatic (e.g. dementia pugilistica)
   Endocrine dysfunction

III. *Atypical Alzheimer's disease*
   E.g. with congophilic angiopathy

IV. *Alzheimer's neuronopathy*
   Normal senescence
   Dementia senilis simplex and presbyophrenia
   Postencephalitic Parkinson syndrome
   Guam-Parkinsonism dementia complex
   Chronic sclerosing panencephalitis
   Other rare organic encephalopathies

V. *Experimentally induced neurofibrillary tangles*

pathological facts. As a basis for further discussion of Alzheimer's disease and related conditions we propose the scheme shown in Table II. Such schemes are only of tentative value and should be replaced as soon as more is known of the aetiology and pathogenesis of these conditions.

SUMMARY

The accepted facts and recent findings concerning Alzheimer's disease are reviewed. The importance of ultrastructural and new chemical and physiological techniques in studying this disease is stressed. Some results from a clinico-pathological investigation of 318 females are reported to outline the concept of Alzheimer's disease and its relation to other neuropsychiatric disorders of advanced age. During their illness they were observed by the same physician. Sixty-eight cases showed the classical features of Alzheimer's disease. Twenty cases were very old and were considered to represent a related but somewhat different entity: Alzheimer-like senile dementia. Brain atrophy was more pronounced in Alzheimer's disease and Alzheimer-like diseases than in the other groups. A study of the occurrence of neurofibrillary tangles in the various disease groups showed that Alzheimer's disease is not simply a presenile form of senile dementia.

Atherosclerosis does not play any part in the pathogenesis of Alzheimer's disease. The significance of other vascular changes of a senile type and of vascular amyloidosis is unknown. The frequency of a Klüver-Bucy syndrome and the high frequency of minor seizures are due to severe damage to the temporal lobes, particularly the limbic structures. The need for further studies on the pathology of organs other than the brain in Alzheimer's disease is stressed.

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DISCUSSION

Roth: Was the diagnosis in your cases made on clinical or on pathological grounds, or was the clinical diagnosis revised on the basis of post-mortem findings?

Sourander: Dr Sjögren has always claimed that the diagnosis was clinical. He has really been able to predict Alzheimer's disease in surprisingly many cases. Of course I cannot exclude the possibility that he consulted the post-mortem records in some difficult cases.

Roth: The pathological data may have led to revision of the initial clinical formulation. The number of cases of Alzheimer's disease in this material also seems exceptionally large in relation to the number of patients

with senile dementia. So the question is whether clinical, pathological or combined criteria were uniformly applied in all cases or whether the high proportion of cases with Alzheimer's disease possibly reflects some special sampling factor. Were these cases representative of those that come to the Göteborg clinic, or was there a tendency to refer cases of Alzheimer's disease to Dr Sjögren?

Sourander: These cases have been collected since the early 1940s. Almost every patient was observed by Dr Sjögren from the time of admission to the terminal stage of the disease. Autopsies were carried out on all who died. If the cases were obvious ones of severe cerebrovascular disease they were only examined macroscopically, but in all those suspected to have presenile and senile dementias the brains were examined histologically. So I think that the material is representative of the patients admitted to Dr Sjögren's department.

Hughes: How is senile dementia distinguished from senility?

Jacob: First of all we have to differentiate senile dementia as a non-specific clinical syndrome and senile dementia as a definable tissue process.

Tomlinson: We must know whether the term senile dementia is being used to refer to a clinical syndrome without any assumed pathological connotations, or to describe dementia in old age with the pathological hallmarks of Alzheimer's disease. If we don't get this agreed now, confusion will reign throughout our symposium.

Roth: Traditionally the distinction between Alzheimer's disease and senile dementia was a clear one. It rested on the occurrence in Alzheimer's disease of focal phenomena: the parietal lobe group of features, the characteristic mixture of apraxia, agnosia, aphasia, spatial disorientation and so on. In senile dementia, on the other hand, a simple amnestic dementia was held to be the principal ingredient of the clinical picture. The condition progresses from amnesia for recent events to more general intellectual deficiencies and personality deterioration without definable focal symptoms or signs. The German workers have recently called this distinction in question. Lauter and Meyer (1968) claim to have demonstrated focal phenomena in the senile cases. In the light of these findings is the distinction valid clinically or pathologically, or are we left with age criteria alone?

Tariska: Alzheimer's disease and senile dementia could nearly always be distinguished according to the presence or absence of focal symptoms seen clinically, and argyrophilic changes seen on pathological examination. Delay and Brion (1962) reported that in senile dementia different clinico-pathological groups could be distinguished.

Roth: At present the pathological criteria are not qualitatively specific. Pathologically, the difference is apparently a quantitative one. But at what point are we to speak of senile dementia or Alzheimer's disease? No-one can provide a precise answer yet, although there may be rough agreement.

Sourander: Fig. 5 (p. 19) shows the occurrence of tangles in Alzheimer's disease and senile dementia in corresponding age groups. The principal clinical difference there is the occurrence of focal neurological symptoms in Alzheimer's disease and the absence of focal neurological symptoms and signs in cases regarded as senile dementia.

Hughes: I can't distinguish the 70–79 year age group with Alzheimer's disease from the 70–79 group with senile dementia.

Roth: Dr Sourander states that if focal neurological findings were clearly present at some stage, the cases in question were diagnosed as Alzheimer's disease, but you regard the distinction as spurious, Dr Hughes?

Hughes: Would an epileptic fit be sufficient evidence of a focal neurological lesion in this context?

Sourander: No.

Tomlinson: At this moment I am only pleading that the term senile dementia should be used either to mean dementia in old age, without inferring any particular cerebral pathology, or to mean dementia in old age with the pathological features of Alzheimer's disease. If it were used in the latter way then at least we should all know what is meant.

Roth: The term senile dementia was never intended to cover the whole range of pathological processes causing dementia in old age.

Corsellis: It is nevertheless sometimes used in this way, but it would be better to have it more clearly defined. I doubt whether this can be done until we can distinguish clinically a group with senile dementia, that is with progressive dementia not on a vascular basis, and establish the underlying pathology.

Terry: Physiologically there may be some reason to doubt the specificity of the focal changes. As people age the frontal lobes may become more susceptible to these changes, and frontal lobe changes may conceal the symptoms arising from the parietal regions.

Roth: So you would like to have a purely chronological criterion, with senile dementia referring to any progressive dementing process which occurs after the age of 65?

Terry: Yes. If there are severe Alzheimer-like changes we should indicate this in our discussion. And there are other types of senile dementia

such as vascular, simple atrophy, and so on. At least now the words will fit with what the dictionary says about senile and about dementia.

Roth: A classification based on pathological criteria is certainly clear, although it leaves a number of problems unresolved. Clinical and pathological data must be kept separate, as Dr Corsellis said, because this is where the confusion enters.

Tomlinson: It would be acceptable if people referred to arteriosclerotic dementia, or the Alzheimer form of senile dementia, or the mixed arteriosclerotic and Alzheimer types of dementia.

Sourander: I am a bit suspicious about this type of classification. One should clearly differentiate between processes which are characterized by progressive dementia and arteriosclerotic processes which are characterized by exacerbations and remissions.

Jacob: Surely the time factor in the development of clinical syndromes and of basic tissue processes requires careful investigation.

Strich: How many of your patients had congophilic angiopathy and what was the age distribution, Dr Sourander?

Sourander: It depends on what you mean by congophilic angiopathy. We labelled five cases as congophilic, but only one was regarded as Alzheimer's disease. The mean age at death of these five patients was 78 years. We have not been using the new fluorescence method with thioflavine S (Kelényi, 1967), which would perhaps reveal more cases of this kind.

Friede: I like to emphasize that dementia is not a pathological diagnosis and should not be used as such. The pathological diagnoses for clinically demented patients are Alzheimer's disease, Pick's disease, senile atrophy, cerebrovascular disease, etc. Alzheimer's disease refers to characteristic microscopic findings which, as far as I know, are identical for its variants, e.g. presenile, senile and others, as listed by Dr Sourander.

Roth: So that is a pathological diagnosis?

Friede: Yes, it is.

Pratt: Do you still find a peculiar geographical variation in the relative frequency of Alzheimer's and Pick's diseases in various parts of Sweden (Sjögren, Sjögren and Lindgren, 1952), Dr Sourander? And is the frequency of Pick's disease in Sweden equal to that of Alzheimer's disease? These findings were rather different from those in other countries.

Sourander: In Göteborg I have only seen four cases considered as Pick's disease and more than 50 cases of Alzheimer's disease. I would say that Alzheimer's disease is more common also in other parts of Sweden than Pick's disease but I have no substantial evidence for this.

Terry: I am convinced that in New York Alzheimer's disease is at least

a hundred times more common than Pick's disease, which is very rare. This incidence is based not on focal clinical signs but on pathological criteria.

Tomlinson: In Table I (p. 18), Dr Sourander, you don't include any group in which the changes in senile patients with Alzheimer's disease were associated with severe cerebrovascular disease. Is this because the obvious cases of cerebral softening were not examined histologically? If you examined all cases of dementia in old age histologically, then some cases with cerebrovascular disease would also be found to have severe disease of Alzheimer type. If so the contribution towards the total dementia may well come from both these types of cerebral pathology.

Sourander: From a clinical point of view there is little overlapping between presenile-senile dementias and cerebrovascular diseases. Less than 10 per cent of our patients with Alzheimer's disease showed significant arteriosclerotic changes in the brain at autopsy. On the other hand, as seen from Fig. 4 (p. 18), plaques and tangles were absent in most cases of cerebrovascular psychoses.

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ALZHEIMER'S DISEASE AND RELATED CONDITIONS
G. E. W. WOLSTENHOLME & MAEVE O'CONNOR

THE LIMBIC AREAS IN ALZHEIMER'S DISEASE AND IN OTHER CONDITIONS ASSOCIATED WITH DEMENTIA

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THE pathological process in Alzheimer's disease is not confined to one part of the brain. Although in most cases the cortex of the frontal and temporal lobes is more affected than that in the parieto-occipital region, any marked tendency to localized atrophy is rare.

It may therefore seem pointless to pick out the limbic grey matter for special attention when the rest of the cerebral mantle is as likely to be affected. There are, however, two reasons for doing this. First, many workers over the years have drawn attention to certain peculiarities of the tissue changes in the limbic areas. Secondly, there is the current, although far from new, idea that the limbic pathways have a particularly important role to play in the "mechanism of emotion, memory, learning and the organization of behaviour" (Smythies, 1966)—disturbances of which form the cardinal features of the dementia seen in Alzheimer's disease.

The first thing to be considered, therefore, is the nature of the pathological process in the limbic areas, or more precisely in their temporal components, which are the hippocampus and the adjacent cortex, the uncus, and the amygdaloid nucleus. The following observations are drawn partly from the extensive literature on the subject and partly from a recent analysis of 20 cases of Alzheimer's disease (14 women and six men). The mean age of these 20 cases at death was 64 years; the estimated duration of the illness ranged from two to 14 years, with a mean of five years. In all 20 cases the diagnosis had been made or suspected during life and was confirmed histologically after death. Degeneration of the large cerebral vessels was minimal in 18 of the 20 cases; in the remaining two it was moderately severe but was without evidence of focal necrosis.

The part within the limbic areas that has been most often studied is the hippocampus. According to von Braunmühl (1957) Simchowicz had already in 1911 remarked on the peculiar sensitivity of this region to the changes associated with ageing. Several subsequent studies (Morel and

Wildi, 1955; Brion and Schonback, 1966) have shown that senile plaque formation, Alzheimer's neurofibrillary tangles, and granulovacuolar degeneration (Woodard, 1962) are all particularly intense in the strip of cortex which curves round from the resistant and largely unaffected part (h2), through the h1 field to continue into the subiculum. Plaques and neurofibrillary tangles tend to be much less prominent as the resistant sector and the end folium (h3) are entered on the one side, and the lateral part of the subiculum is reached at the other end (Fig. 1). The extent of this hippocampal degeneration is reflected by the neuronal devastation

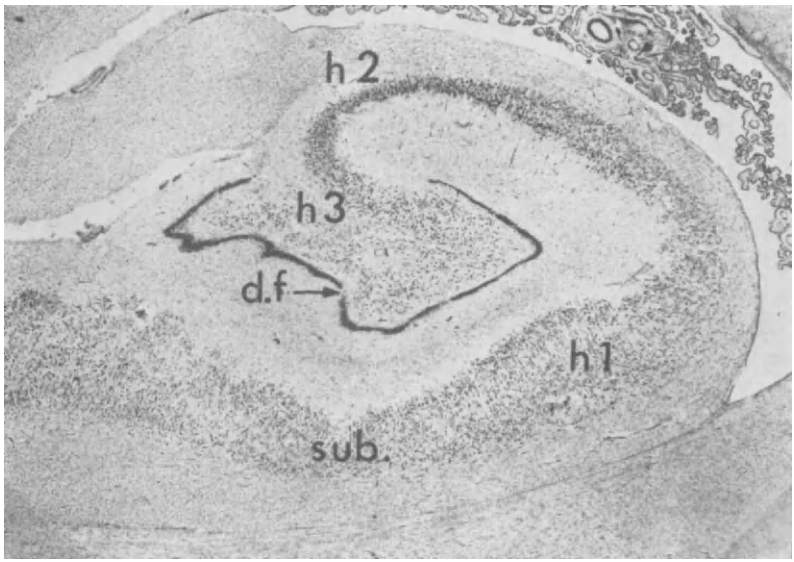

FIG. 1. Normal hippocampus showing the conventional anatomical subdivisions. d.f. = dentate fascia; sub. = subiculum. × 11·5, cresyl violet.

and the gliosis in this area (Fig. 2), for in the great majority of cases of Alzheimer's disease the loss of nerve cells in the h1 field and the adjacent part of the subiculum (Fig. 3) is exceptionally marked. It is, moreover, always bilateral although there may be some variation in intensity on the two sides. The pattern of the damage, with the heavy glial reaction along each edge of the pale atrophied cortical ribbon, has a peculiarly distinctive character and it differs from the hippocampal necrosis, and eventual sclerosis, that may occur as the result of hypoxia and in epilepsy in which the end folium and dentate fascia also tend to be severely affected (Fig. 4). As this degenerative process tracks forward towards the uncus, the neuronal loss and the fibrous gliosis continue to affect the pyramidal cell layer of the

hippocampal "pes" but the damage becomes less marked as the ribbon of cells runs up towards its junction with the amygdaloid nucleus (Fig. 5).

Jamada and Mehraein (1968) have recently studied quantitatively the extent to which the "limbic system", as against several other cortical areas, is affected in Alzheimer's disease. This was done by counting both the number of plaques and the number of cells with neurofibrillary tangles. The amygdaloid nuclei were found to be severely affected in nearly all cases, with counts that were significantly higher than in any of the six cortical areas examined. In a few instances the changes were concentrated

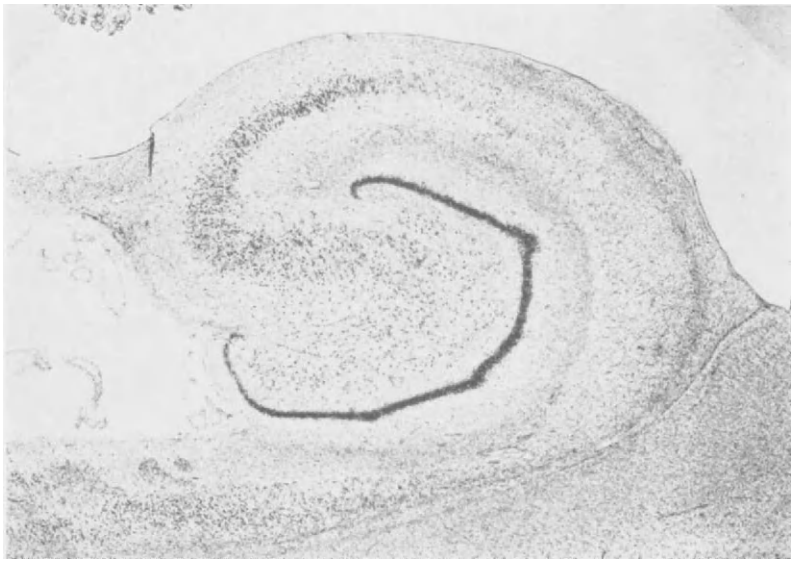

FIG. 2. Hippocampus in Alzheimer's disease to show the marked neuronal loss in the h1 field and subiculum, accompanied by a generalized heavy gliosis. × 12·5, cresyl violet.

on, if not confined to, the amygdaloid nuclei and Jamada and Mehraein questioned, as Grünthal (1928) had done, whether this was not the most susceptible area to plaque formation in the brain. In this connexion, the distribution of the plaques and of the neurofibrillary tangles is of interest, since the more medial and central parts are usually considerably more affected than the lateral group of neurons (Fig. 6). This marked tendency may be related to the fact that the cortico-medial complex in the human embryo differentiates much earlier than the basolateral group (Humphrey, 1968).

The cytoarchitecture in the amygdaloid nucleus changes rapidly from

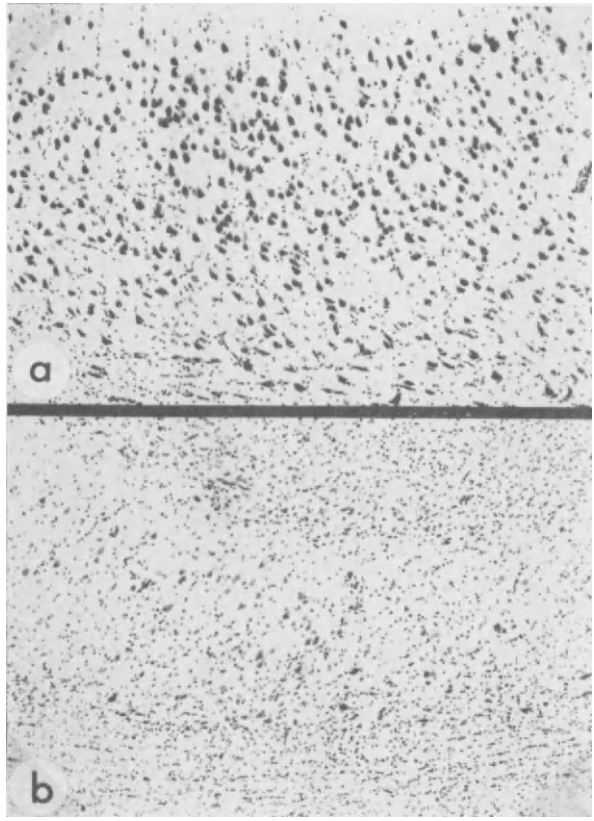

FIG. 3*a*, *b*. Massive neuronal loss in the subiculum in Alzheimer's disease (*b*), compared with normal (*a*). × 31, cresyl violet.

one level to the next, and variations in cell population are not always easy to assess. Neuronal loss certainly appears to be much less obvious than that in the hippocampus but it may nevertheless be considerable. As with the plaque formation it is usually more noticeable in the medial half of the nucleus (Fig. 7*a*, *b*) and it is then accompanied by a heavy fibrous gliosis. Another well-established feature, which Jamada and Mehraein also emphasized, is the exceptionally marked lipochrome content and ballooning of the nerve cells in the amygdaloid nucleus even when compared with other areas prone to develop similar changes.

No special histological features appear to have been identified in other parts of the limbic areas. The mamillary bodies may occasionally contain a moderate number of senile plaques, and a few neurons may show neurofibrillary damage, but any marked degeneration is exceptional. The

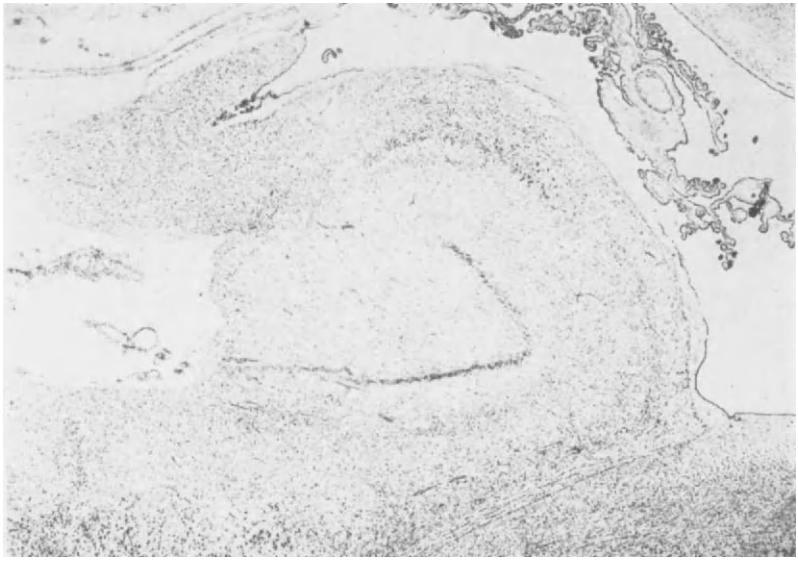

FIG. 4. The pattern of hippocampal scarring after hypoxia and in epilepsy differs constantly from that seen in Alzheimer's disease (see text). × 16, cresyl violet.

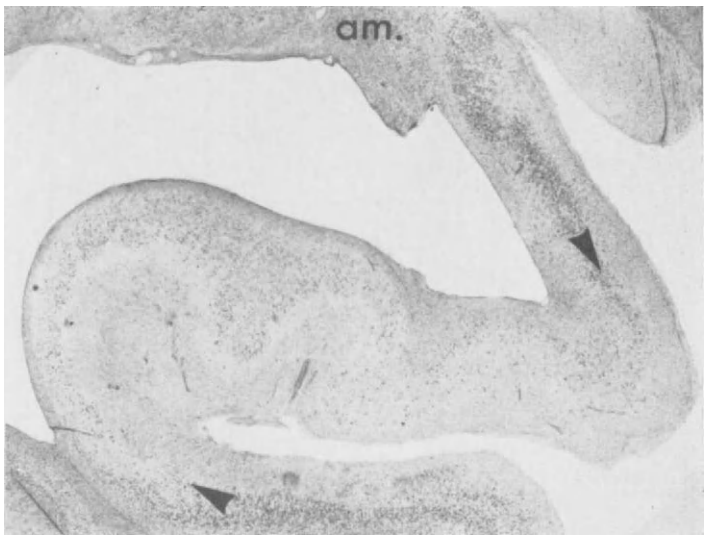

FIG. 5. Hippocampal pes showing the relatively severe neuronal loss in the cortical ribbon between the two arrow heads. The cortex at the junction with the amygdaloid nucleus (am.) is relatively spared. × 5, cresyl violet.

cortex of the cingular gyrus has not been described as particularly vulnerable.

Thus the histological peculiarities of the tissue reaction in the limbic areas are largely confined to the amygdaloid and hippocampal regions.

The second question, therefore, is whether this emphasis of the degenerative process on the medial temporal grey matter in Alzheimer's disease has a particular significance.

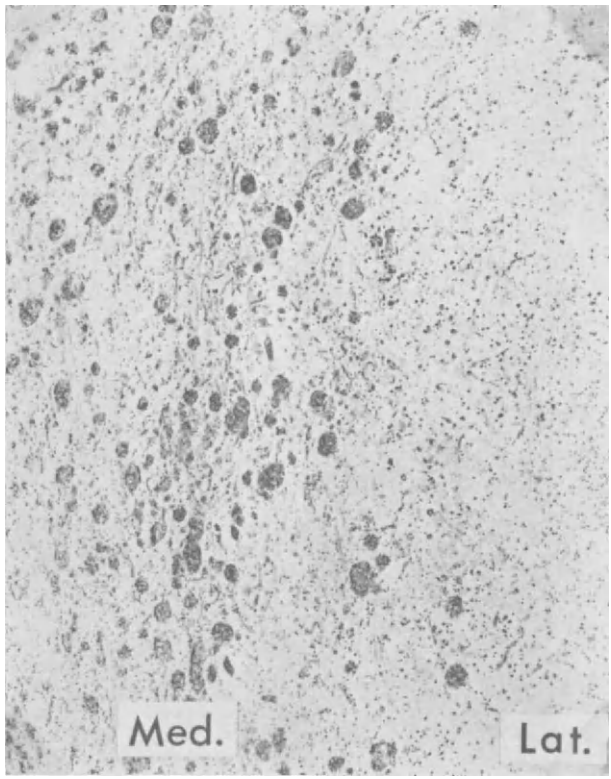

FIG. 6. Senile plaque formation which is considerably more marked in the medial (Med.) part of the amygdaloid nucleus than laterally (Lat.). von Braunmühl impregnation × 50.

When Broca delineated the "great limbic lobe" in 1878, he claimed that it represented the "brutish" part of the forebrain, whereas the rest of the cerebral mantle, which he called the extra-limbic mass, was concerned mainly with intelligence. This concept of a limbic lobe, or even a limbic system, has always aroused both suspicion and enthusiasm. Elliot Smith in 1901, for example, wrote disparagingly about the "strange fascination"

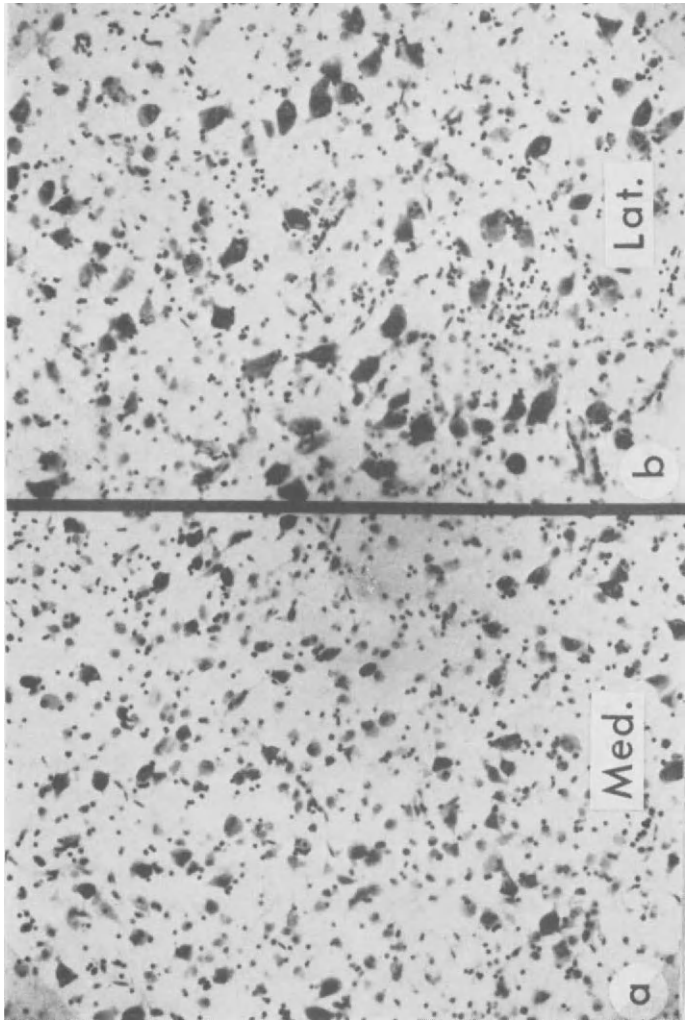

FIG. 74, *b*. Representative sample of neuronal population of amygdaloid nucleus showing the loss of neurons in the medial part (*a*), compared with the less affected lateral area (*b*). × 15, cresyl violet.

that the idea then held for many writers, while nine years later he was talking about the genius of Broca and of returning once again to the concept of the limbic lobe. Since that time, but particularly after the work of Papez in 1937, many different activities have been attributed to the limbic grey matter and its connecting pathways (Smythies). From the neuropathological point of view, the most convincing association so far documented is that between the disorder of recent memory and damage to the hippocampal regions, particularly when the damage is bilateral.

The idea that such an association might exist is far from new. Interest in it, however, has increased considerably during the last 20 years, following the observation that the bilateral, and even at times the unilateral, removal of the medial temporal grey matter (including the amygdaloid nucleus and hippocampus) is followed by a severe defect in recent memory (Scoville and Milner, 1957). There are, moreover, numerous clinico-pathological studies of patients whose main symptom was the loss of recent memory and whose structural abnormality in the brain consisted of damage paramount in the two hippocampi and the adjacent cortex (Grünthal, 1959; Corsellis, Goldberg and Norton, 1968).

It is not unreasonable, therefore, to suggest that the memory defect characteristic of Alzheimer's disease may be related to the particularly marked degeneration in the hippocampal cortex. It is then only a short step to question whether the milder deterioration of memory that may occur with advancing age might not also have a similar basis, since several studies, including a recent one by Tomlinson, Blessed and Roth (1968), have shown that the hippocampal and subicular neurons are selectively prone to degenerate in elderly but non-demented people.

Overshadowing the memory disorder in Alzheimer's disease, however, is the advance of the dementia which is generally seen as a consequence of the diffuse cerebral atrophy. It would therefore be unwise to lay too much emphasis on the role of the limbic damage in this connexion, particularly as, although gross intellectual and behavioural deterioration has been recorded after destruction of the limbic areas, other parts of the hemispheres have also usually been affected in such cases (Grünthal, 1959).

The same argument applies to attempts to link the various manifestations of the Klüver-Bucy syndrome that may occur in Alzheimer's disease to limbic or to temporal lobe degeneration. Some features of this syndrome have been reported in man after the surgical removal or destruction by disease of both temporal lobes, and it is conceivable that severe atrophy of the lobes as a whole, and not merely of the limbic areas, may be instrumental in producing the same effects (Jelgersma, 1964; Pilleri, 1966). Such

an assertion, however, is easier to make than to substantiate, for convincing proof requires a more precise and more persistent analysis of the clinical, psychological and pathological variations in dementia than has so far been attempted. It might then be possible to estimate the relative importance of the focal concentration of a degenerative process even though the process itself is diffuse.

SUMMARY

The limbic grey matter in the temporal lobes (amygdaloid nucleus, uncus and hippocampal regions) is particularly liable to degenerate bilaterally in Alzheimer's disease. Certain peculiarities of the changes in these areas have been described and illustrated.

The possible significance of this tendency to develop intense damage in the limbic areas is briefly discussed in relation to memory disorder and to dementia.

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DISCUSSION

Sourander: As observed by Brown and Schäfer (1888), the symptoms following bilateral temporal ablation may fade away, whereas the Klüver-Bucy syndrome in patients with Alzheimer's disease is permanent. Thus

persistence of the syndrome appears to depend on extensive cerebral lesions.

Corsellis: In Klüver and Bucy's original cases the syndrome faded only partially (Klüver, 1958).

Sourander: In those cases not only the amygdala and hippocampus but also large areas of the temporal neocortex were removed. A Klüver-Bucy syndrome can also be produced when the bilateral lesions are restricted to the temporal neocortex (Akert *et al.*, 1961), after which a gradual return to normality also occurs.

van Bogaert: Is epilepsy always seen when lesions in the hippocampal region are very severe?

Corsellis: Convulsive attacks had occurred in about a third of the cases of Alzheimer's disease that I examined. I found no correlation between the presence or the severity of hippocampal lesions of this kind and the occurrence of epileptic attacks.

van Bogaert: It seems difficult to differentiate the lesions related to the disease and those related to the epileptic fits.

Corsellis: Possibly the explanation lies in the different pathogenesis and the time of life at which the disease develops. The damage in the epileptic patients is thought to be hypoxic in origin and probably occurs early in life. In Alzheimer's disease the lesion is degenerative and is clearly occurring at the other end of life.

Sourander: The question of seizures is important. As I mentioned, we recognized two types of seizures, *grand mal* and minor seizures, frequently of the masticatory type. The latter usually occur rather early in the course of the disease and may be related to changes in the amygdaloid body.

Dayan: Does the incidence of tangles parallel that of plaques in these regions?

Corsellis: Broadly yes, although there are differences within the various hippocampal fields. Morel and Wildi (1955) demonstrated these.

Dayan: My impression is that in normal people of the same age as patients with Alzheimer's disease one does not see this accentuation of damage in the limbic area.

Tomlinson: In a group of non-demented patients dying in an acute general hospital, plaques may be found in the hippocampal complex a decade earlier than elsewhere in the neocortex. In those dying in old age without mental deterioration one does not see severe damage of the type seen in senile dementia of Alzheimer type (Tomlinson, Blessed and Roth, 1968).

Dayan: Plaques and presumably tangles have not often been reported from regions of the brain other than the cortex, and I think this is largely because other areas have not been examined. The problem of possible endocrine consequences of Alzheimer's disease was also raised. There are good accounts of neurofibrillary degeneration in hypothalamic neurons, (Hirano and Zimmerman, 1962).

Corsellis: Ishii (1966) recently studied the distribution of neurofibrillary degeneration (tangles) in the brain stem.

Polak: Is there any relationship in your cases between the lesions and any damage to the vessels? Have you seen fibrillary degeneration in the astrocytes?

Corsellis: I have not found any constant relationship between vascular change and these senile changes. Many people have realized how well preserved both large and small vessels seem to be in Alzheimer's disease, although obviously exceptions occur. I have not so far recognized degeneration in astrocytes.

Pratt: The amnestic syndrome after bilateral temporal lobectomy is not accompanied by dementia. The short-term memory store is normal but there is a failure of long-term memory (Milner, 1966).

Barondes: People aged 70 years or more almost invariably seem to have some difficulty with recent memory. Are there any pathological correlates of this? Does one invariably find some atrophy or tangles in the temporal region? In other words, is some pathological condition which resembles Alzheimer's disease an invariable accompaniment of age?

Corsellis: Tangles and some degree of plaque formation in the hippocampal region are common in what would be called the normal elderly population (Tomlinson, Blessed and Roth, 1968).

Roth: Kral (1962) says there is a benign form of forgetfulness in elderly subjects which is not followed by progressive intellectual deterioration or premature death. Yet in a random population sample in Newcastle (Kay *et al.*, 1968) memory impairment in elderly people not suffering from definite psychiatric disorder was correlated with a higher than normal mortality rate. These old people were not judged to be demented. However, one certainly sees stationary amnestic syndromes in old age occasionally and comparison of the pathological changes in these and in more malignant cases would be very interesting.

Hughes: Dr Corsellis's view is attractive, because clinically Alzheimer's disease begins with amnesia and for a long time remains so. Many things seen in the Klüver-Bucy syndrome perhaps could be stated in terms of para-amnesic phenomena. To me Alzheimer's disease is amnesia

para-amnesia; it is forgetfulness always, and for that reason the idea of a limbic syndrome is attractive.

Corsellis: The role of the limbic system in other dementias is perhaps also of interest. In Huntington's chorea my impression is that the limbic areas are not particularly affected. The hippocampus usually looks remarkably intact to me. Incidentally, I believe that the memory disorder is said to be less marked in Huntington's chorea than in other forms of presenile dementia.

Hughes: I cannot understand the low incidence of Pick's disease in this country as compared with Scandinavia.

In our experience, in 70 to 80 per cent of cases of Alzheimer's disease the presenting symptom is described as a stroke by the general practitioner or the patient's relatives. It therefore seldom comes up for consideration by the neurologist except as a case of cerebrovascular disease.

Terry: What do you mean by a stroke?

Hughes: I mean something which is described by either a relative or general practitioner as such.

Roth: Professor Jacob, can Pick's disease and Alzheimer's disease be clearly distinguished clinically?

Jacob: The main symptom in the initial stage of Pick's disease is loss of activity, while in Alzheimer's it is the amnesia, as it is also in Creutzfeldt-Jakob disease. But the hippocampal region in Alzheimer's disease is often damaged, while in Creutzfeldt-Jakob disease it is not.

Friede: The hippocampus may be involved in Creutzfeldt-Jakob disease (Friede and DeJong, 1964).

McMenemey: We had the case of a 61-year-old woman with a four-month history of the spongiform encephalopathy type of the Creutzfeldt-Jakob group to which I have referred briefly (McMenemey, 1968). There were plenty of tangles in the hippocampus—more, we thought, than could be accounted for by reason of age. Dr Hughes once showed me patients in Bristol who had what he called senile amnesia, with plenty of amnesia but no real dementia. In a brain from one of those cases we found marked changes in the hippocampus, especially in the stratum glomerulosum, but very little else; tangles were more in evidence than plaques.

The point about the amygdaloid nucleus being more involved mesially than laterally is very interesting, Dr Corsellis. Do the two halves have a different blood supply, or is there some question of a different chemical constitution of the neurons as, for instance, in certain parts of the pyramidal cell layers of the hippocampus where the zinc content is allegedly higher than in adjacent parts of that layer?

Corsellis: Not that I know of. The amygdaloid nucleus is mostly supplied by the anterior choroidal artery whereas the contribution from the posterior cerebral artery and its branches comes in further back.

Tomlinson: In a group of demented old people of Alzheimer type our findings (Tomlinson, Blessed and Roth, 1970) are very similar to those Dr Corsellis has described. At autopsy if one sees in a demented old person obvious atrophy of the hippocampus and hippocampal gyrus, often with neighbouring temporal lobe atrophy, one can predict with great accuracy what the histological appearances are going to be. The hippocampal pyramidal neurons in Alzheimer's disease in presenile or senile age groups are often greatly reduced in number, as Dr Corsellis has said. It is worth remembering that hippocampal pyramidal neurons are particularly subject to granulovacuolar degeneration and that this change almost never occurs outside the hippocampus. Far more pyramidal neurons are involved in this change in Alzheimer-type disease than in any other form of cerebral disease.

Sourander: All the cases we considered as Alzheimer-like senile dementias were characterized by the same type of atrophy of the temporal limbic structures as is seen in cases of Alzheimer's disease proper.

Barondes: It is said that we are all continuously losing neurons. Is this true? Is the temporal region particularly involved in this presumed decay?

Dayan: Conflicting evidence has been presented about this, but in general, in areas where nerve cells are particularly clearly displayed, such as the cerebellar cortex in normal man and in normal animals (normal as far as anybody can tell), there does seem to be a fall-out of neurons with age (Harms, 1924; Inukai, 1928; Müller, 1939). More general observations about its extent and localization in the cerebral cortex have been published by Balthasar (1954) and Brody (1955).

Corsellis: Brody (1955) showed a progressive loss of neurons from the cerebral cortex. He used one or two brains from most decades and counted the neurons in several different cortical areas, including the first temporal gyrus.

Barondes: Was the drop especially in the temporal region?

Corsellis: He found a general tendency for the counts to dip but that it was greatest in the superior temporal gyrus. He did not study the hippocampal or limbic cortex.

Roth: We have devoted a good deal of attention to the Klüver-Bucy syndrome and lesions in the limbic system. It is salutary to remember that in recent years a wide range of psychiatric disorders have been described in association with temporal lobe or limbic lesions, or EEG abnormalities

in these areas. They include the schizophreniform disorders of chronic epileptics, aggressive behaviour in psychopaths, disorders with prominent depersonalization, and disturbances of sexual adaptation including fetishism and impotence as well as dysmnesic syndromes.

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ALZHEIMER'S DISEASE AND RELATED CONDITIONS
G. E. W. WOLSTENHOLME & MAEVE O'CONNOR

CIRCUMSCRIBED CEREBRAL ATROPHY IN ALZHEIMER'S DISEASE: A PATHOLOGICAL STUDY

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ALZHEIMER'S disease is a particular type of presenile dementia characterized histopathologically by the presence of numerous argyrophilic plaques and by neurofibrillary degeneration. Atrophy of the brain visible to the naked eye is also not uncommon, although this may be so obscured by terminal circulatory and/or metabolic disturbances that the cortical atrophy and ventricular dilation easily demonstrable by pneumoencephalography before the patient's death cannot be found when the brain is sectioned.

The clinical appearance and the course of the disease are fairly typical, although not pathognomonic: the same macroscopic and microscopic changes occur in other fairly well delineated patterns of illness, leading to uncertainty in their classification. Nevertheless, among patients who died at an advanced age in the National Neuropsychiatric Institute in Budapest, the clinical diagnosis of Alzheimer's disease was verified pathologically in 60 per cent of cases so diagnosed, whereas the parenchymal or "pure form" of senile dementia (Delay and Brion, 1962) was verified pathologically in only 10 to 15 per cent of cases so diagnosed. The clinical diagnosis is based on the history of aphasia, apraxia, agnosia and changes in activity.

The disease is not rare in Hungarian mental hospitals. In a series of 1248 consecutive post-mortem examinations in the five-year period 1955 to 1959, 2 per cent, i.e. 4·4 per cent of those dying at the age of 65 years or over (Tariska, 1967) were cases of Alzheimer's disease. The age distribution of 84 subsequent cases of Alzheimer's disease is given in Table I. Approximately 75 per cent of these died in the presenium, and the remainder in the senium. In this latter group secondary psychiatric symptoms due to circulatory, respiratory, renal, urological or metabolic complications tended to divert the psychiatrists' attention from the possibility of Alzheimer's disease. The younger the patient the greater likelihood there was of a correct diagnosis being made.

TABLE I

AGE DISTRIBUTION OF HISTOPATHOLOGICALLY VERIFIED CASES OF ALZHEIMER'S DISEASE AT THE TIME OF DEATH

Age	*Female*	*Male*	*Total*	
45–49	1	—	1	
50–54	—	3	3	
55–59	5	8	13	62
60–64	9	12	21	
65–69	10	14	24	
70–74	5	3	8	
75–79	5	3	8	22
80–84	2	2	4	
85–89	1	1	2	
Total	38	46	84	

The present study concerns the seven cases in the series of 84 which showed varying amounts of circumscribed cerebral atrophy. Frozen and paraffin-embedded sections of formalin-fixed material were stained by numerous methods, including Bielschowsky's and King's silver impregnations and counterstaining with Sudan III.

Circumscribed cerebral atrophy in Alzheimer's disease has been reviewed by McMenemey (1963), and reported and discussed by Berlin (1949), van Mansvelt (1954), Seitelberger and Jellinger (1958), Delay and Brion (1962) and Tariska (1965).

Delay and Brion (1962) distinguished three groups, as follows: (1) diffuse atrophic lesions with moderate focal accentuation in either the parieto-occipital or fronto-temporal regions; (2) focal lesions in the posterior parts of the brain; (3) pure frontal naked-eye atrophy with diffuse histological changes throughout the cerebral cortex and in the thalamus. They concluded that circumscribed atrophy is really only an accentuation of the general atrophy which occurs most frequently in the parieto-occipital or temporal regions.

Our seven cases (Table II) can be ranged into four groups according to the topography of the macroscopic atrophy, and the neuronal devastation, glial reactions and argyrophilic lesions.

In the first and second groups, the diffuse atrophy is accentuated in the frontal, parieto-occipital and temporal lobes, and it resembles Pick's disease in that (1) the cortical areas of projection tend to be spared; (2) there is asymmetrical cortical atrophy in two of the four cases; and (3) the areas showing most atrophy are of walnut colour and "knife-blade" character. Three of the four brains were of very low weight, as were those in the other groups; Case 2 was not weighed. The frontal and parietal lobes were most severely affected, especially the precuneus, where the walnut colour

TABLE II

SOME DETAILS OF SEVEN BORDERLINE CASES OF ALZHEIMER'S DISEASE WITH CIRCUMSCRIBED CEREBRAL ATROPHY

Case no.	*Sex*	*Age at death (years)*	*Duration of symptoms (years)*	*Brain weight (g)*
1	F	58	6	900
2	F	70	6–7	—
3	M	64	8	1050
4	F	79	3	1150
5	M	68	6	1250
6	F	62	5	1090
7	F	68	1	880

and knife-blade convolutions occurred in two instances. The polar and basal parts of the frontal lobes were markedly affected in all cases. However, the operculum and the perisylvian fronto-temporal convolutions were never so heavily affected as in Pick's disease.

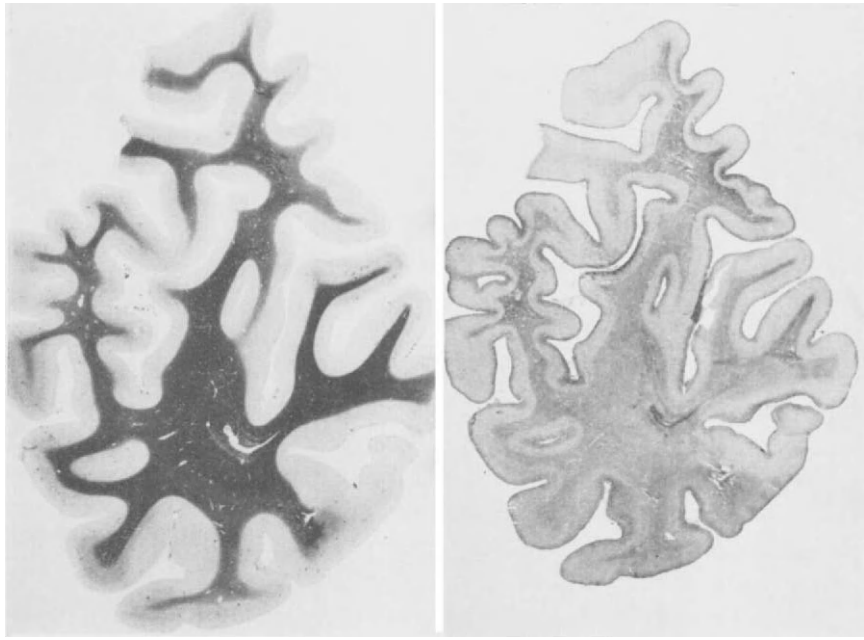
The reduction of the cerebral white matter and the dilation of ventricles were proportionate to the degree of cortical atrophy, while the basal ganglia were approximately normal in size. Slight atheromatosis of the basal vessels and general atherosclerosis were also present with renal (Case 2) or cardiac (Case 3) consequences, but were not relevant in Cases 1 and 4. Non-atherosclerotic cerebrovascular disturbances were found only in Case 1, where they consisted of miliary infiltrative foci associated with spongy demyelination, resembling the picture seen in rheumatic encephalitis. The subdural haematoma in Case 3 was due to a head injury three weeks before death.

Two main differences between the first and second groups could be detected by microscopic examination. Firstly, the convolutions which had appeared normal to the naked eye were less affected than the atrophied areas. This difference was scarcely perceptible in Cases 1 and 2, but was more marked in Cases 3 and 4. Little or no neurofibrillary degeneration was observed in areas 1, 4, 17 and 42, while the argyrophilic plaques tended to be confined to certain lamellae (e.g. IVc in striate cortex). No essential difference was observed in the brain stem, where many argyrophilic plaques were seen in the caudate nucleus and putamen and fewer in the thalamus. Neurofibrillary degeneration was plentiful in the thalamus, periventricular and hypothalamic nuclei, mamillary body and tegmental and tectal nuclei of the pons and mesencephalon.

Secondly, the glial reaction was moderate as usual in the first group but was exceptionally strong in the second group and accompanied by pallor in the convolutional white matter.

In Case 3 the Woelcke preparation shows that the precuneus, the angular

gyrus and the subjacent convolutional white matter are pale, with excessive gliosis which is not confined to the areas with myelin damage but extends in varying degree to the entire occipital lobe (Fig. 1). Three zones could be distinguished at higher magnification in preparations stained for fibroglia: plaque-like figures in the superficial layers, isomorphic gliosis in the white matter, and astrocytosis in the deep cortical layers (Fig. 2).



(*a*) (*b*)

Fig. 1. Case 3. (*a*) Myelin pallor and dilatation of perivascular spaces in angular gyrus and precuneus. Heidenhain-Woelcke × 2·6. (*b*) Fibre gliosis at the same level in the cortex and white matter, out of proportion to the myelin pallor. Kanzler × 2.6.

Similar excessive gliosis, gliotic plaques and a slight myelin pallor were also found in the temporal lobe and to a lesser extent in the middle frontal convolution, in the centro-medial nucleus of the thalamus and in the periventricular zone.

In Case 4 cortical and subcortical gliosis, plaque-like in appearance, was also seen in the second and third temporal convolutions and in the uncus, but it was diffuse and isomorphic in the subjacent white matter; it was very dense in the white matter of the hippocampus, the subpallidal tracts and the external capsule, but less so in the medullated laminae of the globus pallidus and the periventricular thalamic zone (Fig. 3). The mesencephalon,

the posterior lentiform nucleus, pulvinar, periaqueductal grey matter, substantia nigra and basal part of the pons in the midline also showed severe gliosis. (Fig. 4).

The lamination and polarity of the cortical nerve cells were disturbed because of the partial loss of the neurons, neurofibrillary degeneration of

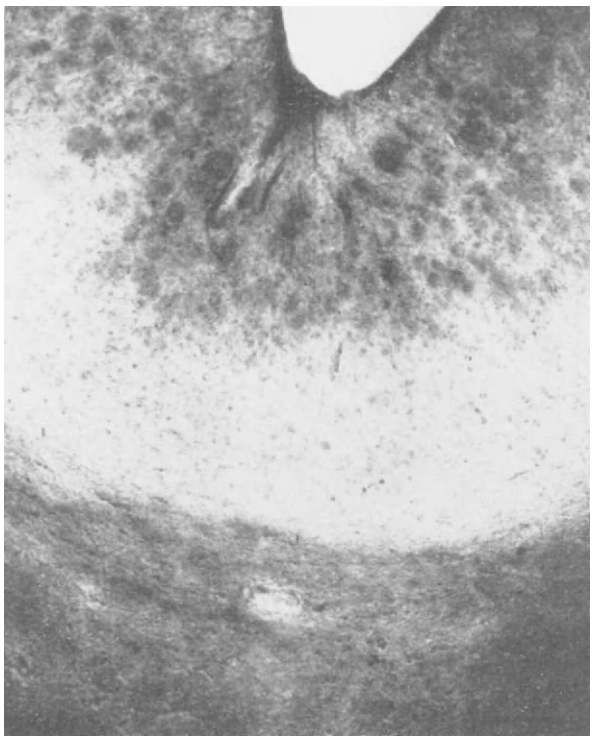


Fig. 2. Case 3. Fibre gliosis in the angular gyrus: plaque-like organization in cortex, isomorphic in white matter. Kanzler × 15.

those still visible and the presence of argyrophilic plaques. The astrocytosis and fibrillary gliosis in the brain stem were out of proportion to the demyelination and nerve cell changes (neurofibrillary degeneration in the thalamus and mesencephalic and pontine tegmentum), and were also pronounced in the medulla and cerebellum where no argyrophilic changes were present.

The third group is characterized (1) by the clear-cut margin of circumscribed atrophy seen in both macroscopic and microscopic examination, and (2) by the neurofibrillary degeneration which was confined strictly to the atrophied areas and was marked in Case 5 but less so in Case 6.

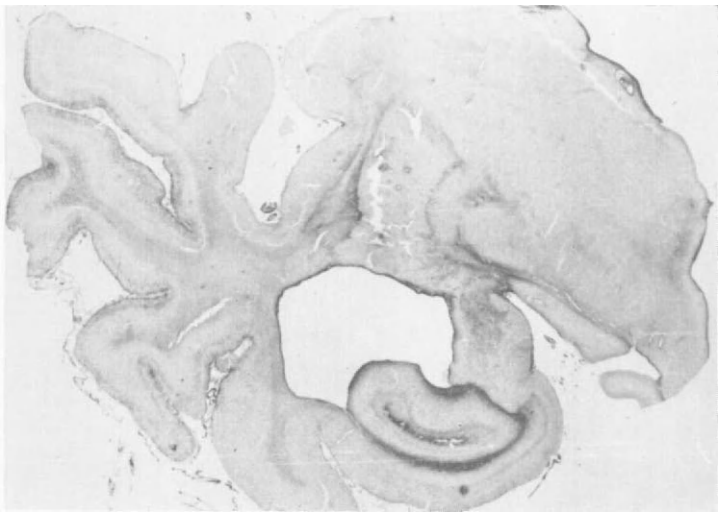


FIG. 3. Case 4. Fibre gliosis (see text). Kanzler × 2·5.

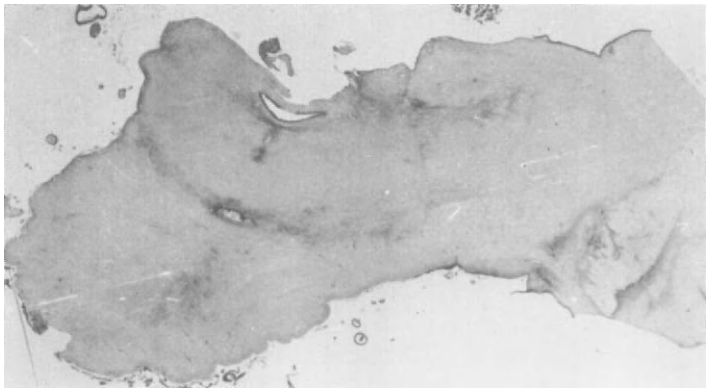


FIG. 4. Case 4. Fibre gliosis (see text). Kanzler × 2·5.

Case 5 (Tariska, 1965) showed asymmetrical atrophy which was severe on the frontal and anterior insular convolutions, moderate on the temporal second and third convolutions and slight on the supramarginal gyrus. The walnut colour and knife-blade character of the superior frontal gyrus and severe atrophy of the operculum were also similar to what is seen in Pick's disease (Fig. 5).

Pathological examination confirmed that the atrophy was circumscribed. The most severely affected areas were spongy in appearance, due to the pronounced loss of neurons in the superficial layers and the compensatory glial network, while in lamina IIIc and V nearly all the remaining nerve

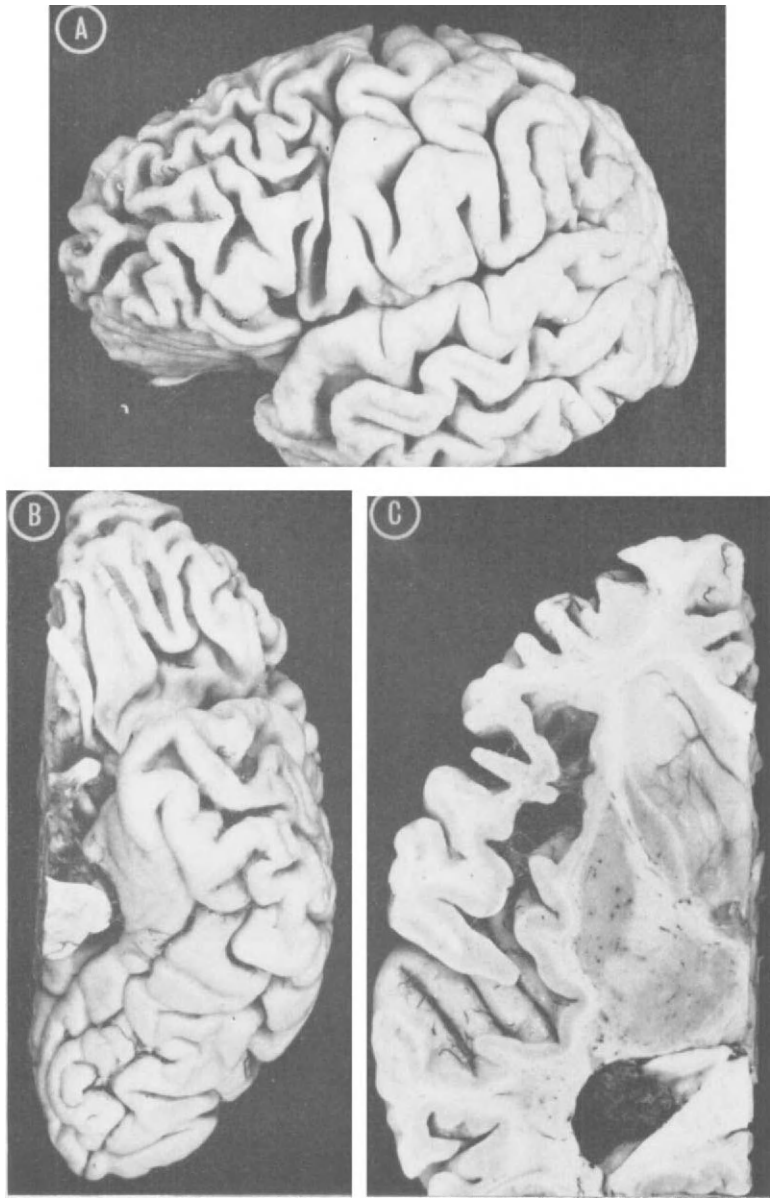
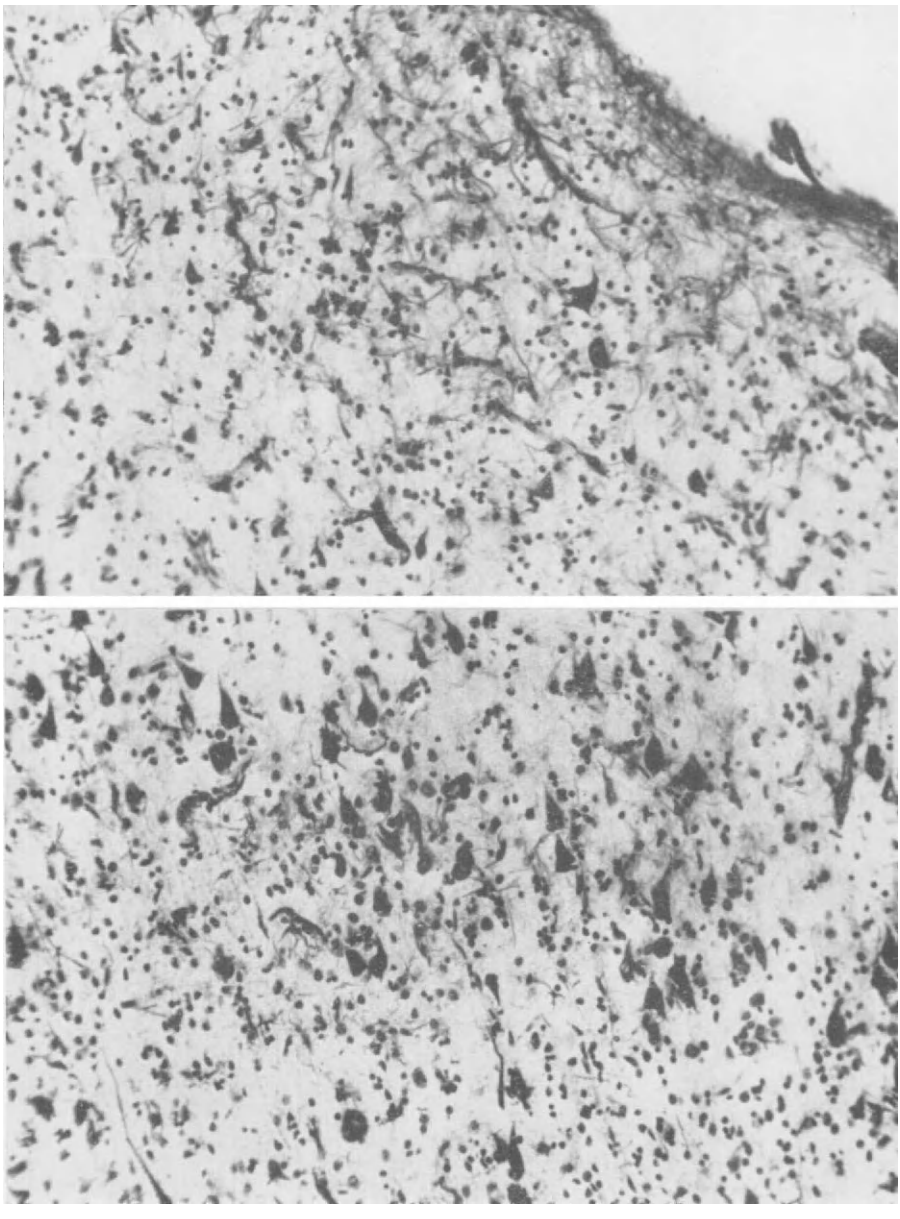


FIG. 5*a*, *b*, *c*. Case 5. See text.



(*a*)

(*b*)

FIG. 6. Case 5. (*a*) Frontal cortex. Dense fibre gliosis and neurofibrillary degeneration. King × 100. (*b*) Neurofibrillary degeneration with a laminar appearance and astrocytosis. King × 100.

cells displayed neurofibrillary degeneration (Fig. 6). Inflated nerve cells, argyrophilic globules and plaques were lacking. Diffuse but slight myelin pallor was seen in the cerebral white matter in proportion to the cortical damage and was associated with relatively slight gliosis. The basal ganglia showed accumulation of lipofuscin in the nerve cells and gliosis. A few nerve cells of the ventral and medial thalamic nuclei and those of the posterior hypothalamic nuclei (including the mamillary body) displayed neurofibrillary degeneration. The left cerebral hemisphere was more severely

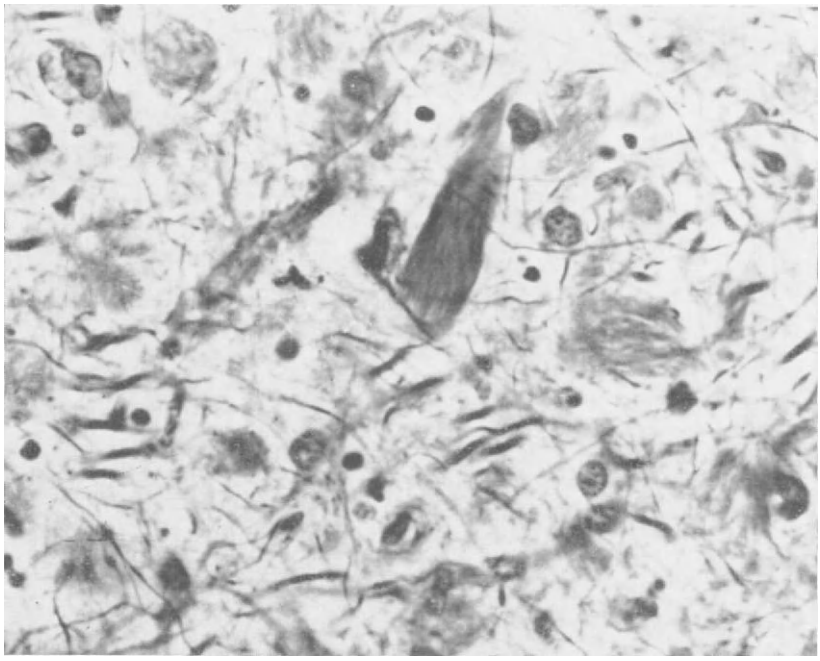


FIG. 7. Case 6. Amygdaloid nucleus. All nerve cells display fine neurofibrillary tangles. Bielschowsky × 400.

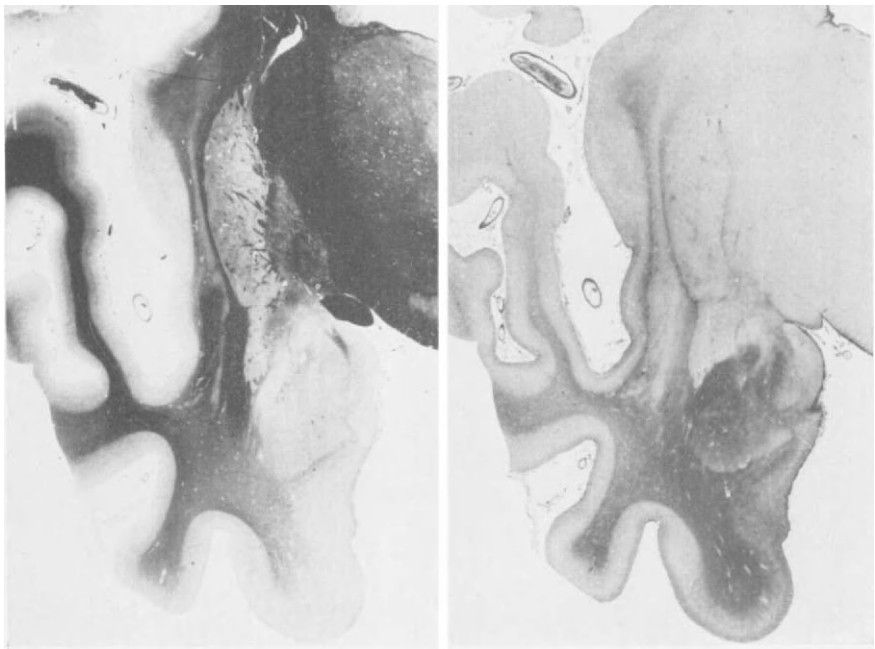
affected than the right; lesions in the hippocampus, i.e. neurofibrillary and granulovacuolar degeneration with astrocytosis, were observed only in the left subiculum and Sommer's sector.

Case 6 showed symmetrical atrophy which was predominantly temporal and insular; atrophy of the frontal lobes was less pronounced, with a clear-cut margin accentuated at the poles. Small fresh and repaired ischaemic foci were present in the centrum semiovale, but the basal ganglia and the internal capsule appeared normal.

The temporo-insular cortex was reduced in thickness by extensive nerve

cell loss with a resulting spongiform fibre gliosis, especially in the superficial lamellae, while the remaining nerve cells displayed neurofibrillary degeneration. The same was observed in the frontal cortex to a lesser degree. In the subiculum, hippocampus, amygdala and temporal neocortex there was a conspicuous excess of fine tangles but few discernible nuclei (Fig. 7). Boutons and granulovacuolar degeneration were also observed.

Extensive but focal pallor of myelin was present, moderate in degree in



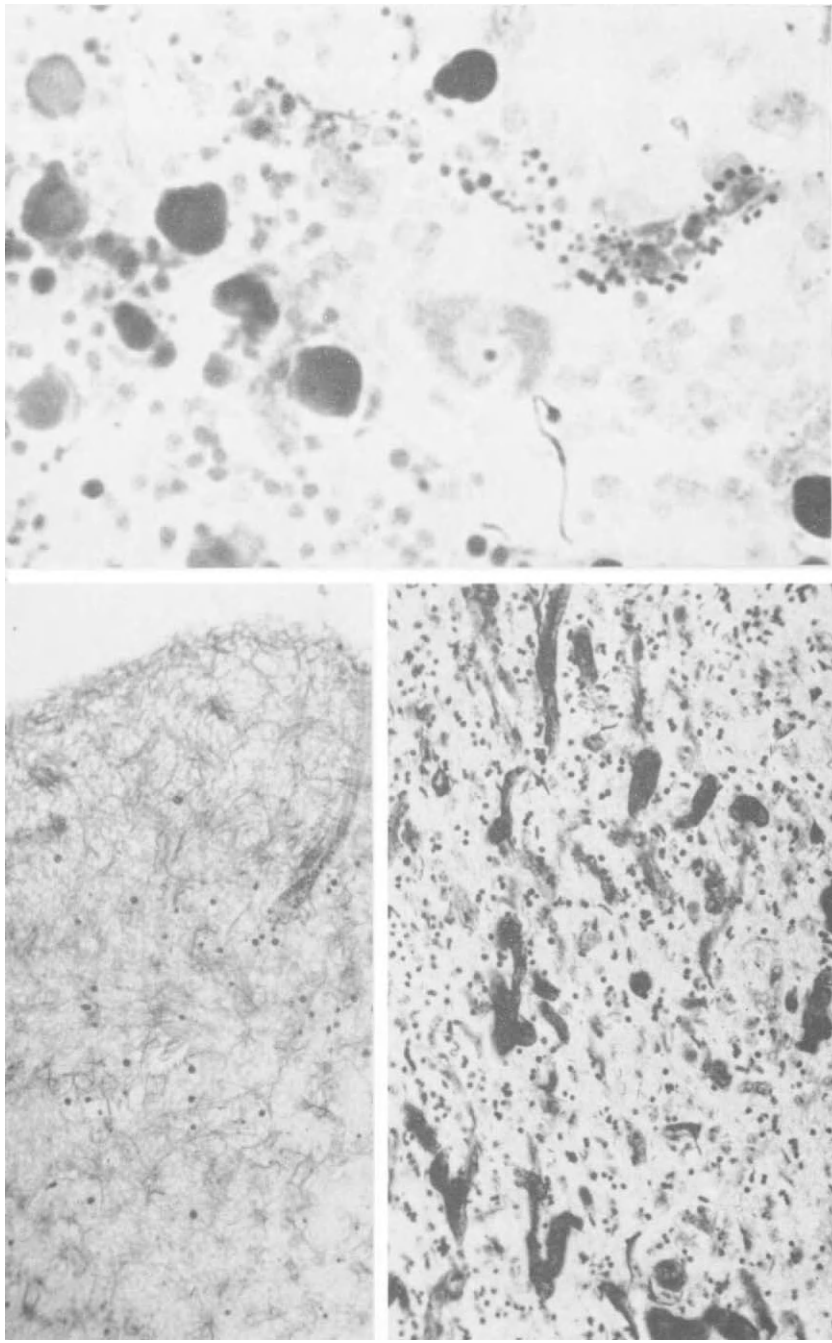
(*a*) (*b*)

FIG. 8. Case 6. (*a*) Myelin pallor in the uncus and temporal white matter and to a lesser extent in the brain stem. Heidenhain-Woelcke × 7. (*b*) Fibre gliosis out of proportion to the degree of demyelination. Kanzler × 7.

the anterior and middle third of the temporo-hippocampal region, in the external capsule and capsula extrema and to a lesser extent in the globus pallidus; in these areas there was also overwhelming and dense fibrillary gliosis (Fig. 8).

In other cortical areas only a few nerve cells, especially in laminae IIIc and V, displayed neurofibrillary degeneration.

In addition, hypertrophic arteriosclerosis was observed in the pial vessels, while Fahr's calcinosis was seen in abundance, deep-seated in the brain stem nuclei and cerebellum (Fig. 9*a*), at the cerebral corticomedullary junction,



(*a*)

(*b*)

(*c*)

FIG. 9. Case 6. (*a*) Glio-capillary calcinosis and globular deposits in the cerebellum. Luxol fast blue B and cresyl violet × 400. (*b*) Fibre gliosis in the temporal cortex. The small black spots represent calcium deposits. Kanzler × 80. (*c*) Increased vascularity with argyrophilic deposits in the vessel walls in the convolutional white matter subjacent to the atrophic temporal pole. King-Sudan III × 80.

and to a lesser extent in the cerebral white matter. There were small ischaemic necroses in the centrum semiovale, sometimes undergoing repair, but in the occipital white matter they were recent, fibrillary gliosis in their vicinity being slight or lacking.

The vascular changes and their consequences were well separated in space from the atrophic and argyrophilic lesions; thus in the temporal lobe only small deposits of calcium were scattered in the cortex, although in the white matter there were angioma-like vessels with deposits in their thickened walls (Fig. 9*b*, *c*).

The one case assigned to the fourth group showed frontal and temporal

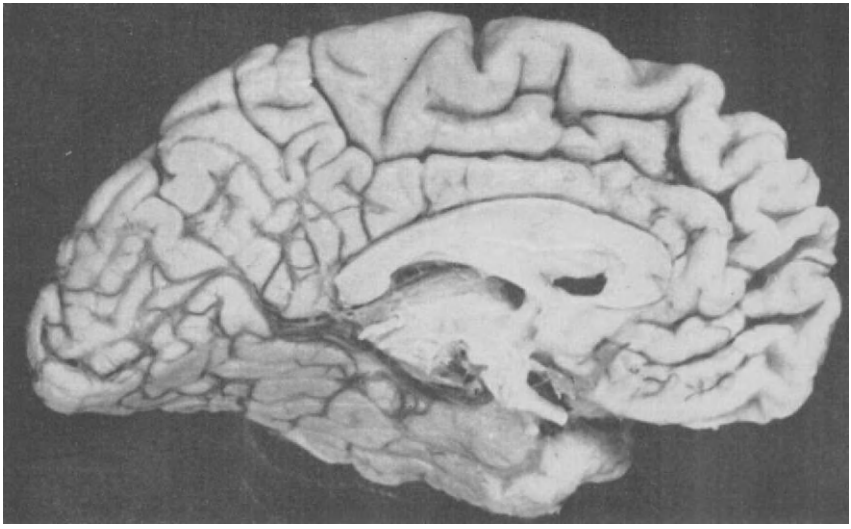


FIG. 10. Case 7. Diffuse cortical atrophy accentuated in the frontal pole.

polar atrophy, a little granular in appearance (Fig. 10) and with an indistinct margin; the atrophy was more marked on the left than on the right side. Microscopically, the normal lamination of the cortex was obscured by an overgrowth and pigmentation of the astrocytes with spongy transformation of the neuropil consequent upon degeneration and loss of neurons. The microscopic lesions were accentuated in the frontal, temporal and occipital lobes, less so in the parietal and central gyri. They were marked also in the caudate nucleus (Fig. 11) and especially in the putamen, but less so in the thalamus. The left side was more severely affected than the right in the temporal, parietal and occipital lobes, in the caudate and even in the

(*a*)

(*b*)

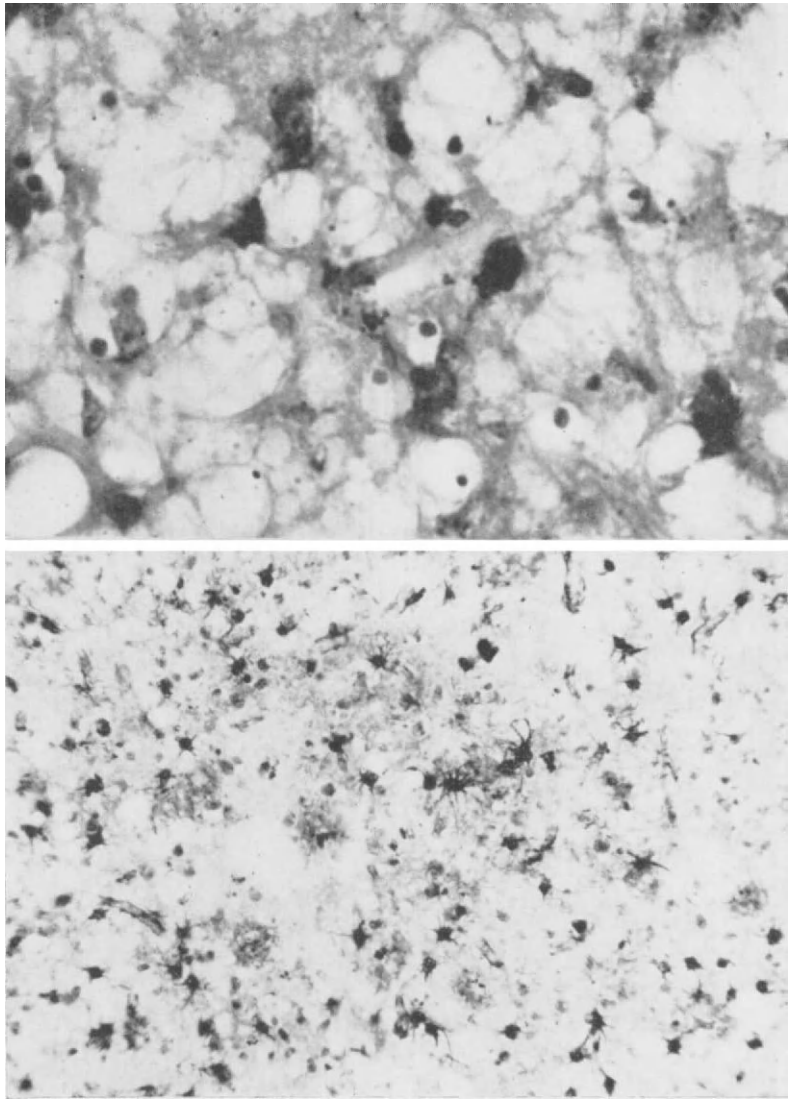
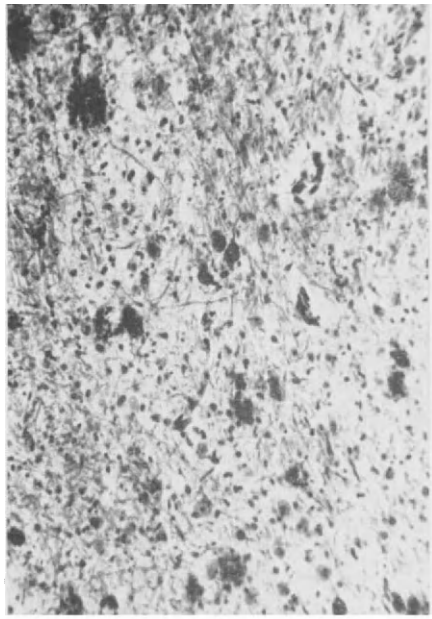
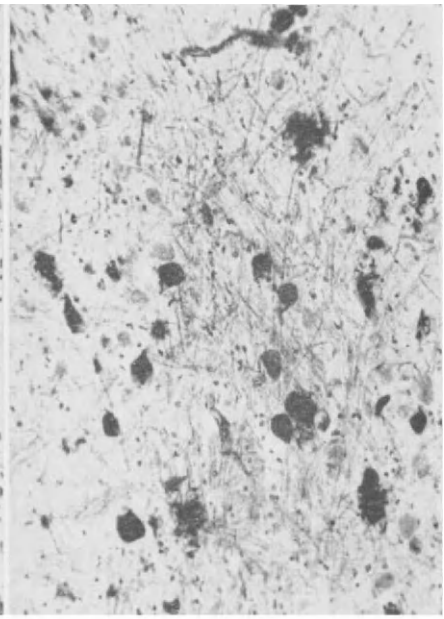


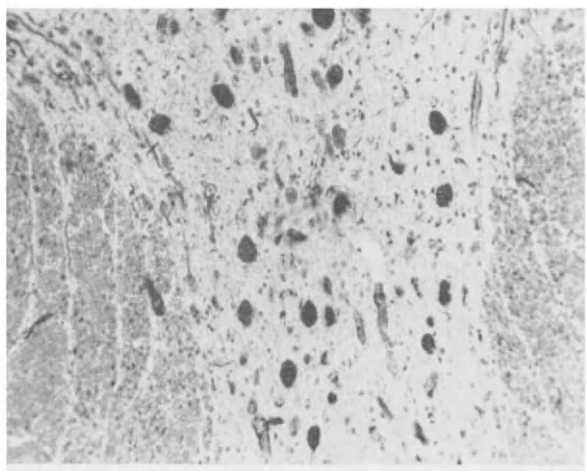
FIG. 11. Case 7. (*a*) Putamen. Bullous and macrospongious form of the spongy degeneration. Luxol fast blue B and cresyl violet × 400. (*b*) Plaques and proliferation of astrocytes in the severely spongy temporal cortex. Cajal-Globus × 400.

(*a*)



(*b*)





(*c*)

FIG. 12. Case 4. (*a*) Small plaques and neurofibrillary tangles in the hypothalamus. King-Sudan III × 300. (*b*) Case 4. Small plaques, neurofibrillary tangles and lipofuscin accumulation in the nerve cells of the pontine tegmentum. King-Sudan III × 300. (*c*) Case 2. Neurofibrillary tangles in the mesencephalic nucleus centralis superior. King-Sudan III × 300.

hippocampus, although lesions of the frontal cortex were equal in severity on both sides.

With silver impregnation, the cerebral cortex revealed moderate numbers of plaques but fewer neurofibrillary changes scattered in different areas, especially in the temporal neocortex. In addition, neurofibrillary changes were found in the mamillary bodies, basal nucleus, prerubral field and periventricular thalamic nuclei (Fig. 12).

DISCUSSION

According to the clinical symptoms, course and duration of the illness, Cases 1, 2, 3 and 4 could be regarded as Alzheimer's disease without reservation, although symptoms appeared suddenly in Case 4, and were seemingly related to a cardiac infarct in Case 3. Case 5 was at first diagnosed as Pick's disease because of reduced spontaneity, lack of insight and changes in moral personality, but later the diagnosis was modified to Alzheimer's disease because of the type of speech deterioration. In Case 6 hypertensive vascular disease was correctly diagnosed.

In Case 7 the rapid course, the symptoms and a single EEG examination did not permit a firm diagnosis. The bilateral delta and theta activity, especially on the temporal tracing, was interpreted (T. Nagy) in favour of Alzheimer's disease, and the abnormalities characteristic of subacute spongiform encephalopathy were not seen. This patient showed an illness with an easily recognizable biphasic course similar to that described by Colle and Miranda-Nives (1967). The spongiform encephalopathy had a prominent cortico-striatal topography, but the argyrophilic changes, although moderate in numbers, were more widely distributed. Presenile psychosis of Jakob type accompanying a few neurofibrillary changes (Jervis, Hurdum and O'Neill, 1942), and polioencephalopathy accompanying a few plaques (Alema and Bignami, 1959), were interpreted as accidental superimposition of two illnesses by Garcin, Brion and Khochneviss (1963). The cortical, striatal and thalamic localization of the argyrophilic changes suggests that in Case 7 Alzheimer's disease might have developed if the patient had lived longer. Nagy (1968), in a study of patients with dementia in advanced age, found the EEG to be of great value in the differential diagnosis of Alzheimer's disease.

I believe that progressive dementia in all cases is due to the loss of neurons and the argyrophilic changes. Congophilic and hyalinous angiopathy in Case 1 and the hypertrophic arteriosclerosis with Fahr's calcinosis in Case 6 are also important in the pathogenesis.

The morphological changes in Cases 1 and 2 could be regarded as typical Alzheimer's disease because of the ubiquity and uniformity of the atrophic and argyrophilic (congophilic) lesions with typical glial reactions throughout the cerebral cortex, even though the changes are intensified in the area showing most atrophy. They are similar to the cases described by Divry (1939), Rothschild and Kasanin (1936), and Divry, Ley and Titeca (1935).

Cases 3 and 4 differ from Cases 1 and 2 by reason of the excessive cortical and subcortical overgrowth of the astrocytes and demyelination. This may have been induced by hypoproteinaemia and hypergammaglobulinaemia in Case 3, and by cold agglutination due to the 17S macroglobulin in Case 4. Both of these "dyscrasias" may induce disturbances in the function of the blood-brain barrier and astrocytes. The topography of the changes resembles that reported by Liebers (1933). The excessive gliosis, which is out of proportion to the underlying demyelination, could be compared with Pick's disease type II (Neumann, 1949), progressive subcortical gliosis (Neumann and Cohn, 1967) or presenile glial dystrophy (Seitelberger, 1968). In this rare form of presenile dementia, loss of the nerve cells in the cortex is not prominent, the neurons are shrunken rather than swollen and there are no argyrophilic inclusions, but the subcortical gliosis is prominent. In Cases 3 and 4 the glial hyperplasia and proliferation is cellular and fibrillar in a plaque-like organization in the cortex, amygdaloid nucleus, thalamus and hypothalamus, but fibrillar and isomorphic in character in the convolutional and intralaminar white matter. I suggest these two cases be designated the *gliotic type of Alzheimer's disease*. Apart from the cerebral changes they show many plaques and much neurofibrillary degeneration in the mesencephalic and pontine nuclei (periaqueductal, raphe and tectum).

Cases 5 and 6 show neurofibrillary degeneration and neuronal loss replaced by gliosis, but no plaques. Schnitzler (1911), Weimann (1921) Goodman (1953) and Raskin and Ehrenberg (1956) described similar cases. McMenemey (1963) considered these as "unusual cases which are seemingly related to Alzheimer's disease". Neurofibrillary changes in the absence of argyrophilic plaques have been described in boxers (Grahmann and Ule, 1957; Ferguson and Mawdsley, 1965). Case 6 with calcium deposits in many places resembles Weimann's case, which was probably the first description of Fahr's disease combined with Alzheimer's disease, although Weimann stressed that "Alzheimer's neurofibrillary changes could only be seen in a few examples of the upper cortical layers", while in Case 6 the changes in the temporo-hippocampal region were profuse.

In addition, cortical and subcortical demyelination and dense fibre gliosis were seen in the same region.

The pathogenesis of focal necroses in the cerebral white matter could easily be attributed to hypertensive disease with hypotensive attacks and Fahr's calcinosis. The neurofibrillary degeneration and atrophic sclerosis in the temporal lobe may be independent of vascular changes, because they are well separated and different in character.

Case 5 is a pure case of circumscribed cerebral atrophy due to nerve cell loss, gliosis and extensive neurofibrillary degeneration, without plaques, which I commented on in 1965 as superimposed Pick's and Alzheimer's disease. It differs greatly from Berlin's (1949) cases in which both plaques and neurofibrillary degeneration were scattered over the entire cerebral cortex, while the inflated cells of Pick's type confined exactly to the focally atrophied areas were also accompanied by demyelination and fibrillary gliosis in the subjacent white matter.

McMenemey (1963) regarded the cases published by Berlin (1949), Liebers (1933, 1939) and Moyano (1932) as examples of Pick's and Alzheimer's double disease. Delay and Brion (1962), on the other hand, accepted only Berlin's cases as "formes mixtes". Lüers and Spatz (1957) also believe in the possibility of double disease. Seitelberger and Jellinger (1958), re-emphasizing the frequent incongruity between the macroscopic appearance and microscopic changes (Liebers, 1933), the variations in the quantity, localization and extent of the changes, and the different histochemical properties of Pick's argyrophilic bodies and Alzheimer's neurofibrillary changes, argue for a strictly dualistic concept of Alzheimer's and Pick's disease. Van Mansvelt (1954) believes the topographical confinement of the lesions to be more important than their quality in the differential diagnosis of Pick's and Alzheimer's diseases. I consider that in the present state of our knowledge it is best to classify similar cases as double or superimposed diseases.

The changes in the brain stem deserve particular consideration, because the frequency of neurofibrillary degeneration in the midline nuclei of the thalamus, hypothalamus, basal nucleus and the mesencephalic and pontine tegmental nuclei does not markedly differ from case to case, although in the seven cases referred to here the number of neurofibrillary changes was less than in the cases reported by Hirano and Zimmerman (1962). It may be thought that this topographical distribution, added to the usual involvement of the hippocampal and temporal region, is responsible for the clinical syndrome. I agree, following Miskolczy (1934, 1938, 1959), that the frequency of neurofibrillary degeneration is a decisive factor in Alzheimer's dementia, together with the loss of nerve cells.

SUMMARY

Circumscribed cerebral atrophy and uneven distribution or restriction of the microscopic changes to certain areas were studied in seven out of 84 cases of Alzheimer's disease investigated during the last 15 years.

Unequivocal Alzheimer's disease was diagnosed when neuronal depletion and numerous argyrophilic changes (i.e. plaques, neurofibrillary and granulovacuolar degeneration) were observed throughout the cerebral cortex and the brain stem, although certain locations, especially the areas of cortical projection, tended to be spared.

The gliotic type of Alzheimer's disease is suggested as a designation for those instances where naked-eye atrophy resembles that in Pick's disease in its topography, while the abundance of plaques, neurofibrillary changes and nerve cell loss are characteristic of Alzheimer's disease. The excessive overgrowth of astrocytes in a plaque-like organization in the cerebral cortex and subjacent white matter, with relatively slight myelin damage, is compared with the progressive subcortical gliosis of Neumann and Cohn (1967) (Pick's disease type II). The overwhelming gliosis is regarded as an exogenous complication in the otherwise endogenous atrophying process of "Alzheimerization", and it is due to hypoproteinaemia or cold agglutination disease associated with the 17S macroglobulin.

Superimposed or double Pick's and Alzheimer's diseases are recognized in two instances in which neurofibrillary degeneration was found in the atrophic convolutions only. In one case complications due to Fahr's gliocapillary calcinosis were also observed, with ischaemic foci scattered in the white matter. The atrophic changes and the vascular consequences have a completely different topography.

Subacute spongiform encephalopathy accelerated the course of the illness in one case.

The argyrophilic changes in the limbic system and thalamic, hypothalamic and mesencephalic nuclei are thought to be responsible for the symptoms in Alzheimer's disease.

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DISCUSSION

Roth: I was interested in the high incidence of other physical illnesses such as myocardial infarction or nephrosclerosis in your cases, Dr Tariska. In presenile and senile dementias, the causes of death are often given in vague terms—death seemingly occurs not with a bang but a whimper—largely because extracerebral disease tends to be inconspicuous and those affected die for reasons closely linked to the cerebral degenerative process.

Corsellis: I think most patients with an organic dementia of a non-vascular type do tend to fade out, usually dying with a terminal bronchopneumonia. Only a minority have malignant disease or a precise cause of death.

Tariska: Certainly in that patient (Case 3) the cardiac infarct was quite coincidental.

Shelanski: This question of death in patients with cerebral diseases puzzles me. In physiology we were taught that if certain key areas in the brain stem still functioned the person would continue to function. If those areas are not much involved in these diseases, what is the mechanism by which these people die?

Terry: Doesn't fading out mean simply that they don't have any dramatic episode? They get pneumonia for the most part.

Roth: There is more at issue than an undramatic mode of death. The functional disorders of old age, that is the affective disorders and paraphrenias, have a relatively low mortality rate but a high prevalence of extracerebral physical disease, and, in the patients who die, these specific disorders generally prove to be the causes of death. In contrast, in patients with senile and arteriosclerotic dementias, who have a high mortality rate, causes of death are often described in vague terms such as "myocardial degeneration" or "senility" which are little more than confessions of ignorance (Kay and Roth, 1955; Kay, 1962). However, Dr Tariska's findings suggest that there may be some variation within the "organic" groups and perhaps the time has come for further systematic studies of extracerebral disease in the presenile, senile and arteriosclerotic psychoses. The role of the regulating centres of autonomic function in the hypothalamus has already begun to attract attention.

If senile and arteriosclerotic psychoses are taken together, the expectation of life is only one-quarter to one-fifth of that expected in the general population (Kay, 1962). There is something interesting here, possibly related to problems of ageing but not understood at the present time. Dr Tariska, were the various conditions such as heart disease, nephrosclerosis, hypertension and so on, the cause of death in your cases?

Tariska: Many patients died with several illnesses at the same time, but the clinical course of the psychiatric illness was in most cases that of Alzheimer's disease and not that of somatic disease, except for Case 6.

McMenemey: Alzheimer's disease has been described in conjunction with Simmonds' disease (Schob and Güntz, 1932). Some patients get extremely emaciated and one wonders if there is a relationship between this and the quantitative involvement of the neurons in the hypothalamic nuclei.

Hughes: I think a typical case of Alzheimer's disease would always show extreme emaciation if the patient didn't die first from intercurrent disease.

Sourander: Extremely rapid decrease in body weight apparently unrelated to cancer and to inadequate feeding, according to Dr Sjögren (to be published) is a common finding in the terminal stages of Alzheimer's

disease. As well as the changes in the hypothalamic nuclei shown by Morel and Wildi (1950) one should consider the relation between the limbic structures and the hypothalamic centres. If the limbic structures are destroyed this might affect the hypothalamic functions and nutritional state of the patients.

Tomlinson: If one wants to investigate wasting and its morphological counterparts, cases of Alzheimer's disease present the best opportunity. The body weight in many cases of Alzheimer's disease is as low as 30 kg by the time of death, and 26 to 30 kg is about the lowest that the ordinary adult coming to autopsy ever reaches.

Hughes: Could emaciation be due to amnesia? Unless these patients are fed they won't ask for food.

Roth: But there are Alzheimer cases that eat everything they can lay hands on.

Hughes: They certainly do that in the stage of locomotor activity. But in the final stages, with this complete emaciation, I always got the impression that they forgot.

Roth: The question remains unanswered as to whether the wasting is more frequent and pronounced than that occurring in chronic diseases outside the brain that prove fatal within a few years of onset.

Strich: The congophilic angiopathies with plaques that I shall describe (pp. 105–124) followed a catastrophic illness like a subarachnoid haemorrhage or head injury. Is the natural history of Alzheimer's disease a decline in steps, or is it a smooth decline? Are there precipitating factors?

Terry: Every disease has had this sort of precipitating factor indicated at times.

Strich: I don't mean precipitating factors only at the onset of the disease, but during the disease. Do they get worse every time they have a cold?

Roth: I have the impression that, in a minority of patients with cerebral degenerative disease, a rapidly downhill course follows ventriculography or air encephalography.

Hughes: After they had cortical biopsies, three patients of mine actually improved in behaviour. Whether it was a leucotomy effect or coincidence I don't know.

Terry: That has been our experience too, a couple of times.

Jacob: Sometimes the first injection (e.g. barbiturate) can be dangerous: occasionally we can observe the first delirious state in these circumstances.

Hughes: After vitamin injections some patients seem to have improved too.

McMenemey: Malamud and Lowenberg (1929) described two cases

with remissions of one and four years respectively. (The second was the famous case of the seven-year-old boy whose psychosis followed scarlet fever and proved fatal 17 years later.) Rothschild (1934) mentions a woman (case 3) whose mental state deteriorated when she was given radium therapy for carcinoma of the cervix but there was no progression of the psychosis in the last three years of life. More reports on arrested cases or cases with remissions would be welcome. Lowenberg and Rothschild's case 2 (1931) whose illness began in the last weeks of pregnancy had an eight-year remission during which a second child was born. The case could, I think, have been a toxic psychosis because there were no tangles but only plaques. This does raise the question, however, of what is the effect of pregnancy on Alzheimer's disease?

Roth: In three recently described cases, improvement appeared to follow the administration of cortisone (Chynoweth and Foley, 1969).

Dayan: What happens to neurons that bear tangles? Do they inevitably disappear? Are there other causes of the cerebral atrophy of Alzheimer's disease, generalized or localized? Dr Tariska, did you see any particular features in your cases that might tell us what has happened to these neurons?

Tariska: The amygdaloid nucleus in case 6 showed an abundance of tangles and relatively few nuclei. In the hippocampus it is common to find tangles without nuclei.

Terry: That is a very common statement by light microscopists. They are able to look at many more tangles than electron microscopists, but we must have seen many hundreds of tangles in the electron microscope and never once has one been extracellular. I suspect that the light microscopist is looking at a neurite full of the elements that make up an argyrophilic mass rather than at a cell body. I really don't believe that tangles remain after the cell dies.

Roth: You consider them all to be intracellular?

Terry: Yes. They are all surrounded by a plasma membrane.

Roth: And they start from intracellular neurofilaments?

Terry: Yes, or at least they start from the twisted tubules that are more or less specific to this group of changes.

Taylor: What effort has been made to look at other tissues in someone who has just died of Alzheimer's disease, on the assumption that the protein in these tangles is not confined to the nerve cell? Is there any suggestion of tangles of tubules in mitotic cells?

Terry: One should certainly discover whether all microtubules have been changed in this way. I know of no such investigation yet.

Dayan: Schwartz (1965) has suggested that plaque-like material may

occur in other tissues, particularly the pancreas, but his results were obtained by capricious methods of light microscopy.

Gonatas: Dr Terry, is the origin of the twisted or helical tubules of Alzheimer's disease from altered microtubules or filaments a proven fact or a working hypothesis?

Terry: There is no evidence for a transformation from a normal microtubule to a twisted tubule. It is a working hypothesis.

Tomlinson: Dr Tariska, your cases all showed an exceptional degree of gliosis. I have only seen one comparable case of gliosis in the temporal lobe in Alzheimer's disease. Do you think gliosis of this severity can be demonstrated in many cases of Alzheimer's disease?

Tariska: I think this was exceptional.

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ALZHEIMER'S DISEASE AND RELATED CONDITIONS
G. E. W. WOLSTENHOLME & MAEVE O'CONNOR

MUSCULAR TWITCHINGS IN ALZHEIMER'S DISEASE

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THE starting point of this investigation was a histologically confirmed case of Alzheimer's disease which, in spite of a history of progressive loss of memory and aphasia over a period of four years, we had misdiagnosed as Creutzfeldt–Jakob disease because of persistent muscular twitchings, partly localized and partly generalized. However, we were later able to diagnose correctly two cases of Alzheimer's disease with myoclonus, one of which was hereditary, with several other members of the family presenting the same muscular phenomena. In the meantime we had found that ten cases of Alzheimer's disease with persistent muscular twitchings had been described in detail in the literature; six of them were heredofamilial in type and some had other sibs with muscular twitching.

Barrett (1913) reported a case of Alzheimer's disease of four years' duration in a 37-year-old woman who had fibrillary and myoclonic twitchings on one side of the body. Cuel (1924) described his case as having choreiform twitchings. Divry and Moreau (1934) described the muscular twitchings in a 56-year-old patient, whose disease had lasted for 15 years, as follows: ". . . muscular twitchings of the face and sudden jerkings of the limbs. Sometimes he mouths a word, without making a sound; at other times he moves his tongue very quickly from left to right for several seconds." Jelgersma (1964) described generalized myoclonus in a 41-year-old female who suffered from the disease for 15 years. The heredofamilial cases described by Lowenberg and Waggoner (1934) resemble our own familial case: of five affected probands three had "muscular twitchings of an irregular myoclonic type, the muscles twitched constantly and made walking and even sitting impossible. There were irregular variable myoclonic movements of the muscles, particularly of those of the face and of the fingers of the left hand." Van Bogaert, Maere and de Smedt (1940) described a family of seven cases of whom two suffered from irregular clonus (*secousses cloniques*). McMenemey and co-workers (1939) described a family in which the 80-year-old grandfather suffered from "twitching of

the tongue and drooping of the eyelids", and his grandson who died at 51 during the five-year course of the disease experienced "shock-like contractions of the legs and arms sufficient to spill water, when holding a tumbler which was but three parts full." Grünthal and Wenger (1939, 1940) in their description of a family afflicted with Alzheimer's disease referred to fit-like states of rhythmic extension and flexion of legs and "finally twitching of the whole body." Von Braunmühl (1957) described in his heredofamilial cases epileptiform fits and twitchings as well as frequent short tonic twitchings of the whole body including the face. Lüers (1948), describing a heredofamilial case of a 43-year-old woman with a history of 20 years duration, wrote: "Over the whole body there are almost continuous wave-like muscular twitchings without much effect on movement."

Detailed histories of our own cases are given at the end of this paper, but in summary we are concerned with a woman patient (case III) (He.M. 953/61) who fell ill at the age of 47 with progressive mental deterioration leading later to loss of speech, and in whom myoclonus was an early feature. She died after the disease had lasted five years. In another woman, now 61 years old (case II), the disease, otherwise classical in type, has so far lasted four years. We are not sure when the muscular twitchings, which occur especially in the mornings, first began. In the family we studied (case I), six females from three generations had Alzheimer's disease (Fig. 1). In

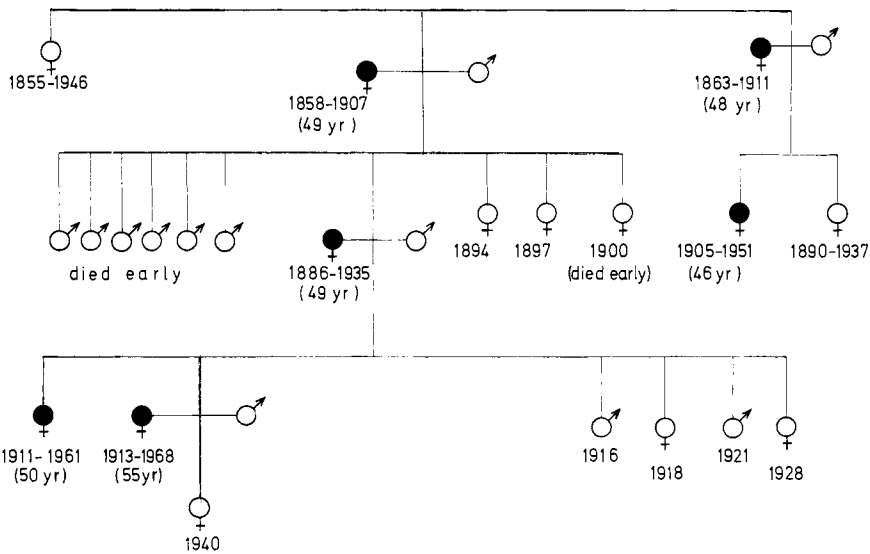

FIG. 1 Family of case Fa.E. Black circles indicate the six females in three generations who had Alzheimer's disease with muscular twitching.

five of these myoclonus probably developed with the initial stage of amnesia; in the sixth it began with a major epileptic fit.

Before describing the myoclonic features in Alzheimer's disease, I should like to refer to the possible causes of myoclonus as generally defined in the Anglo-American literature. Table I, which lists the possible aetiological

TABLE I

POSSIBLE AETIOLOGICAL FACTORS FOR MYOCLONUS

Infectious diseases:
- General and infantile paralysis
- Lues cerebri
- Encephalitis lethargica
- Typhus encephalitis
- Smallpox encephalitis
- Encephalomyelitis of malarial origin
- Herpes zoster myelitis
- Subacute sclerosing leukencephalitis
- Inclusion encephalitis
- Infectious mononucleosis
- Tuberculous meningitis
- Brucellosis
- Multiple sclerosis

Degenerative diseases:
- Huntington's chorea
- Cerebello-olivary atrophies
- Olivo-ponto-cerebellar atrophies
- Dyssynergia cerebellaris myoclonica
- Hallervorden–Spatz disease

Traumatic lesions:
- Acute cerebral trauma

Intoxications:
- Encephalopathia alcoholica (delirium tremens)
- Lead poisoning
- After tranquillizers
- Minamata disease

Metabolic disorders:
- Encephalopathia uraemica
- Hepatoencephalopathia
- Basedow's (Graves') disease
- Poliodystrophia cerebri progressiva (Alper's disease)
- Cerebral lipidoses
- Maple sugar disease

Presenile and senile atrophies:
- Alzheimer's disease
- Pick's disease
- Pernicious involutional psychoses
- Creutzfeldt–Jakob disease
- Bilateral symmetrical degeneration of thalamus

Hypoxic encephalopathies:
- E.g. cardiac arrest

factors for myoclonus of neurological origin, is based on Aigner and Mulder's (1960) schema (and see Bonhoeffer, 1936; Stertz, 1936; Hassler, 1953; Weingarten, 1957; Lance and Adams, 1963; Schenck, 1965; Sherwin and Redmon, 1969). These factors include varieties of acute and chronic meningoencephalitis, infections and intoxications, metabolic disorders of many types, heredodegenerative conditions and diseases of old age. From a random sample of such case histories, it soon becomes apparent not only that what we see is myoclonus in its strict sense, but also that very complex muscular phenomena are frequently involved. This applies particularly to the group of involutional presenile cerebral processes presented in Table II, which are of special interest to us and which include Alzheimer's disease, Creutzfeldt–Jakob disease and a few other less well-defined conditions, as well as the pernicious involutional psychoses with terminal

TABLE II

PERCENTAGE OF CASES SHOWING EPILEPTIC SEIZURES AND MYOCLONUS

	Epileptic seizures		*Myoclonus*
	%	*Authors*	
Alzheimer's disease	60	Sjögren and Sourander, 1962	Rarely
	16	Lauter, 1968	
Cerebrovascular diseases	8	Sjögren and Sourander, 1962	—
Creutzfeldt–Jakob disease	21	Siedler and Malamud, 1963	46%

pathological brain changes I described in 1960 (Table III). A comparison of these diseases shows that the statement by Nevin and co-workers (1960) on "the relative paucity of major epileptic phenomena in contrast to the almost constant occurrence of myoclonic jerks" is valid not only for Creutzfeldt–Jakob disease but also for those cases of Alzheimer's disease in which myoclonus occurs (see Sjögren, Sjögren and Lindgren, 1952). Of the 13 cases referred to here (ten from the literature and our own three cases) five had major epileptic fits (Table IV). The same is true of other types of presenile diseases.

The muscular twitching syndrome of Creutzfeldt–Jakob disease resembles that of Alzheimer's disease. In their Case IV Nevin and co-workers (1960) described the clinical phenomena in Creutzfeldt–Jakob disease as follows: ". . . there were occasional rapid jerk-like movements of short duration and variable distribution involving the right arm and shoulder . . . At times slight rhythmic flexion-movements were noticed at the left wrist persisting for 5 to 10 seconds. On one occasion the eye and head were turned to the right and remained in this position for 1 to 2 minutes . . . Involuntary movements continued and though varying from time to time, consisted of isolated myoclonic twitchings, involving proximal limb segments, mainly the arms. These movements were increased by handling

TABLE III

SUBACUTE PRESENILE ENCEPHALOPATHIES (MIXED FORMS OF MUSCULAR TWITCHINGS)

Authors	*Case*	*Age*	*Sex*	*Duration of final stages of disease (months)*
Alajouanine and van Bogaert, 1950	I	61	M	1
	II	62	F	2
Garcin *et al.*, 1950	I	55	F	3
	II	66	M	1
	III	52	F	2
Poursines, Boudouresques and Roger, 1953	I	54	M	4 (12)
Delay, Brion and Sadoun, 1954	I	57	F	5
Jacob, 1960 (pernicious involutional psychosis)	I	60	F	3 (15)
	II	50	F	6 (48)

Figures in parentheses: duration of whole course of disease.

TABLE IV

THIRTEEN CASES OF ALZHEIMER'S DISEASE WITH MUSCULAR TWITCHINGS

Authors	*No. of probands with muscular twitching*	*Epileptic seizures*
Familial:		
Lowenberg and Waggoner, 1934	3 of 5	+
Van Bogaert, Maere and de Smedt, 1940	2 of 7	+ ?
McMenemey *et al.* (1939)	2	−
Grünthal and Wenger, 1939, 1940	1	+ ?
Von Braunmühl, 1957	1	−
Lüers, 1948	1	−
Case I (Jacob)	6	+ (3 times)
Sporadic:		
Barrett, 1913	+	+
Cuel, 1924	+	−
Divry and Moreau, 1934	+	−
Jelgersma, 1964	+	−
Case II (Jacob)	+	−
Case III (Jacob)	+	−

of the patient. Occasionally all limbs were involved synchronously. Less often localized rhythmic movements, e.g. flexion and extension of the wrist or thumb or pronation and supination at the elbow, persisted for a fraction of a minute." Case V was reported thus: "The myoclonic jerking would involve either one or more individual digits or the limb as a whole ... Occasionally rhythmic movements were noticeable immediately following any disturbance of the patient, especially handling of the left arm." Case VII: "At intervals there occurred variable clonic jerks of the muscles of limb or face either in rhythmic succession or in random fashion and they were especially liable to occur in association with periods of irregular breathing" (see also Kirschbaum, 1968).

The histories of our patients affected by Alzheimer's disease include the following description of case He.M. Several years before admission to hospital jerky twitchings of the right arm, producing sudden arousal at night, were noticed in addition to the slowly progressive disorders of memory. During clinical study persistent but different types of motor discharges of varying duration were observed. Besides the continuing jerky flexor movements of the right arm, irregular twitchings of the body and limbs occurred, occasionally including the eyelids; usually the twitchings were predominantly on the right side. At times the twitchings appeared as solitary flash-like motor discharges in rapid succession, for instance in one or more fingers, the whole hand or in the feet. Occasionally mass discharges occurred, affecting whole muscle groups in the region of

the extremities and trunk, but solitary twitchings predominated. Sometimes considerable variations in the intensity of these muscular phenomena were noticed. Eighteen months after the onset of the disease we observed in this hereditary case involuntary jerky twitching in the left arm, especially during voluntary movement. All the time the patient was in hospital jerky movements lasting several seconds were observed in the left arm and sometimes also in both arms. At other times irregular muscular twitchings seemed to affect various muscle groups and could be intensified on touching the patient and also under psychological stress. Sometimes these twitchings had no marked motor effects, yet at other times there were strong massive motor discharges. Both during speech and independently of it, hiccup-like twitching occurred, with sudden contractions of the chest and expulsion of air giving rise to further speech disturbance. Later on contractions and jactitations of the whole body occurred, accompanied by a piercing scream; when the patient was seated these movements were sufficient to pull her backwards. The intensity of these muscular twitchings varied considerably and only subsided when the patient calmed down.

Apparently the structural complexity and the general course of Creutzfeldt–Jakob disease and Alzheimer's disease are reflected directly in the quite typical muscular twitching syndrome. In contrast the rapid muscular phenomena recorded singly are often described in the literature as choreiform, tetanoid, Jacksonian, or as a form of electrical chorea or Kojevnikoff's epilepsy. Even fascicular twitchings have occasionally been mentioned. Van Bogaert, Radermecker and Titeca (1950) pointed out the occasional connexion between myoclonus and *Fasciculationen mit Bewegungserfolg* (in French *signe de l'index*, and in English contraction-fasciculation). Denny-Brown and Pennymaker (1938) and Denny-Brown (1949) interpreted these electromyographically. Quite apart from the many possible variations of single types of twitching, the extent to which natural motor activity can be irritated strikes one again and again. One is tempted to quote Virgil's moving description from the *Aeneid* (X, 395–396):

> "te decisa, Laride, dextera quaerit semianimesque micant digiti ferrumque retractant"
> [For you, Larides, searched the severed right hand, almost lifeless, the fingers twitching, still grasping the sword]

Electroencephalographic and electromyographic analyses are certainly of special importance in the classification of muscular twitching. However, even the EEG patterns do not often differ in a uniform way, and a specific pattern of excitation seldom correlates with the whole of the complex

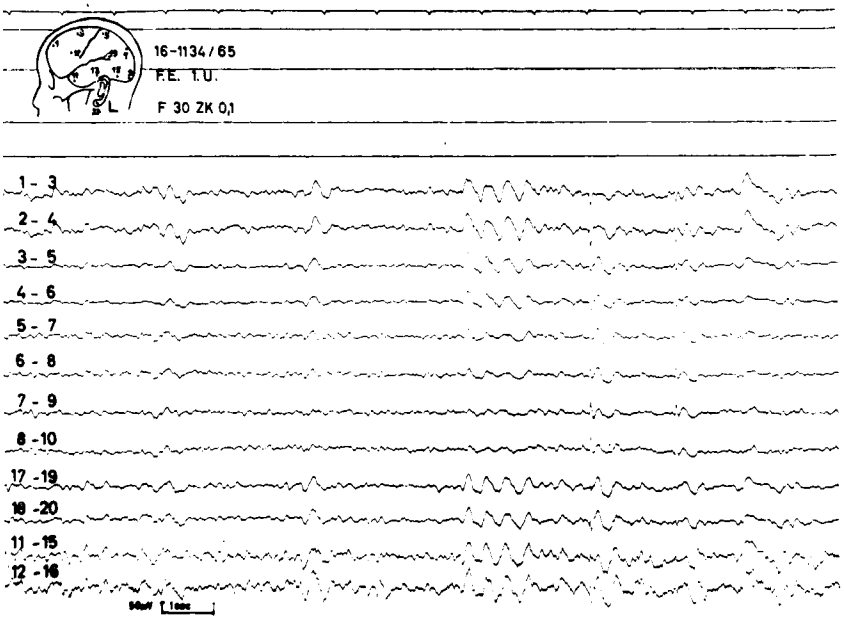

FIG. 2 Case Fa.E. 1965. EEG No. 16-1134/65 (Dr Lütcke). Irregular θ-waves, 5 to 7 Hz, with irregular α-activity predominantly from the lower α-frequency region, with slight superimposed β-activity. Repeated occurrence of single spike and wave complex, as well as intermittent δ-rhythm at a frequency of 2·5 Hz, both accentuated in the frontotemporal region, but no evident focus.

muscular twitching syndrome. Figs. 2 and 3 show the varying patterns seen in our familial case, Fa.E. Regarding attempts at classification, we should keep in mind Hughling Jackson's (1878) warning: "We must not classify on a mixed method of anatomy, physiology and psychology, any more than we should classify plants on a mixed natural and empirical method, as exogens, kitchen herbs, graminaceae and shrubs." By this I mean that classification of motor phenomena according to the EEG and EMG patterns is merely one possible form of classification, though an important one (see Nevin, 1967; Gordon and Sim, 1967).

A different approach would be to correlate the duration of muscular twitching and the structural changes throughout the course of the disease with all other neurological and neuropsychiatric syndromes. Schottky (1932) made the first attempt to compare the various motor disorders with the peculiar disintegration of speech. As is well known, the aphasic syndrome in Alzheimer's disease (and in my opinion this applies also to Creutzfeldt–Jakob disease) is characterized by "indistinct focal disturbances"

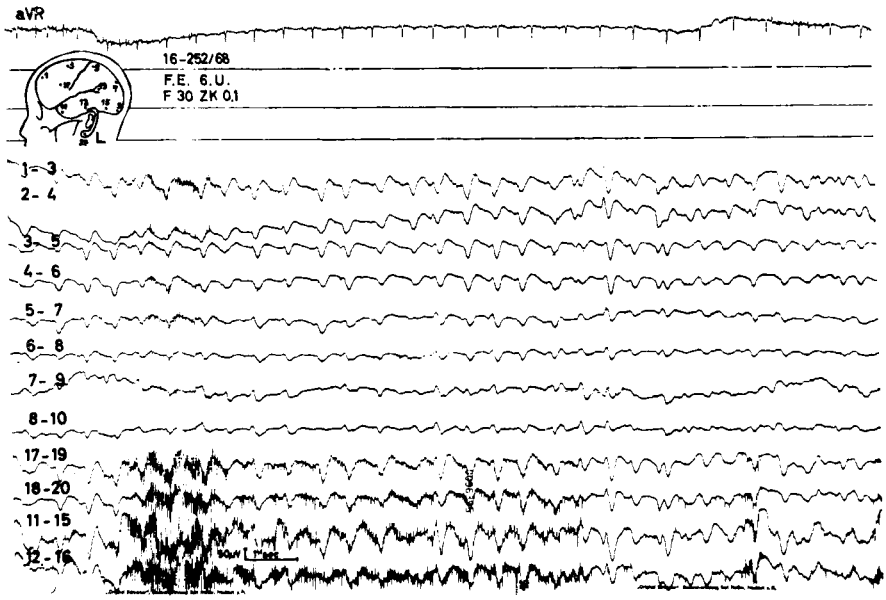

FIG. 3 Case Fa.E. 1968. EEG No. 16-252/68 (Dr Lütcke). Predominantly of δ-waves, 2 to 3 Hz, and slow θ-waves persisting as background activity with frequent interruption by complexes of triphasic waves most marked in the frontotemporal region or sharp-and-slow waves at a frequency of 2 Hz, again with no localization.

when compared with the classical focal types of aphasia. Schottky believed that "if one were really critical, one could even talk of indistinct focal disturbances in the field of motor disorders." This would agree with van Bogaert's reference to a mixture of different types of muscular twitchings. Moreover, the aphasic and muscular twitching syndromes vary in intensity and extent during the progress of the disease. Occasionally patients who are apparently completely unable to speak suddenly utter short sentences and understand simple orders. In the same way a patient whose muscular twitchings have been sufficiently severe to preclude any regular motor activity may sometimes, if only momentarily, be free to carry out normal movements. We know however that psychomotor disturbance can intensify myoclonus, which is why van Bogaert spoke of "excitomotor phenomena."

Finally, in the initial phase of the disease, cases with sporadic muscular twitching present similarities which were described by Mallison (1947) as "transitory activity and disturbances of inhibition", i.e. there may be transient disorders of verbal expression and at first also reversible breakdown

of automatic activity, or paroxysmal memory disorders ("transient global amnesia"; Fisher and Adams, 1964) which Mumenthaler and van Roll (1969) have recently described may occur. Such isolated "pace-maker" syndromes may indicate that all specific patterns indicating disintegration of function are to some extent determined from the very beginning, even if they are at first reversible. Obviously this also applies to the muscular twitching syndrome.

One further point, as indicated by Schottky (1932), makes the motor phenomena comparable with both the aphasic and apraxic phenomena of Creutzfeldt–Jakob disease and Alzheimer's disease. What Goldstein (1926) and Bürger-Prinz (1930) had discussed under the concept of "cerebro-organic functional disorders", for example as in the amnestic syndrome, is certainly also valid for aphasic and motor disorders. The impairment of vocabulary and alalia as well as the motor aphasia may result from an increasing loss of spontaneous speech, but they may also be due to a disturbance of normal relations between processes of thought and speech. Loss of vocabulary and speech is therefore not due to just one basic disorder, but can be explained by several functional disorders affecting different systems and at times overlapping (Jacob, 1968). Both the structural analysis and the EEG analysis of the muscular twitching syndrome in Alzheimer's disease and in Creutzfeldt–Jakob disease would be incomplete without consideration of these factors.

We should therefore concentrate on finding the anatomical basis for these clinical phenomena. Adams, van Bogaert and van der Ecken (1964) have emphasized that in a disease such as striato-nigral degeneration the general degenerative processes must be differentiated from more local and specific ones, while Walter Spielmeyer (1926) has taught us to speak merely of "disease centres", because more general tissue changes are usually present in the background. This is particularly true of Alzheimer's and Creutzfeldt–Jakob diseases, and makes it possible to understand the many disintegrative factors at work and the resulting clinical syndromes which may resemble each other. Both because the tissue damage is general and because multifocal clinical phenomena cannot be attributed to any "additional" destruction of one or other functional centre, it is better to assume that the topical relationship is due to an "interfunctional" pattern of disintegration. With this in mind the remarkable differences in symptoms, compared with the "pure" focal disease, become much clearer (see Jacob, 1966).

However, the question remains how far such special functionally related disorders correspond to the many different EEG patterns, as Nevin (1967) and Gordon and Sim (1967) have shown.

CASE HISTORIES

Case I: Fa.E.

Born 15th July 1913, died 4th December 1968; duration of the disease five years.

Fig. 1 refers to the family in which females in three generations were afflicted from the maternal side in the fifth decade. Muscular twitchings were probably already present during the initial amnestic period but one patient had epileptic seizures. Reports from relatives suggest that altogether six members of the family were afflicted with the disease.

At the age of 51 in 1964 this patient began to lose interest and initiative and showed progressive disorders of perception and memory, diminution of spontaneous speech, with halting and stumbling over words, dysgraphia, dyslexia, a progressive indifference to her surroundings, disorientation, weakness and moods of depression. By 1967 transient anxiety states developed, accompanied by delirium and hallucinations.

Twitchings were seen about six months after the first changes in behaviour; they occurred several times a day, especially in the left arm on voluntary movement, but did not amount to true myoclonus. Six months later she had her first episode of unconsciousness, with facial pallor and sweating but no convulsions; this attack was followed by confusion and irrational groping movements. Similar episodes occurred repeatedly in the next three years, and during her last year of life she had general convulsions on about twelve occasions.

Throughout her time in hospital (1967–68) flash-like jerky movements lasting one second were observed in the left arm, but sometimes in both arms, and these increased noticeably before and after the occasional epileptic seizures. In addition irregular twitchings seemed to involve a variety of muscle groups and could be intensified by disturbing the patient. Occasionally they occurred without full contraction of a muscle, but at other times strong contractions indicated massive motor discharges. Sometimes, especially when she was attempting to speak, hiccup-like twitchings with clonic contractions of the chest resulted in expulsion of air and interference with her attempts at speech. Later on contractions and jactitations of the whole body occurred, accompanied by a piercing scream; when the patient was seated the movements could be so violent as to pull her backwards. Periods of unmotivated withdrawal alternated with periods of euphoria and a certain attention to people around her, when she would temporarily regain her normal voluntary movements and even be able to speak in short spontaneous sentences. During such periods of remission her gait improved and she felt unsteady only when turning; the spontaneous

muscular twitchings also seemed to subside and localized twitching occurred only rarely.

The neurological signs included exaggerated deep tendon reflexes with occasional foot clonus, and at times reduced movements of expression and poor mimicry, a fine jerky tremor of the fingers, unsteady gait with very short steps, and hypertonia.

Pneumoencephalographic and radiological findings indicated well-established atrophy of the cerebral cortex and dilatation of the ventricles, particularly in the frontotemporal and anterior parietal regions. The cerebrospinal fluid was not remarkable.

Figs. 2 and 3 show the electroencephalographic findings in 1965 and 1968.

Her sister, *Neu.R.*, from the age of 45 in 1956 had muscular twitchings which occasionally caused her to drop things. Twitchings usually occurred in the mornings soon after rising but it also happened when she was still in bed. Sometimes these caused sudden collapse of the whole body but no loss of consciousness. About a year later she became increasingly forgetful and disorientated, losing all sense of time. She was dysphasic, slower in her movements and responses and often tired, sleeping a lot. Her first epileptic seizure occurred towards the end of 1959. Initially she had retained some insight, and her euphoria and affective receptiveness had been remarkable, in spite of a very poor memory. Speech disturbances with paraphasia increased, as did perseveration and the amnestic syndrome, which was accompanied by confabulation and still more marked disorientation. During this period, in addition to the jerky movements she had attacks lasting for almost an hour, in which she would lie awake in bed with "glassy eyes and smiling", with the head arched backwards and without uttering a sound. The muscular twitchings persisted but in 1960 there were frequent epileptic seizures. Despite the general worsening of the aphasic, agraphic and alexic disturbances, on occasion she could answer simple questions and name objects. She could at times go for short walks, in spite of occasional great difficulties in walking and standing. Her confusion increased progressively until she died.

Pneumoencephalographic findings indicated distinct atrophy of the cerebral cortex and dilatation of the ventricles.

The EEG showed a mixed series of irregular θ-waves, 4 to 7 Hz, interspersed in varying frequency with δ-waves, 2 to 3 Hz, at an amplitude of 25–50 μV, and frequently interrupted by complexes or short series of highly accentuated polymorphic δ-waves at a frequency down to 1 Hz. In the occipitoparietal region inactive biphasic sharp waves appeared at a

frequency of 2 Hz, and high-density small single spikes on one side or the other.

The neuropathological findings (Pathologisches Institut Stuttgart) were in every respect typical of Alzheimer's disease.

Case II. Ste.A.

Born 16th June 1908; present duration of the disease four years.

In 1965 at the age of 57 this patient showed distinct behavioural changes but hardly noticeable amnesia. She was unstable and occasionally irritable or depressed. She lost all interest in the household and became generally indifferent. She became increasingly forgetful and hallucinated and in 1966 was diagnosed as having a severe amnestic syndrome with personal, place and time disorientation. She said that sometimes it seemed that everything had disappeared and she no longer knew what to say. She could not solve a simple arithmetic problem. She acted in a helpless and embarrassed way and had a vacant look. Sometimes she was delirious and restless. No unusual neurological symptoms were noted.

Pneumoencephalography indicated atrophy of the cortex and ventricular dilatation, slightly more emphasized on the left side.

During 1967 she had moods of depression and delusions of poverty, and gradually lost all initiative. Spontaneous speech ceased; she mostly answered "yes" without understanding anything. She confused things, as in asymbolia. She could not dress herself and at times appeared sensory-aphasic, with disturbances in comprehension and perseveration, particularly after a change of tasks. Scattered muscular twitchings, localized in character, occurred especially in the mornings, but it is not certain when they first appeared.

The EEG in 1967 showed low density and rapid irregular activity in the α and β bands, superimposed on θ activity, 4 to 6 Hz, with occasional acceleration in the upper θ and lower δ bands. There were dense short single or double spikes, either generalized or more marked in the frontotemporal region. Occasionally biphasic sharp waves were accentuated in the occipitoparietal region. There was no indication as to side or focus.

Case III: He.M.

Born 14th April 1910, died 8th January 1962. In the family history only the suicide of the patient's brother is worth noting.

Apart from a period of several hours of unconsciousness and a temporary loss of speech after an accident she remained well until her 47th year, when she experienced gradually increasing disorders of memory. Three years later relatives noticed an occasional jerky twitching of her right arm.

In the middle of 1961 she took to her bed, refusing food without reason and speaking little and unintelligibly. She was admitted to hospital in August and at first was still able to give her history, even if somewhat incompletely. She told of vague sensations in her heart, of sudden arousal at night connected with the twitchings in her right arm, of which she was quite aware, and of the sudden cessation of thoughts. Her speech became slower and halting, and broke down completely, mostly at the end of a sentence but also under stress, when verbal and literal paraphasia would occur. Considerable amnestic disorders with disorientation as to time and place were apparent right from the beginning. During the six-month terminal stage, besides the progressive loss of voluntary activity, there occurred loss of interest and perception, amnesia, decreased ability to think, dysphasia and perseverations. There were episodes of restlessness, anxiety and depression, and paranoid hallucinations with fear of dying and starvation. The degree of speech disturbance varied according to the episodes of restlessness. Persistent but very different types of muscular twitchings were observed. Besides the jerky flexor twitchings of the right arm which were already present in 1960 she developed widespread twitchings of the trunk and limbs, with occasionally frequent blinking of the eyelids. These twitchings, which seemed to predominate on the right side, were solitary, flash-like motor discharges affecting one finger or all fingers on one hand, or even the feet. Occasionally there appeared to be massive discharges involving whole muscle groups, particularly in the extremities and the trunk, but solitary twitchings predominated. In spite of regular treatment with Zentropil (diphenythydantoin sodium) the intensity of these muscular phenomena varied from barely perceptible to strong. Apart from the limitation and difficulty of movement she was unsteady in her gait and walked with very short steps, while increased deep reflexes could be elicited in all extremities; ankle clonus could also be elicited.

Pneumoencephalography and arteriography indicated distinct cortical atrophy and ventricular dilatation. The cerebrospinal fluid had a total protein content of 14 mg/100 ml (globulin 5 mg/100 ml, albumin 8 mg/100 ml, protein quotient 0·75), and 1/3 lymphocytes.

The EEG showed low density, rapid frequency activity from α and β bands, with δ waves, 4 to 6 Hz, superimposed. There were frequent outbursts of very dense irregular δ- and β-waves, either generalized or more marked in the frontotemporal region, and preceded by or dispersed with sharp short spikes. Short generalized single spikes or sharp waves were also accentuated in the occipitoparietal area. There was no indication as to side or focus.

The neuropathological findings were in every respect typical of Alzheimer's disease.

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DISCUSSION

Nevin: Myoclonus is a multi-aetiological phenomenon arising at different levels in the nervous system, and I think it would be difficult to define the origin of any one type of case or EEG change. In other cases of Alzheimer's disease with myoclonus one might see quite a different pattern.

Jacob: Which aetiological and topical factors are present when you don't see the pattern we saw?

Nevin: I have seen cases of Alzheimer's disease with myoclonus with only a flat record, and isolated spikes and sharp waves. The pattern in your case is a very abnormal and unusual one.

Jacob: Perhaps it is also a problem of the stage of the process. The three examples of EEG patterns in our familial case date from 1965 (Fig. 2), 1967 and 1968 (Fig. 3).

Roth: Dr Nevin, would you regard these EEG patterns as similar to your own findings in subacute spongiform encephalopathy?

Nevin: Not completely. The pattern is more variable, not as highly synchronized and not as sharp; in fact it is slower. The patterns are not identical but to a certain extent homologous.

Sourander: Could you comment on the value of the EEG in the differential diagnosis between Alzheimer's disease and Pick's disease, Dr Nevin?

Nevin: In Pick's disease the EEG is within normal limits whereas in Alzheimer's disease it is abnormal in all except the earliest cases. The amyloid degeneration in the vessels that will be described here by Dr van Bogaert and Dr Strich may have an important bearing on the clinical interpretation of the EEG (Gilbert, 1968).

It is well known that in most Alzheimer cases the EEG is characterized by a low voltage record with diminution of the α activity and some

increase of θ activity (Roberts, 1962). A typical case of three years' duration showed very diminished α activity with random θ potentials of 3 to 6 Hz. In another of seven years' duration a very low voltage EEG showed θ potentials at 4 to 7 Hz to be the dominant activity. Another pattern has, however, been emphasized by Liddell in a paper in 1958 in which bilaterally synchronous δ potentials occurred paroxysmally. These were elderly patients and I thought that the correlate of this finding of high amplitude δ potentials might be amyloid or congyloid degeneration of the vessels. This was in fact present extensively at autopsy in one patient showing this EEG pattern and studied personally. This change was also found in other cases with a build-up of δ potentials. In one of these with myoclonic jerks amyloid degeneration of the vessels was easily recognized at biopsy. In another case the diagnosis of Alzheimer's disease and amyloid degeneration of the vessels was made on the clinical picture and the presence of the δ activity on the EEG. In a series of 29 cases of Alzheimer's disease pathologically proved, 18 showed the usual well-recognized EEG pattern with no amyloid in the biopsy. One case two years later was still negative at autopsy, and four cases one to five years later showed some amyloid changes. Four cases showed the second pattern, with high-voltage δ potentials, and in the biopsy material the amyloid degeneration of the vessels was easily recognized. However, in four cases where the EEG indicated possible amyloid degeneration, no amyloid in vessels was found in the biopsy, but I don't think this necessarily rules out the postulate that vascular changes resulting from amyloidosis may cause these δ potentials, because biopsy samples are very small.

In a patient with a 17-month history there was marked amyloid vascular degeneration at post-mortem, and here the EEG corresponds to the picture in Dr Jacob's cases—a picture which I would correlate not with typical Alzheimer degeneration but rather with amyloid degeneration of vessels.

This experience is small and the viewpoint expressed may not be supported by further observation but so far it has enabled us to make with confidence the clinical diagnosis not just of Alzheimer's disease, but of Alzheimer's disease with amyloid degeneration of the vessels.

van Bogaert: In a case of Alzheimer's disease of the familial and precocious type (van Bogaert, Maere and de Smedt, 1940) twitching was limited to the feet and the hands, and there were never any big movements. But towards the end of life there was very severe myoclonus, not like that in epilepsy but tending in the same direction. Is that also your impression, Professor Jacob?

Jacob: In our familial case the mother and sister of the proband showed

the first sign of muscular twitching in the upper extremities. Later they showed increasing and generalized myoclonus.

van Bogaert: In our second family (van Bogaert, Maere and de Smedt, 1940) the three cases all began with a little twitching in the fingers. But in another family described in the same paper twitching began in patients aged 35 or 36. They had severe myoclonus in the last stages of the disease. The difference may be due to some genetic factor added to the rest of the process. In both families there were severe neurological symptoms, in one family without myoclonus and in the other family with myoclonus. In a new family we are studying now we find eight or ten cases of Alzheimer's disease, some of the precocious form and some of the classical form. In this family even in the classical form the elderly patients always begin by showing twitching.

Jacob: In the family I studied, twitching began at the age of 45 years and was continuous during the whole course of four to five years. I think it is a more malignant sign than the convulsive states.

Roth: There appears to be a pronounced genetical factor in causation with direct transmission down the generations. Such very clear pedigrees are only rarely seen in Alzheimer's disease.

Pratt: I regard these syndromes as quite distinct from Alzheimer's disease, even though there may be histological resemblances. Something that is clinically so typical within itself, and so different from the ordinary picture, must be regarded as a distinct disorder.

Jacob: But in our cases the pathological appearance was typical.

Pratt: It is clinically atypical of ordinary Alzheimer's disease in that each member of the family showed the movements.

Terry: How unusual is it really? Two of the three cases we reported in 1964 (Terry, Gonatas and Weiss, 1964) had muscular twitchings and we have now had at least two more cases. These involve both the familial and the sporadic types. Twitching may really be quite common in Alzheimer's disease, and related to alterations in the cortical synapses or something like that. I certainly would hesitate to implicate a whole new disease if the overlap is so extensive.

Jacob: I think the topistic tissue pattern is the same in all these cases.

Pratt: If it were a question of overlap one would expect to get a similar variability within the family group, which is not the case.

McMenemey: Dr T. Mandybur (unpublished) found myoclonus in three out of 15 cases of Alzheimer's disease.

Sourander: Dr Sjögren saw symptoms of myoclonus in 54 out of 68 cases in the terminal stage of Alzheimer's disease (Sjögren, 1970).

Hughes: Continual tapping, most annoying to other patients, is a characteristic sign of Alzheimer's disease which I have not seen in other conditions. This might account for some of those cases, Dr Sourander. Another movement, rubbing, is further from myoclonus but also characteristic. Tapping has not been described in the literature, though rubbing has. Is that myoclonus?

Roth: The EEG phenomena suggest an epileptic kind of event. The spikes are unmistakable. They could not have arisen from a voluntary movement.

Hughes: The cases of aphasia which Professor Jacob described had a shortened form of speech. The healthy brain can make up sentences as the person is speaking—some of us may have a lot of difficulty but finally we come to the end of the sentence! But the patient with Alzheimer's disease seems to start a sentence and forget the end. The information is perhaps lying about in the brain but only the prefabricated sentences are picked up.

Jacob: It is one of several kinds of aphasia in Alzheimer's disease. But a similar aphasic disorder may result from an increasing loss of spontaneous speech.

Tariska: About 10 per cent of our cases of Alzheimer's disease showed muscular twitchings of the myoclonic type in the terminal phase. In a few cases, twitchings were observed over the whole course of the disease but only from time to time, for a few days at a time. In some of them Creutzfeldt-Jakob disease was suggested, and only at post-mortem could we confirm that it was purely Alzheimer's disease. But we have never seen EEG changes similar to those demonstrated by Professor Jacob, although the EEG abnormalities one can see in the course of Alzheimer's disease are so constant that they assist the differentiation between Pick's and Alzheimer's disease and between Alzheimer's disease and angiopathic dementias.

Jacob: But myoclonus is an important clinical sign for the differential diagnosis of Creutzfeldt-Jakob and Alzheimer's diseases. Myoclonus is one of the leading symptoms in Creutzfeldt-Jakob disease.

Nevin: It is not always present.

Jacob: It is present during the initial stages of 50 per cent of cases with Creutzfeldt-Jakob, but in Alzheimer's it generally occurs in perhaps 10 per cent in the terminal phases.

McMenemey: Can anyone really locate the site of activity of myoclonus in these predominantly cerebral diseases?

Roth: In view of the clear hereditary transmission and the striking EEG

changes, has a possible relationship with Unverricht's myoclonic epilepsy been investigated?

Sourander: The cases of progressive myoclonic epilepsy described by Unverricht and Lundborg were not examined pathologically. In subsequent non-Scandinavian cases inclusion bodies of Lafora type were frequently seen. In the three Scandinavian cases we examined (Haltia, Kristensson and Sourander, 1969) no inclusions were observed. In these cases cerebellar cortical degeneration was predominant. Perhaps attention should be paid to the cerebellum in Alzheimer's disease, particularly in cases with myoclonic activity.

van Bogaert: The question of the site of origin of the myoclonic activity is a difficult one. In Alzheimer's disease there are extensive lesions of the cortex, and in some cases one finds them in the dentate nucleus and in the pallidum. These may be responsible for some myoclonus in the extremities. But physiologically it is impossible to say where the myoclonus begins.

Roth: The possibility has been raised that towards the terminal stages electrolyte disturbances may lead to changes in neuromuscular excitability. But myoclonus in Professor Jacob's patients was not confined to the terminal stages of the illness. These biochemical problems would appear to merit more systematic investigation in Alzheimer's disease.

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ALZHEIMER'S DISEASE AND RELATED CONDITIONS
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CEREBRAL AMYLOID ANGIOPATHY AND ALZHEIMER'S DISEASE

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THE title of this contribution may be misleading. Cerebral amyloid angiopathy is not peculiar to Alzheimer's disease but historically it was on material from patients with this disorder that Professor Paul Divry (Fig. 1) carried out his work, so important at the time, and which Professor McMenemey has asked me to mention.

Everyone now agrees that cerebral amyloid angiopathy is not specific to any type of presenile or senile degeneration, for it can be found in common cerebrosclerosis (Gerhard, 1969), dementia pugilistica (punch-drunkenness) (Brandenburg and Hallervorden, 1954), in different types of illnesses (Bertrand and Koffas, 1946; Jervis and Soltz, 1936) and, finally, in ageing animals (von Braunmühl, 1957).

Psychiatrists differentiate senile dementia from Alzheimer's disease by specific symptoms which, in each case, overlie the same background of dementia. In spite of lesions of similar quality, the two diseases differ from each other in their intensity and localization. Calendar age is of little importance; what matters in these types of involutional disorder is biological age. This is why we think that certain attributes, which I shall mention later, are common to both.

In the history of cerebral angiopathy, the work of my fellow citizen Divry provides us with a lesson in methodology: subsequent and more detailed studies have been made by other workers as and when technical advances permitted.

For a long time the cortical vessels were considered to be normal in senility and such changes as had been noted were attributed to the age of the patient, though the absence of histological changes did not in itself imply normal vascular function (McMenemey, 1941).

Sixty years ago, Oppenheim (1909) described a hyaline vascular change, metachromatic to toluidine blue, and associated it with the presence of senile plaques. At the time, fine radial networks were shown to be implanted in the walls of the affected vessels, penetrating from them into the

parenchyma (Fischer, 1910). Lowenberg (1925) was struck by the moniliform appearance of these changes. A little later it was suggested that the senile plaques were associated with the precipitation of para-proteins (Hechst, 1929).

In 1927, Divry stated that the nucleus of the senile plaque consisted of an

FIG. 1. Professor Paul Divry, 1889–1967. Professor of Psychiatry and Neurology, University of Liège.

amyloid nodule, of spherocrystalline structure. Divry and Florkin (1927) described the optical properties of the substance as follows: "the amyloid substance in it is practically isotropic and becomes clearly birefringent after treatment with Lugol and even more so with Congo red."

In 1934, Divry claimed that "Alzheimer's neurofibrillary degeneration

is nothing but an amyloid infiltration of ganglion cells" and that not all senile plaques necessarily contained amyloid, since they did not all have a nucleus.

Regarding the mechanism which leads to amyloidosis, Divry agreed with the idea prevalent in pathology that it was the result of a precipitation within the affected organ and that there was neither degeneration nor pathological metaplasia.

Recalling the concept that Lewy (1914) had developed regarding neurofibrillary degeneration in Parkinson's disease, Divry (1940) added that "in the precipitation of amyloid, humoral changes are necessary, but a local tissue factor should be considered". More recently, Schlote (1965) pointed out again the importance of a blood plasma factor in the genesis of cerebral amyloid angiopathy.

In a series of studies (1927, 1934, 1940, 1941/42, 1947), a synthesis of which he presented in 1952, Divry described cerebral amyloid angiopathy in the vessels of the leptomeninges and pointed out that impregnation of their walls often occurs in zones which are sometimes very localized. He noted that in arterioles and larger vessels amyloidosis may assume the appearance of brush marks. In smaller vessels it gave rise to swellings and eventually to moniliform figures.

Divry showed that the three characteristic phenomena (senile plaques, neurofibrillary degeneration of the neurons and cerebral amyloid angiopathy) of Alzheimer's disease and other similar disorders are closely related to one another. They are produced by precipitates from the colloid media, the dispersed stages becoming coarser because of the progress of senility, particularly when this amounts to a pathological senescence. Even granulovacuolar degeneration in the cells (Simchowicz, 1911) suggests a notion of colloid condensation (Divry, 1952). It indicates inversion of the protein quotient, i.e. hyperglobulinaemia and other ill-defined changes in the humoral medium. There must also intervene, Divry adds, "factors peculiar to the organ's metabolism, giving rise to amyloid and para-amyloid precipitations".

Divry's account (1927) of the curious property that plaque nuclei have of acquiring clear birefringence, including a polarization cross, after treatment with Lugol's iodine or better still Congo red, was noted by Scholz (Scholz, 1938; Scholz and Nieto, 1938). At about the same time, Scholz had studied the pathology of meningocortical vessels intensively in elderly people. He differentiated vascular fibrosis, with its proliferation of dense connective fibrils staining bright red with van Gieson and poor in cells, from hyalinosis of the wall, with its less pronounced thickening, relatively few nuclei and

sub-intimal deposits of a substance staining yellow with van Gieson's technique. This hyalinosis tended to undergo fatty degeneration with foam cells and be accompanied by an adventitial lymphocytic reaction, and even by atrophy of adjacent parenchyma of the nervous system, with gliosis and haemosiderin debris. The hyaline substance may disappear and be replaced by an annular connective network. Of course, Scholz's lesions were in no way connected with the fibrosis of the small vessels, probably collecting veins in the sub-pial and sub-ependymal regions (Scholz, 1938). These vessels may be subject to a serious degree of fibrosis independent of hypertension (Recondo and Lansberg, 1967) and coincident with arteriosclerosis (Peiffer, 1969).

In contrast to these two types of changes, Scholz described "vessels in which a deposited substance, staining yellow with van Gieson, was localized external to the elastic lamina, resulting in oedematous structure-less degeneration of the media." This deposited substance has no affinity with fats. It brings to mind the substance that makes up the senile plaques and gives rise to their argyrophilia (Gellerstedt, 1933; Scholz, 1938). Scholz outlined its metachromatic qualities in relation to aniline dyes, its affinity for iodine and for Congo red, and its birefringence in polarized light. The substance takes on a crystalloid appearance so that the vessel containing it looks as if it has stripes around it, like a ribbon made up of a regular series of annular thickenings. He showed that it could go through the adventitia and even through the glial membrane, to spread into the neighbouring parenchyma. Such alteration of the arterioles could also be observed in the capillaries; the nuclei often disappeared, the lumen was somewhat narrowed and the wall was often greatly swollen. This change was to be found wherever there were plaques. It was not observed in the pial arteries. He called the change *drüsige Entartung der Arterien und Kapillären*. As Morel and Wildi pointed out much later (1952), translation of this expression is difficult in other languages. One could talk of "crystalloid degeneration" or "grumous degeneration" (Lafora, 1952). Scholz left open the problem of the relationship between this substance and the amyloid found, for instance, in renal blood vessels, whereas Divry accepted the idea, as early as 1941/42, that it was a true cerebromeningeal vascular amyloidosis.

Later on, Jacob (1939) related this amyloid transformation to the perivascular senile plaques described by Struwe (1929). However, these perivascular plaques are found mostly around larger arterioles, there is no glio-adventitial symphysis (Pantelakis, 1954), reaction to Congo red is often negative and the characteristic radial spicules are absent (Agostini and

Benincasa-Stagni, 1953; Arab, 1954; Corsellis and Brierley, 1954; von Braunmühl, 1957; Agostini, 1958; Surbek, 1961).

If arteriosclerosis of the basal vessels is associated with ageing and that of the mainly temporal-parietal-occipital convexity is associated with hypertension, it follows from Scholz's work that vascular hyalinosis can be found in both states.

Between 1943 and 1952, Morel and his colleagues, returning to the theory of a disorder in permeability (Divry, 1934; Scholz, 1938; Benedek and McGovern, 1949), suggested changing the term *drüsige Entartung* to *angiopathie dyshorique*. The word "dyshoria" was coined by Schürmann and MacMahon (1933) to describe a primary disorder in permeability of the vascular endothelium and its basal membrane. It is claimed that it permits a migration of substances from the plasma into the media, an imbibition which determines medial or even adventitial necrosis with precipitation of a protein substance.

The purpose of the designation suggested by Morel was to draw attention to the presence of an exudate in the nervous parenchyma, i.e. one which had gone through both the adventitia and the glial membrane, as had already been mentioned by Divry and Scholz. Morel also found the lesion in the visual area (Lowenberg, 1925). The clogging up process in the arterial and capillary walls, which begins in the media, often spreads to the adventitia and the nervous parenchyma in the form of extracellular fibrils (Surbek, 1961). However, the preferential localization (or pathoclisis) is not necessarily limited to the occipital region as had often been reported earlier (Divry, 1952). These changes may be found in the parietal and frontal lobes, the cerebellum and the hippocampus. The deposit may be fibrillary in nature, spicular or stuck in a homogeneous mass like a button to the outer wall of the vessel. Whatever its shape, the deposit is not penetrated by reticulin fibres. It can be observed around the venules but their endothelium always remains intact.

Divry, Scholz and Morel all interested themselves in the mechanism of production of this deposit. According to Scholz and Nieto (1938), the hyaline substance migrates outwards from the intima and accumulates in the media, as seen mainly in the meningeal and perforating arterioles. Dyshoric angiopathy seeks out the arteriole and capillary networks in susceptible zones and affects mainly the most distant part of the vascular tree. It should be noted from this that, in a number of instances, these two disorders of permeability affect different areas of the brain.

There are cases—reported by Scholz in 1938—where the imbibition is limited to the vessel wall and where it affects the meningeal rather than

the perforating arterioles. It was in order to characterize this variety that Pantelakis (1954) suggested the designation of *angiopathie congophile*.

When these contributions, made over 20 years, are taken as a whole, one sees that in senile processes, and therefore in Alzheimer's disease, an abnormal vascular permeability occurs which allows the production of substances, probably complex, which in some cases possess some features attributable to the amyloid substance. *If the deposit is limited to the wall* only, it is referred to as "congophilic angiopathy"; if it *overflows into the adventitial space*, welding it to the glio-adventitial structure (Morel, 1943) and even penetrating into the nervous parenchyma, under various forms, it is called "dyshoric angiopathy". These discontinuous deposits tend to occur in areas differing from those of hyalinosis, a difference also claimed to be due to a disorder of permeability. Both are often formed in the same brain (Arab, 1959) but in different parts. However, hyalinosis may be closer than is generally thought to the congophilic material (Stochdorph, 1969). Hyaline deposits have been reported edged by a thin congophilic layer (Arab, 1959; Schwartz, Kurucz and Kurucz, 1964). Differences in preferential localization and in the size of deposits are probably dependent upon anatomical and functional peculiarities of the nervous parenchyma at the vessel wall concerned, but the biological origin of the deposits has not yet been elucidated. It is a curious fact that newly formed vessels arising inside a vessel distorted by amyloid degeneration do not accept the amyloid in the same way as the original vessel (Stochdorph, 1969), as if the newly formed tube did not possess the same aptitude for pathological imbibition as the old vessel. The amyloid deposit may gradually take the form of a chalk-like mass (Götze and Krücke, 1942) in a case of granular cortical atrophy. This shows some relationship between the mechanism of both processes, and also that there is a possible biochemical affinity.

The presence of adventitial cellular reactions in the hyaline deposits and their paucity or even absence in amyloid deposits (Stochdorph, 1969) have led workers to liken the first process to an inflammation, and the second to the phenomena of a serous inflammation (*seröse Entzundung*).

Taking into account the concepts formulated by Divry, Scholz and Morel, we are led to think that amyloid uptake may be limited to the vessel wall (congophilic angiopathy), may extend to the adventitial space up to the glio-adventitial barrier (dyshoric angiopathy) and may even spread into nervous parenchyma (*drüsige Entartung*).

In the literature on Alzheimer's disease, there is a dearth of information on amyloid infiltration of vessels other than those of the terminal end of the

vascular tree, where it can be observed in all sectors. Hardly anything is known of amyloidosis in large vessels.

Characterization of hyaline and amyloid substances is thus based on staining procedures and on their properties of refraction in polarization microscopy. It does not determine in any way the composition of the deposits, which is now the province of present-day histochemists and neurochemists.

From recent studies based on polarization techniques, Schlote (1965) has shown that in fully developed cases of congophilic angiopathy, four layers can be identified with aniso-diametric particles in an alternating perpendicular arrangement. He showed that the staining or optical characteristics of the deposits are similar to those observed in generalized amyloidosis. The nuclei of senile plaques and the vessel wall have a common feature, i.e. the appearance of filamentous amyloid components in parallel or radial deposits exhibiting a peculiar crystalline precipitation. The same substance may penetrate the parenchyma and gives the appearance of a crush. Deposits accumulate on either side of the vessel wall, which restricts the meaning of the expression congophilic angiopathy and characterizes only one phase of the process.

Do the precocious adult and even juvenile forms, with a hereditary or family background, with more pronounced neurological symptoms, and a mental picture that is often atypical or hardly outlined, belong to Alzheimer's disease? Are they special diseases?

Their independence has been severely criticized. Few observations have been accepted (Jervis and Soltz, 1936/1937). We do not refer here, of course, to the Alzheimer aspects of advanced mongolism (Bertrand and Koffas, 1946).

A number of these atypical and precocious cases include cerebellar or spastic symptoms (Worster-Drought, Hill and McMenemey, 1933; Worster-Drought, Greenfield and McMenemey, 1940, 1944; van Bogaert, Maere and de Smedt, 1940), sometimes accompanied by amimia resembling that of extrapyramidal states, shaking and dysarthria. This is a most peculiar set of symptoms, if one remembers that vessels in the white matter of the cerebellum or in the mesencephalon seldom exhibit congophilia (Gerhard, 1969). Some of these cases show fairly important lesions in the centrum semiovale.

At the Congress of Neuropathology in Rome (1952), McMenemey suggested that there was a special kind of senile insanity whose pathology is essentially concerned with para-amyloidosis. He stressed the importance of lesions of the centrum semiovale and of the corpus callosum, the former being also mentioned in another English observation (Corsellis and

Brierley, 1954) on the argyrophilic retraction of the vessels. He pointed out that the lesions particularly affected the cerebellum and hippocampus, that there were only a few senile plaques and that few cells were affected by neurofibrillary degeneration.

Although in the first observations on these precocious and atypical forms the substances which impregnate the vessels and sometimes the parenchyma were compared to those making up the senile plaques (van Bogaert, Maere and de Smedt, 1940; Lüers, 1948; Worster-Drought, Greenfield and McMenemey, 1944), it was quickly agreed that there are deposits of amyloid or of a substance approaching amyloid (Divry, 1952; Corsellis and Brierley, 1954). American authors write about "an amyloid transformation of the artery wall" (Neumann, 1960).

The work of Worster-Drought, Greenfield and McMenemey (1944) brings out a new notion: that of the frequency of neurological disorders with psychiatric components in forms where heredity plays a striking part.

This is not the place to discuss whether these additional neurological symptoms are due to systemic changes. There would be nothing exceptional if general or organic metabolic disorders like these included an associated abiotrophic type of degeneration. What is important is that the same lesions are found in these precocious neurological forms as in other manifestations of Alzheimer's disease and senile dementia. We have re-examined, from this point of view, our personal observations and some of the material was sent to P. Schwartz, to whom I am indebted for some magnificent slides. In our material, we again found the same changes as in the Anglo-American cases and in the classic instances of Alzheimer's disease. We do not believe that the differences in staining characteristics reported in two cases in one family (Surbek, 1961) are sufficient to enable us to talk of a biological type different from other examples of classical Alzheimer's disease.

We feel that these atypical forms are the same illness as in presenility, that is an organic metabolic illness, capable of being precipitated by outside causes (Surbek, 1961) or even, in some families, of being started by genetic factors. Cerebral amyloid angiopathy is only one element of this disorder.

We are confirmed in our opinion by the fact that in senile dogs, congophilic angiopathy showed the same features as in man (von Braunmühl, 1957; Dahme, 1957, 1962; Osetowska, 1966), i.e. the same staining characteristics, the same linear filamentous structures, and the same localizations in the smooth muscles of meningeal and cerebral arterioles. In dogs, the changes appear to be similar to those reported in vessels of other organs and in particular of the heart (Dahme, 1962).

SUMMARY

Cerebral amyloid angiopathy is most frequently found in Alzheimer's disease. It is also found in senile dementia and to a lesser degree in ordinary senility. It probably also exists in the same form in generalized or systemic amyloidosis, though this has been less well studied.

It appears as a sign of general humoral disturbance and of local tissue changes, which obviously cannot be clarified by histological methods. It is but one of the visible elements of the physicochemical alterations which characterize normal involution and which present the same appearance in adult pathological states and in presenility. These pathological states are partly due to hereditary and family predispositions but other outside factors or concomitant dystrophies may also play a part in their genesis.

The history of vascular amyloidosis shows how much the evolution of physiopathological concepts is influenced by changes in methods. Further enlightenment must now come from research work in the basic sciences.

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[For discussion see pp. 124–135]

ALZHEIMER'S DISEASE AND RELATED CONDITIONS
G. E. W. WOLSTENHOLME & MAEVE O'CONNOR

ATYPICAL ALZHEIMER'S DISEASE WITH CONGOPHILIC ANGIOPATHY PRESENTING WITH DEMENTIA OF ACUTE ONSET

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THIS paper describes the clinical and pathological findings in six patients in whom an intracranial or metabolic disturbance was closely followed by profound and permanent mental changes. Examination of the brains of these patients showed widespread amyloid degeneration (congophilic angiopathy) and the presence of senile plaques. This raises the possibility that in typical Alzheimer's disease and in senile dementia systemic precipitating factors similarly affect the deposition of senile plaques.

HISTOLOGICAL METHODS

Brains were fixed in 10 per cent formalin. Large blocks (12 cm^2 or so) from both hemispheres were embedded in paraffin wax. Frontal, parietal, temporal and occipital regions, two levels of basal ganglia, cerebellum, and several levels of brain stem were taken from each case, with additional blocks as required. Frozen sections were impregnated with silver for nerve fibres (von Braunmühl's stain), microglia or astrocytes. Paraffin sections were stained with the usual neurohistological techniques and with Congo red, or the periodic acid-Schiff reaction (PAS) counterstained with Luxol fast blue (LFB). Senile plaques were counted in cortex and basal ganglia in 14 μm thick paraffin sections stained with PAS/LFB (Fig. 3*b*, *c*). Translucent squared paper (Plastitone CH 21, squares 1 mm^2) was stuck on the under-surface of the slide (Fig. 1). Thus the lines were not in the same plane of focus as the section and did not interfere with viewing, but provided a grid in which microscopic fields could be sampled. Two counts were done on each slide at a magnification of $\times$ 120. Free and perivascular plaques and plaque fragments of all sizes were counted in the microscope field

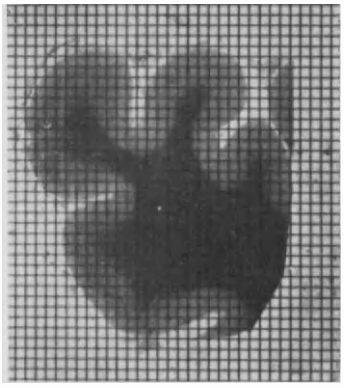

FIG. 1. Section of parietal lobe with squared paper attached to under-surface of slide, as used for plaque counting.

overlying every second square in every third row (horizontal rows in one count, vertical in the second). The ocular had a concentric ring etched in it and only those plaques more than half of which lay within the circle (area 1·1 mm^2) were counted. No correction for shrinkage of the tissue was made. The average number of fields of cortex sampled in each slide was 240. Fields with the highest plaque counts were marked with a felt-tipped pen (Figs. 7, 8). The score for each field was recorded and histograms such as that in Fig. 9 were prepared.

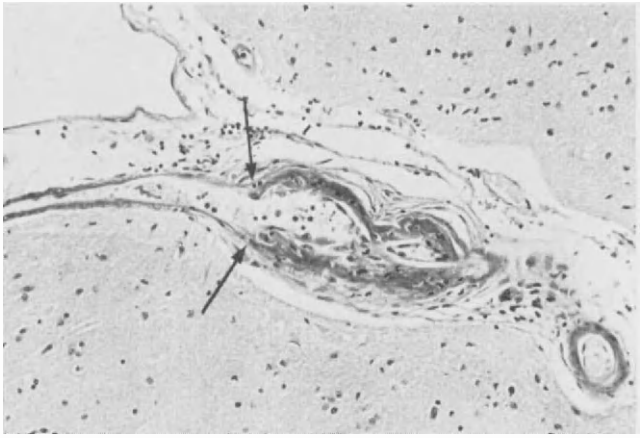

FIG. 2. Longitudinal section through a meningeal vessel showing abrupt change (arrows) from the normal thin-walled structure to the thickened portion containing amyloid. PAS/LFB × 120.

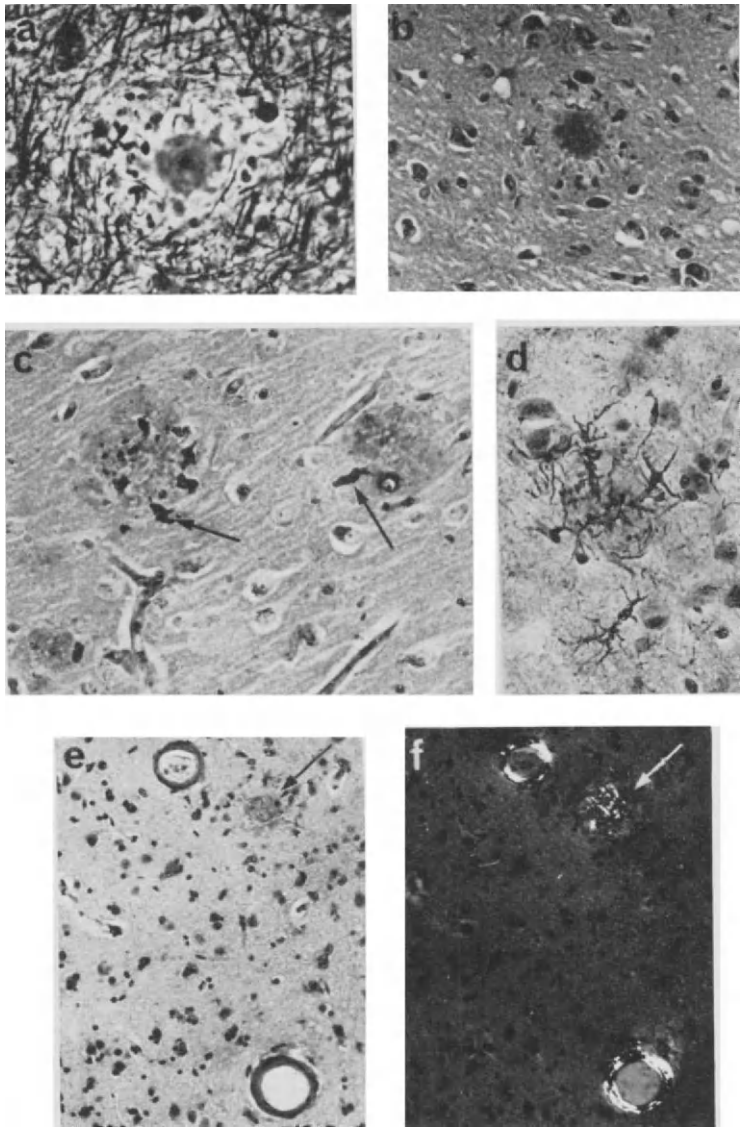

FIG. 3. (*a*) Senile plaque with core. The surrounding tissue contains argyrophilic granules and fibrils. Bielschowsky × 400.

(*b*) Senile plaque with PAS-positive core. There is a faintly granular surround. PAS × 195.

(*c*) Plaques without cores. Note microglial cells filled with PAS-positive material (arrows). PAS × 195.

(*d*) Microglial cells surrounding and near to a plaque. Weil-Davenport. × 290.

(*e*) Two vessels with structureless walls and a senile plaque containing congophilic fibrils. Congo red × 120.

(*f*) Same field as (*e*) with polarized light.

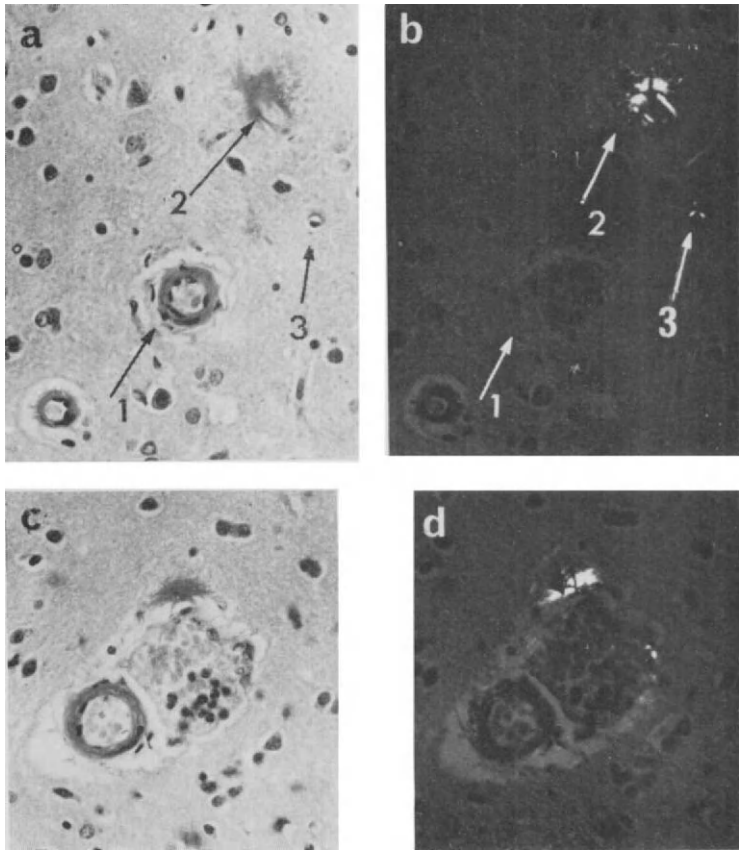

FIG. 4 (*a*) Cerebral cortex stained with Congo red showing (i) normal vessel. (ii) Capillary with wall partially replaced by amyloid which is spreading into the parenchyma. (iii) Capillary with congophilic wall. × 195.
(*b*) Same field as (*a*) with polarized light.
(*c*) Plaque adjacent to unaffected vein. Congo red × 130.
(*d*) Same field as (*c*) with polarized light.

Histology of main cerebral lesions

Widespread and severe congophilic angiopathy and typical senile plaques were found in all six cases, and nerve cells with neurofibrillary tangles were seen in four. These changes differed in no way from those described by previous workers. The major cerebral arteries were not affected. Smaller arteries as well as capillaries in meninges and cerebral cortex were patchily involved (Figs. 2, 3*e*, 4*a*, 5), but veins were often spared. The affected segment was structureless and was replaced by amorphous material. The wall of the vessel was frequently thickened

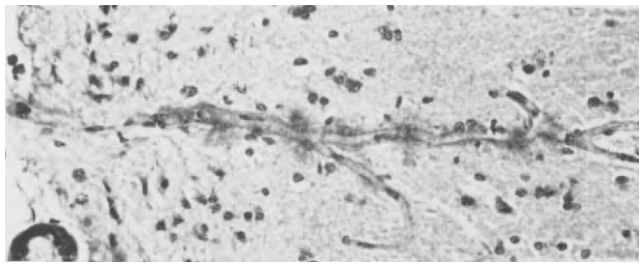

FIG. 5. Small vessels in cortex with numerous excrescences (perivascular plaques) of PAS-positive material extending into the parenchyma. PAS/LFB × 200.

and sometimes the lumen was narrowed or even occluded by the abnormal material. This substance, like that in many senile plaques, had the staining characteristics of amyloid: it was eosinophilic, dirty yellow with van Gieson's mixture, birefringent, strongly congophilic (Figs. 3*e*, *f*; 4) and PAS-positive (Figs. 3*b*, 5). Sometimes the substance in the vessel wall was continuous with small masses in the parenchyma (Figs. 4*a*, 5), such masses often constituting the cores of senile plaques. Plaques were also seen adjacent to normal vessels (Fig. 4*c*). All varieties of senile plaques described

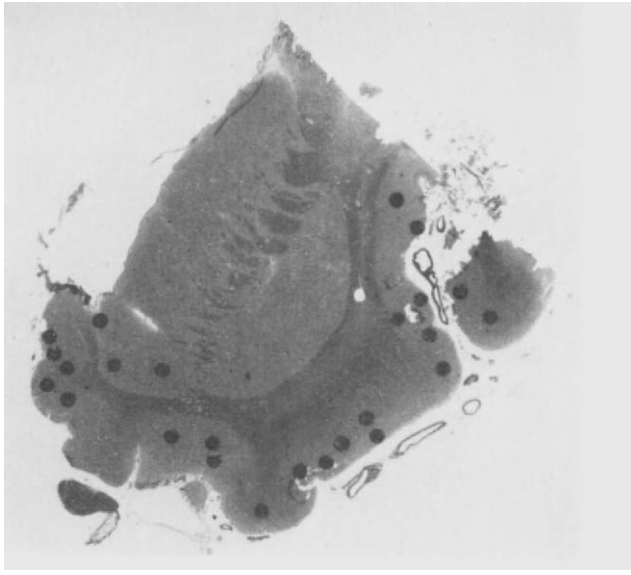

FIG. 6. Case 1. Section of anterior basal ganglia. Each dot indicates a field with over 15 senile plaques. No plaques were seen in the upper two-thirds of the caudate nucleus and putamen. PAS/LFB, × 1·5.

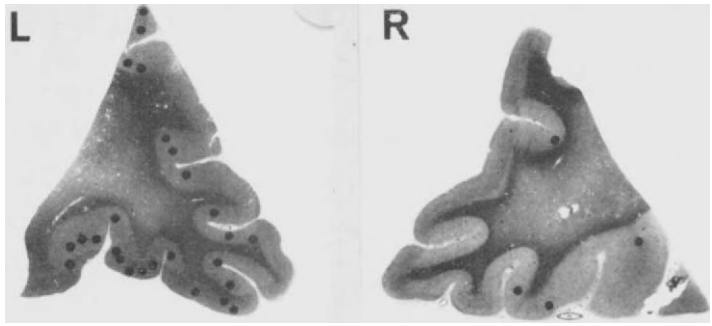

FIG. 7. Case 2. Frontal lobes. Dots indicate fields with more than 10 plaques. There are more plaques in the left lobe (L) than in the right (R). White matter shows loss of myelin. PAS/LFB, natural size.

in Alzheimer's disease were encountered (Figs. 3, 10). Many had congophilic cores or contained congophilic fibrils (Fig. 3*e*). Many microglial cells were present in the cortex, especially near plaques (Fig. 3*d*). Another feature present to a greater or lesser extent in all cases was loss of myelin in the centrum semiovale (Figs. 7, 11) with astrocytic gliosis, increase in microglial cells, but usually no neutral fat. No congophilic vessels were seen in the white matter except in Case 6. Much lipochrome was seen in the nerve cells. The distribution of the lesions will be described with the individual case reports.

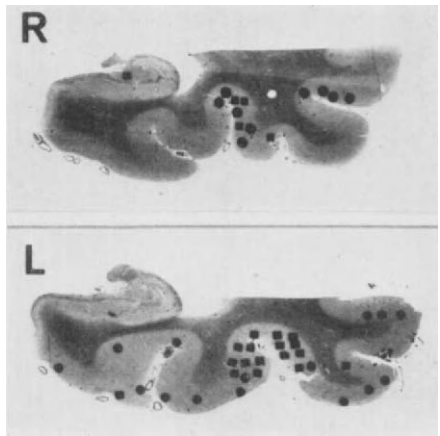

FIG. 8. Case 4. Temporal lobes. Dots indicate fields containing 15–30 plaques, squares mark fields with over 30 plaques (R) right, (L) left. Notice that cortex around inferior temporal sulci contains the most plaques. The hippocampi are relatively spared. PAS/LFB, natural size.

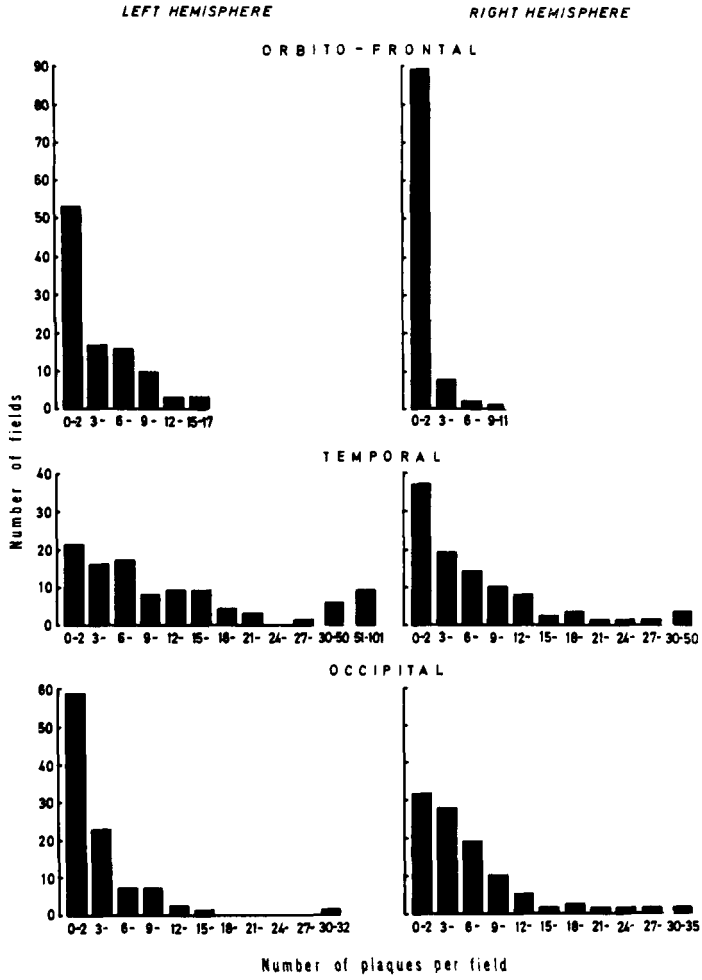

FIG. 9. Case 4. The frequency distribution of senile plaques per field in a total of 100 fields. Histograms showing the difference in distribution in various cortical areas.

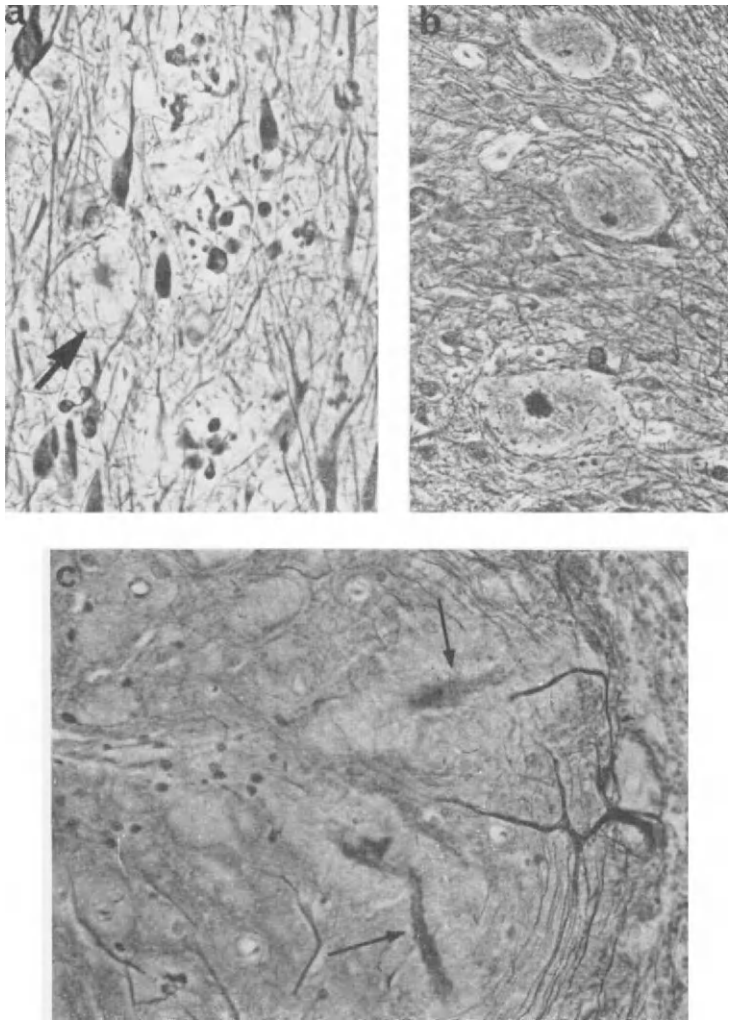

FIG. 10. Case 6. Death seven days after cardiac surgery.

(*a*) Hippocampus. A senile plaque (arrow) and clusters of abnormal nerve fibres. Palmgren × 225.

(*b*) Case 6. Hippocampus. Typical senile plaques, one with core. Palmgren × 150.

(*c*) Molecular layer of cerebellum. Abnormal congophilic capillaries (arrows) surrounded by altered parenchyma. Many plaque-like structures are also seen. Palmgren × 200.

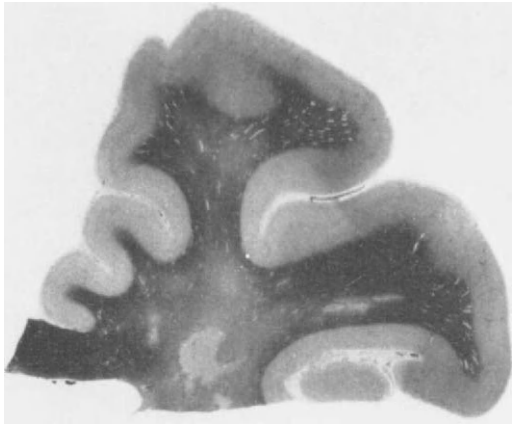

FIG. 11. Case 6. Frontal lobe showing diffuse loss of myelin and many areas of infarction in white matter. LFB/Nissl, × 1·5.

CASE REPORTS*

Case 1 (No. 1956)

This 69-year-old masseuse was working in a beauty parlour until three years before death. There was no family history of mental illness. She was subject to fainting attacks but no epilepsy was ever observed. According to her sister, with whom the patient was in close contact, there was no evidence of mental deterioration before her accident. She was found unconscious at work, having apparently slipped and hit her head. She recovered consciousness in about half an hour but was then confused. There was a left temporal haematoma but no fracture of the skull. Some hours after admission to hospital she answered questions rationally; she remembered going to work but had no recollection of the accident (post-traumatic amnesia at most six hours). She knew her address and that of her ex-husband but gave her age incorrectly. Over the next few days she became more confused and forgetful, without being more drowsy. She was incontinent and often just lay there smiling. The only abnormal neurological sign was defective upward and outward gaze of the left eye. One week after the accident she became unconscious for 40 hours. During this time her blood pressure fell from 130/90 to 100/50 mm Hg for 18 hours. Bilateral angiograms suggested frontal space-occupying lesions. Frontal burr holes were made and some clear fluid under tension was released on the left. Biopsies were taken. She recovered consciousness over the next 24 hours but

* More details will be found in Hollander (1968).

was then, ten days after the head injury, unequivocally demented. She lived in the past and was disorientated, her memory was poor, she was restless, facile and acted inappropriately. She was transferred to a mental hospital where she deteriorated slowly and died $3\frac{1}{2}$ years after the head injury. *Biopsy* eight days after the accident showed, in retrospect, congophilic capillaries, and several senile plaques in putamen or caudate nucleus.

Autopsy findings. Patchy myocardial fibrosis. *Brain:* weight 1170 g; moderate cerebral atrophy and some atheroma of basal vessels. Slices showed marked symmetrical ventricular dilatation, smooth-walled cysts in frontal lobes (old needle tracks). *Histology:* fibrosed meninges; severe loss of myelin in both frontal lobes; congophilic angiopathy in meninges over both hemispheres and in cerebral cortex; neuronal loss in frontal lobes; numerous senile plaques in all lobes (see Table I) but few in hippo-

TABLE I

NUMBER OF SENILE PLAQUES PER HUNDRED FIELDS, IN FIVE CORTICAL AREAS

		Case 1	*Case 2*	*Case 3*	*Case 4*	*Case 5*	*Case 6**
Frontal	R	852·6	238·4	64·4	54·0	3·3	0
	L	715·0	575·1	100·4	19·3	1·1	0
Prefrontal or Motor	R	588·2	174·8	257·7	—	—	0
	L	585·1	284·5	616·6	—	—	0
Parietal	R	592·2	—	147·3	32·8	0·0	0
	L	647·5	143·7	235·8	31·6	0·6	0
Temporal	R	817·2	69·0	687·2	40·1	4·6	Some
	L	443·0	246·9	1384·9	25·7	11·3	Some
Occipital	R	568·5	50·0	550·8	37·1	1·0	0
	L	446·1	103·6	285·7	80·7	1·2	0

* Case 6 had senile plaques in the cerebellar cortex.

campi; plaques in lower third of putamen and caudate nucleus anteriorly (Fig. 6) and in mamillary bodies and amygdaloid nuclei; no plaques in other basal ganglia or thalami; neurofibrillary tangles in cortical cells; one congophilic vessel in cerebellum; no amyloid in other organs.

Case 2 (No. 2586)

This woman was a retired nursing orderly aged 62. She was admitted to hospital for investigation of headaches, blurred vision and a right third nerve palsy. She was a secretive, solitary person who lived on her own, but she gave a good account of herself and her previous life and was correctly orientated in time and place. Blood pressure 130/80 mm Hg. A carotid angiogram showed an aneurysm on the right posterior communicating

artery. The day after this procedure she had a fit, followed by severe occipital headache and neck stiffness, and she became drowsy. A diagnosis of subarachnoid haemorrhage was confirmed by lumbar puncture. Next day she was deeply comatose and remained so for one week. She gradually recovered and after a month was able to respond to simple commands and to make grunting noises but she never spoke again coherently (a tracheostomy was present). The plantar reflexes remained extensor. She died unexpectedly four months after the subarachnoid haemorrhage.

A niece, aged 59, has had several subarachnoid haemorrhages and is demented.

Autopsy findings. Early bronchopneumonia. *Brain*: weight 1276 g; adhesions between arachnoid and dura at base of brain, yellowed leptomeninges; yellow material (old blood clot) in Sylvian fissures and cisterna ambiens; aneurysm at the junction of right posterior communicating and carotid arteries; flattening of cerebral convolutions. Coronal slices showed marked symmetrical ventricular dilatation, due in part at least to obstruction of cerebrospinal fluid outflow by adhesions. No gross brain damage. *Histology*: Widespread congophilic angiopathy in meninges over cerebral hemispheres and in the cerebral cortex; major cerebral arteries and aneurysm not affected; excess of microglial cells in the cortex, particularly in relation to plaques (Fig. 3*d*) and in white matter; some loss of myelin (Fig. 7); many senile plaques (decreasing in number from front to back), far more numerous in the left hemisphere than the right (see Table I and Fig. 7); plaques sometimes occurred in clumps; no neurofibrillary tangles; no amyloid in other organs.

Case 3 (No. 2639)

The patient was a 65-year-old electrician. There was no family history of mental illness, and his previous personality was good. His first subarachnoid haemorrhage occurred when he was aged 54 years. He was left with a partial lesion of the right third nerve but was able to work as a messenger in the City where he learnt his way about. Six years later he had a second subarachnoid haemorrhage. Angiography showed an aneurysm on the right posterior communicating artery. Trial compression of the right carotid artery resulted in loss of consciousness, but the following day the patient withstood compression of the artery for ten minutes without ill effect and it was ligated. After this he developed dysphasia and left-sided spasticity and weakness. Next day he was disorientated and failed to recognize his children. The hemiparesis and dysphasia improved but he remained disorientated and childish and he lived in the past. A year later

he developed epilepsy which was poorly controlled with anticonvulsants. The following year he had a third subarachnoid haemorrhage. He became increasingly restless, had frequent epileptic fits and had to be admitted to a mental hospital. Apart from severe dementia he was noted to have slight Parkinsonism, and a complete right ophthalmoplegia. Blood pressure, 130/90 mm Hg. He died ten months after admission, three years after the carotid ligation.

Autopsy findings. All organs were very small. The left carotid artery was larger than the right, which was occluded by the ligation. *Brain*: weight 1360 g; old softening in the right fronto-parietal region; basal vessels showed no atheroma; aneurysm 0·5 cm in diameter on right internal carotid artery. Section showed old softening in the territory of the right middle cerebral artery, and two pigmented cysts in the white matter on each side, the right one involving the corpus callosum. Slight ventricular dilatation. *Histology:* healed infarcts as seen with the naked eye, also numerous small, scattered cortical infarcts; thickened leptomeninges containing iron pigment; widespread congophilic angiopathy in meninges over both cerebral hemispheres and in cerebral cortex; no amyloid in aneurysmal wall; marked arteriosclerosis of small meningeal and intracerebral vessels; the vessels so affected were not congophilic and some were occluded; subpial gliosis; some senile plaques (see Table I) in all cortical areas examined, also in amygdaloid nuclei; marked loss of myelin in frontal and parietal white matter, with hyaline but not congophilic blood vessels; inclusion bodies (Lewy type) in substantia nigra and in locus ceruleus; right pyramidal tract degeneration.

Case 4 (No. 4731)

This 66-year-old spinster lived on her own. Family history not available. In the past four years she had had several "drop" attacks. Two years before death she began to complain of headaches and blurred vision in the left eye. A pituitary adenoma was diagnosed but no treatment was advised until a year later when hormone replacement was started. A carcinoma of the breast was discovered at this time. The patient was noted to be confused and anxious. Blood pressure 125/80 mm Hg. A total mastectomy was performed and the pituitary tumour (chromophobe adenoma) was partially removed a month later. After this there was rapid mental deterioration so that within two months she was severely demented and incontinent. There were no abnormal neurological signs, and a diagnosis of multiple cerebral secondary carcinomatous deposits was made. She died six months after the craniotomy.

Autopsy findings. There was no recurrence of carcinoma of the breast, and no secondary deposits were seen. Bronchopneumonia. The pituitary fossa was enlarged and contained some tumour (chromophobe adenoma). The tumour compressed the optic chiasm. *Brain*: marked yellow pigmentation (old blood) in leptomeninges over both hemispheres; slight ventricular dilatation; a few small healed cortical infarcts. *Histology*: severe widespread congophilic angiopathy with marked thickening of vessel walls; some neuronal loss in cortex and a few cortical scars; numerous senile plaques, many perivascular; uneven distribution of plaques, varying in different regions and from side to side (Fig. 9); high density of plaques in cortex lining the inferior temporal sulci (Fig. 8); plaques in mamillary bodies and amygdaloid nuclei; loss of myelin in white matter; many neurofibrillary tangles in cortex and in nerve cells in midbrain and pons; cerebellum unaffected; no amyloid in other viscera.

Case 5 (No. 2680)

This man was a 60-year-old retired chauffeur. He had a long history of chronic bronchitis and emphysema and led a life of semi-invalidism. Three months before death, after a hospital admission for pneumonia, he began to have episodes of confusion. He would be "irrational at times but periodically quite normal". A week before his death he assaulted his wife and then climbed over a fence. On admission he was fully conscious but confused and excited. His memory was stated to be poor. He was cyanosed and in severe respiratory failure. Blood pressure 95/65 mm Hg. The patient remained uninhibited and obstreperous but apart from some nystagmus there were no abnormal neurological signs. He died suddenly seven days after admission.

Autopsy findings. Heart weight was 390 g, with hypertrophy of the right ventricle. Severe emphysema and some bronchitis. *Brain*: weight 1285 g; normal externally and on section; slight atheroma of basal arteries.

Histology: widespread congophilic angiopathy in the meninges and cerebral cortex, especially marked in the right frontal region; some nerve cells with neurofibrillary tangles; only a few plaques, mostly without cores, the largest number being in amygdaloid nuclei. Organs other than the brain showed no amyloid.

Case 6 (No. 5652)

This 55-year-old woman was admitted for replacement of aortic and mitral valves. She had had rheumatic fever when 12 years old. For the previous two years she had noticed increasing breathlessness. On examination she was mentally alert. She was in heart failure and had auricular

fibrillation. Blood pressure 160/80 mm Hg. Replacement of the diseased valves by prostheses was carried out under cardiopulmonary by-pass, total perfusion time being four hours 21 minutes. The operation proceeded smoothly but there was some difficulty in maintaining the blood pressure at the end of the operation. The patient was kept on artificial respiration for two days. She never recovered consciousness and died seven days after the operation.

Autopsy findings. There was oedema of all four limbs. The heart weighed 730 g, with marked hypertrophy of the left ventricle. There was a recent infarct in the wall of the left ventricle, minimal atheroma of the coronary arteries, and no occlusion. Spleen weight was 210 g; the liver showed chronic passive venous congestion. *Brain*: weight 1250 g; normal externally apart from minimal atheroma of the basal vessels. Slices showed many small softenings in basal ganglia and white matter and a few small recent and old haemorrhages in the hemispheres and cerebellum. *Histology:* congophilic angiopathy of small arteries in the meninges in all areas but of exceptional severity over the cerebellum; some vessels occluded; severe congophilic angiopathy in cerebellar cortex. Amyloid appeared to spread from vessels into the parenchyma in a fibrillary radiating fashion; large numbers of senile plaques, with and without cores in cerebellar cortex, mainly in molecular layer. Many cores may represent destroyed capillaries (Fig. 10*c*). Infarcts a few days old in cerebellar cortex; a few congophilic vessels in white matter. Cerebral hemispheres showed some congophilic arteries and small recent infarcts in cerebral cortex. Severe congophilic angiopathy in centrum semiovale with diffuse loss of myelin and many small recent infarcts (Fig. 11); congophilic vessels present in necrotic areas, but newly sprouted capillaries in the organizing edge looked unremarkable. Typical senile plaques (Fig. 10*a*, *b*) and clusters of fragmented, twisted and knobbly nerve fibres (Fig. 10*a*) in hippocampi; many neurofibrillary tangles in both hippocampi; no plaques or neurofibrillary tangles elsewhere in cortex; some vessels occluded by embolic material; small old scars present in cerebrum and cerebellum. Occasional congophilic vessels in brain stem. Other organs showed no amyloid change.

DISCUSSION

Congophilic angiopathy with perivascular and free-lying senile plaques was first described by Scholz (1938) who found it in the brains of 12 per cent of a mental hospital population, all the affected patients being over 70 years old. The condition has since been reported in demented old people in other

series, notably those from the Bel-Air Clinic (Morel and Wildi, 1952; Pantelakis, 1954; Surbek, 1961). Congophilic angiopathy in younger patients is very rare. There have been familial cases (Worster-Drought, Greenfield and McMenemey, 1940, 1944; van Bogaert, Maere and de Smedt, 1940; Lüers, 1948; Surbek, 1961) and a few sporadic cases (Benedek and McGovern, 1949; Corsellis and Brierley, 1954; Neumann, 1960), often presenting as presenile dementia with unusual clinical signs. All cases have shown a progressive dementia of insidious onset.

The six patients with congophilic angiopathy reported here differ from previously reported cases in the apparently rapid onset of the dementia, which was closely related to some intracranial catastrophe or general metabolic disturbance (see Table II). It is impossible to date the onset of a

TABLE II

SUMMARY OF CLINICAL FINDINGS

Case	*Sex*	*Age at onset*	*Duration of mental illness*	*Precipitating factor*
1	F	66	3 years	Head injury
2	F	62	4 months	Subarachnoid haemorrhage
3	M	65	2¾ years	Subarachnoid haemorrhage, carotid ligation
4	F	68	6 months	Mastectomy, removal of pituitary adenoma
5	M	60	3 months	Respiratory insufficiency
6	F	55	7 days	Cardiac surgery
I.W.*	M	65	15 months	Osteotomy

* Case of Mandybur and McMenemey (1969, personal communication.)

dementing process precisely, since insidious intellectual deterioration may be skilfully concealed by the patient and his associates. However, the patients in this series were at work or coped adequately at home before the precipitating illness and were severely or completely incapacitated shortly afterwards—that is, often within hours or days. The acute illness therefore probably played a part in the sudden mental deterioration even if slight mental changes had already been present.

The most striking abnormality in the brain in all six cases was the presence of widespread congophilic angiopathy and of typical senile plaques, and it seems reasonable to assume that the deposition of congophilic material in the brain was connected with some event occurring during the acute illness.

The abnormal substance in vessel walls and in the plaques in our cases, as in those of other workers, had the same staining reactions and optical properties as the amyloid which is found in systemic amyloidosis (Divry, 1934), and electron microscopy (Terry, Gonatas and Weiss, 1964; Schlote,

1965) has confirmed that the material has a fibrillary structure similar to amyloid. With few exceptions (Marinesco, 1931; Götze and Krücke, 1942; Haberland, 1964) the deposition of amyloid in the central nervous system has not been associated with massive systemic amyloidosis. Congophilic change in the blood vessels of other organs as well as in the brain has been seen by some workers (van der Horst, Stam and Wigboldus, 1960; Schwartz, Kurucz and Kurucz, 1964) though not by others (Sperr, 1957; Pantelakis, 1954; Surbek, 1961). No amyloid was seen outside the central nervous system in our cases.

The rapidity with which amyloid degeneration can apparently occur is remarkable. In Case 6 severe changes were present seven days after the operation. In Case 1 the angiopathy and senile plaques were seen in a biopsy of the striatum taken during a period of unexplained coma eight days after a head injury. In view of the rarity of the disease and the normal pre-traumatic mental state of the patient it seems unlikely that the lesions were already present before the accident. Whether the amyloid change seen in our patients *post mortem* occurred in one episode or continued over the months cannot be determined. Clinical evidence is not available since in the absence of formal testing it is difficult to decide, in restrospect, whether the dementing process was truly progressive.

As in previously reported cases the vascular disease was confined to the meninges, the cerebral cortex and the basal ganglia, the latter however being only slightly affected. There was no accentuation of the angiopathy in the occipital cortex as described by Scholz (1938) and others. Case 6 was exceptional in that severe congophilic angiopathy was present in the cerebellar cortex and its meninges and in the white matter of the cerebral hemispheres. In cases with generalized systemic involvement amyloid masses are found under the ependyma and in the meninges (Krücke, 1950; Haberland, 1964). There is no explanation for these differences in distribution of the amyloid degeneration.

Typical neurofibrillary tangles were present in four cases. In addition to perivascular plaques all the varieties of senile plaques seen in Alzheimer's disease or in senile dementia were found in each of our cases. Thus they have pathological features in common with classical Alzheimer's disease but whether the pathogenesis of the lesions is always the same remains to be determined.

It is difficult to assess the contribution made by the various pathological changes in the brain to the clinical picture; this was dominated by the mental changes and there were remarkably few abnormal neurological signs. The vascular alterations may have borne the main responsibility for

the dementia but it is not clear by what mechanism cerebral function was disrupted. There was surprisingly little structural alteration in the surrounding parenchyma, infarcts were few and there appeared to be little neuronal loss, though the latter has to be severe before it becomes obvious. The senile plaques and neurofibrillary tangles may have affected the mental state, but these changes were absent or scanty in some of our cases. Blood vessels in the white matter were not congophilic, except in Case 6, though they were sometimes hyalinized. Nevertheless, there was loss of myelin in the centrum semiovale in all cases, possibly because its supply arteries were affected in their intracortical portions. Such loss of myelin has been commented upon in previous case reports and may have contributed to the organic dementia of our patients and other patients with congophilic angiopathy.

No attempt was made to quantitate the amount of vascular involvement, or to correlate it with the number of senile plaques. Plaques were counted in different areas of the brain, the purpose being to determine the *pattern of distribution* of plaques within a given brain rather than to compare absolute plaque counts in different brains. A simple standardized sampling technique was used on paraffin sections stained with PAS/LFB. (This method rather than a silver impregnation was chosen because it is more reliable.)

There were marked differences in mean plaque count between different regions of a hemisphere and in some instances between similar regions in the two hemispheres (see Table I). By mapping microscopic fields with a high density of plaques we have found that even within a given area the distribution of plaques is far from uniform. In Case 1, for example, numerous plaques were found in the entorhinal cortex and the overlying lower third of caudate nucleus and putamen (Fig. 6), whereas no plaques were seen in the upper two-thirds. It may be significant that the two regions have a different blood supply (upper two-thirds—middle cerebral artery; lower third—anterior choroidal artery). In Case 4 many plaques were found in the cortex lining the inferior temporal sulcus of both temporal lobes (Fig. 8). This may have been the boundary zone between the territories of the middle and posterior cerebral arteries in this patient, though the inferior temporal sulcus is more medial than the textbook position of the boundary zone. That the distribution of plaques in a given area of cortex is not random is further demonstrated by plotting the plaque counts per field in histograms (Fig. 9). The shapes of these histograms were frequently irregular and varied from section to section. When counting plaques it seems advisable to employ systematic sampling of the section rather than rough scanning.

It is impossible to compare the absolute plaque counts of different authors (Blessed, Tomlinson and Roth, 1968) because of differences in preparing and staining the sections, differences in sampling, etc. Comparison of regional variations in plaque counts may be more meaningful. Differences between various areas of brain and between the two sides of the brain have been noted (Simchowicz, 1924; Grünthal, 1926; Jamada and Mehraein, 1968) but the pattern is not consistent. An odd distribution of plaques has on occasion been reported. Thus the case of Hemphill and Stengel (1941) showed generalized Alzheimer's disease with much more severe atrophy and more senile plaques and neurofibrillary tangles in the right hemisphere. In 1952 von Braunmühl described a patient with cerebral syphilis in whose brain the only region to contain senile plaques and neurofibrillary change was the right hippocampus. More detailed mapping of plaque distribution within cortical areas might yield interesting information.

Single or multiple head injuries, as in boxing, have on occasion been followed by a slowly progressive dementia with the pathological finding of Alzheimer's disease (Brandenburg and Hallervorden, 1954; Corsellis and Brierley, 1959; Grahmann and Ule, 1957). Only one of these patients, the boxer reported by Brandenburg and Hallervorden, also showed congophilic angiopathy. At present nothing useful can be said about the pathogenesis of Alzheimer's disease after head injury.

It is known that patients may become demented after a subarachnoid haemorrhage (Storey, 1967; Logue *et al.*, 1968). Senile plaques or neurofibrillary tangles have not been reported in such cases, perhaps partly because these changes cannot be seen unless special stains are employed.

None of our patients was hypertensive. One (Case 3) showed some arteriosclerosis of small blood vessels, but curiously arteriosclerosis and congophilic angiopathy were not seen in the same vessel.

Several conditions which might have affected the cerebral circulation were present in our patients during the acute episode which preceded the onset of mental change. Such conditions include periods of hypotension and reduced cerebral blood flow (Adams *et al.*, 1966), subarachnoid haemorrhage which can produce vasospasm (du Boulay, 1963) leading to infarction (Crompton, 1964; Smith, 1963), severe CO_2 retention, and prolonged slowing of the cerebral circulation which has been demonstrated after head injury in man (Taylor, 1966) and in monkeys (Ommaya, 1966). All this suggests that changes in the cerebral circulation should be considered when discussing the pathogenesis of congophilic angiopathy and of senile plaques, though it may of course turn out that other factors are more important.

Most patients with Alzheimer's disease or senile dementia are admitted to hospital when the disease is already of long standing. Significant illnesses or trauma which may have precipitated or accelerated the dementia may have been forgotten or overlooked by the informants. Even when the dementia is apparently of insidious onset and steadily progressive it could prove useful to enquire for precipitating factors which may play a role at the beginning or during the illness.

SUMMARY

Six patients are presented in whom a catastrophic illness such as subarachnoid haemorrhage, head injury or cardiac surgery was followed, within hours or days, by severe and permanent mental changes. The main pathological findings were widespread congophilic angiopathy and typical senile plaques in all cases and neurofibrillary change in nerve cells in four. The angiopathy was most severe in the cerebral cortex and in the meninges over the hemispheres, but in one case the cerebellum and the centrum semiovale were also affected.

There were marked differences in the number of plaques in different areas of the same brain and even in a given area the distribution of plaques was not uniform.

It is suggested that generalized metabolic or vascular precipitating factors should be taken into account in the aetiology of typical Alzheimer's disease.

Acknowledgements

This work was supported by grants from the Mental Health Research Fund and from the Research Fund of the Bethlem Royal and Maudsley Hospitals.

ADDENDUM

Since this paper was written the case I. W. of T. I. Mandybur and W. H. McMenemey (1969, personal communication) has come to our notice. This 65-year-old man was in good mental health until an operation for osteoarthritis. Post-operatively he was excited and confused and it became clear that he was demented. He had several strokes, suffered from epileptic fits and died 15 months after the operation. The brain showed many infarcts, widespread congophilic angiopathy and numerous senile plaques.

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DISCUSSION

Gonatas: The tissue culture studies of Bari, Pettengill and Sorenson (1969) have indicated that there is new formation of amyloid in splenic explants, where obviously no serum factor or blood vessel is present.

Cohen in his recent review on amyloidosis has indicated that thioflavine S staining is notoriously non-specific for amyloid (Cohen, 1967). Also, metachromasia and congophilia are not absolute markers for the identification of amyloid. Ultrastructurally, however, amyloid fibres clearly have a fine structure distinguishable from neurofilaments, microtubules, Alzheimer filaments, fibrin elastin, collagen and many other things. Dr Schwartz's paper (1965) on amyloid in the blood vessels in Alzheimer's disease made us look again with the electron microscope at five brain biopsies from patients with Alzheimer's disease. Even though we have so far had only a few large vessels in the subarachnoid space we saw many vessels with muscular walls, probably venules or arterioles, without any ultrastructural evidence of amyloidosis. I would like to make a plea for some reconsideration of the criteria for identifying amyloid.

Terry: We know for example that cotton fibres are strongly congophilic. They stain well with thioflavine S and are birefringent. Fibrin has exactly the same reactions, so these optical methods for amyloid are grossly unreliable. When Dr Wiśniewski and I wrote the abstracts for this symposium, we said that we hoped to produce cerebral amyloidosis experimentally, because we had already produced strikingly congophilic vessels in rabbit brain. They were the most splendid preparations of that sort I have seen, but each and every vessel turned out to have fibrinoid degeneration when seen in the electron microscope. They were not at all affected by amyloid. In our biopsy studies our sampling methods may have caused us to lose many blood vessels which would perhaps have amyloid infiltration. On the other hand of many thousands of pictures only a single one is of an amyloid vessel, and even this is not a whole vessel. The tangles certainly are not amyloid, unless we are totally unable to identify amyloid by ultrastructural methods.

Roth: Are the ultrastructural phenomena you have described specific for amyloid?

Terry: They are characteristic of amyloid as seen in kidney, spleen and heart muscle, in animals and in humans. It is exactly the same as the material within the plaque.

Roth: But there is no clear evidence at present to suggest biochemical identity.

Terry: Suzuki, Katzman and Korey (1965) found increased amounts of mucopolysaccharide in the brains of patients with Alzheimer's disease, but pure amyloid has not been isolated and this we hope to do. My one picture of an amyloid vessel shows the knuckle of a vessel, that is without the lumen, with a much-duplicated basement membrane investing

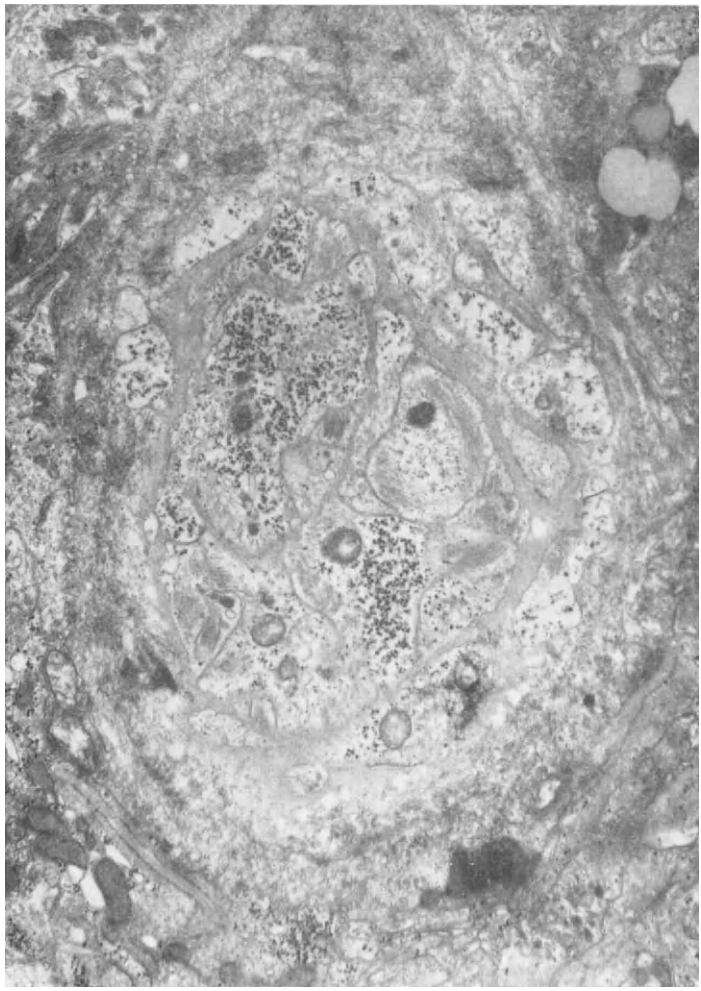
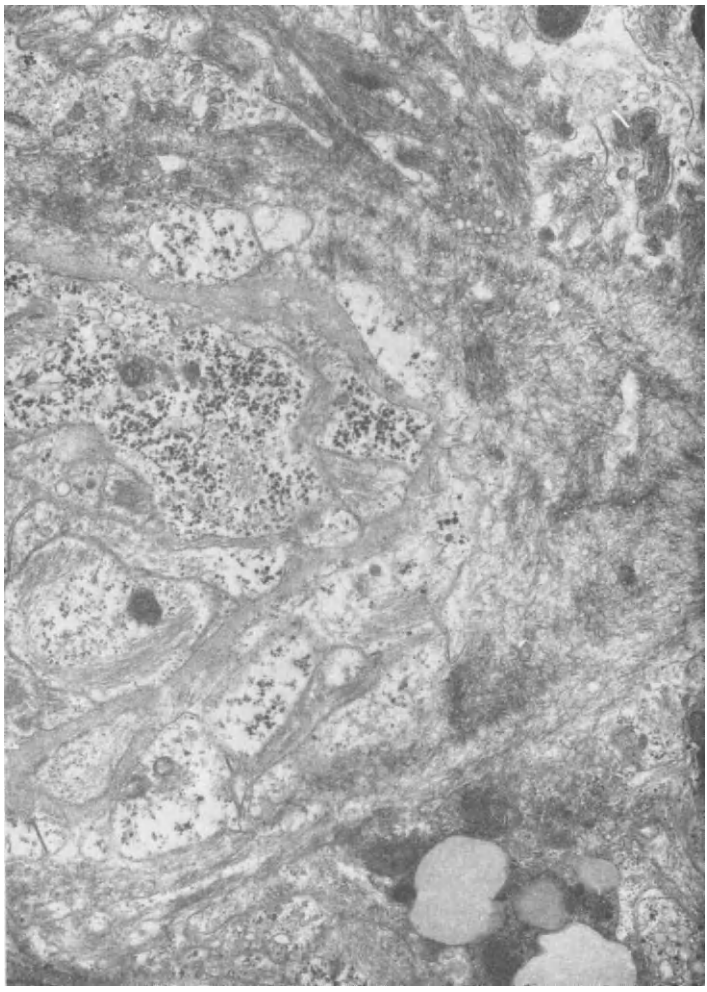


FIG. 1 (Terry). Tangential section through a proliferated, amyloid-invested vessel. The basement membrane is much reduplicated and encloses several astrocytic elements containing glial filaments and glycogen particles. Amyloid filaments are attached to the outer layers of the basement membrane, and are less orderly than the intracellular glial filaments. (*a*) × 10 000. (*b*) × 13 200.

glial cells or astrocytes as well as perithelial elements (Fig. 1). Coming out of some of this basement membrane is extracellular filamentous material typical of amyloid and quite separate from the intracellular glial filaments.

Gonatas: Amyloid in blood vessel walls is intermixed with basement membrane material and I think your amyloid seems to be outside the basement membrane.



(*b*)

Terry: No, it is quite intimately attached to the basement membrane material.

Gonatas: If higher magnifications show that the amyloid is mixed with basement membrane, I agree that this is good evidence for amyloid in the blood vessel wall rather than around the blood vessel.

Shelanski: The microtubules, whether they exist in the mitotic spindle or in the neuron, are highly birefringent without any staining being necessary. I cannot comment on cerebral amyloid but we have made electrophoretic studies, under conditions which determine only the

molecular weights of subunits, of amyloid from kidney and from heart. On a molecular weight basis we cannot discern any difference between them.

Gonatas: The orientation of the molecules of the stained material, not its chemical specificity, is what causes metachromasia.

Kidd: My electron microscopic experience with amyloid is the same, but I once found a picture like the one Dr Terry showed us, but in a very demented old man with multiple myeloma, not Alzheimer's disease, who was one of Dr Hughes' patients. There was an expansion of what appeared to be amyloid arising in the basement membrane layer, pushing right out and surrounded by cell processes. It might have been microglial. This was a capillary-sized vessel.

Strich: Is electron microscopy the only way to decide whether a substance is amyloid or not?

Terry: I would rather put it the other way. The Congo red stains are not the way to ascertain that it is amyloid.

Strich: What about other stains?

Terry: I don't know any that are specific.

Martin: I have been able, although not in the central nervous system, to make good correlations between Congo red staining with green birefringence, thioflavine S yellow fluorescence and the ultrastructural demonstration of generalized amyloid (Martin *et al.*, 1970). Green birefringence after Congo red is in favour of an arrangement of this cotton dye between filaments. On the other hand, isolated amyloid is made up of a doughnut structure as well as filaments.

Terry: The doughnut structure now appears to be something else altogether.

Shelanski: There are two schools of thought: one group feels there is a difference between the doughnut and filaments, and the other that they are the same.

Friede: Congophilic angiopathy may have a predilection for the occipital lobes. How often was it found in other lobes of the brain?

Martin: In samples I gave Dr P. Schwartz from frontal and occipital lobes from eight cases of Creutzfeldt-Jakob disease who were between 55 and 68 years old, he found approximately equal amounts of amyloid in both regions.

Strich: In the cases I described the degree of angiopathy was the same front and back.

Tariska: I have studied the incidence of congophilic angiopathy in different parts of the brain from five cases. This was apparently not

restricted to the occipital lobe, but was usually spread through the whole brain, accentuated—like the plaques—in the depths of the sulci. Probably in a few cases it can also be found in the brain stem but never in the medulla.

Friede: Dr Terry, do you make a diagnosis of congophilic angiopathy, or does the uncertainty of identification of amyloid keep you from using it?

Terry: I do not imply that nothing that stains with Congo red is amyloid. I just say that not everything that stains with Congo red is amyloid.

Roth: The abrupt development of Alzheimer's disease or senile dementia which was such a striking feature in Dr Strich's cases has been described previously. Some years ago Bedford's (1956) cases of dementia developing suddenly and rapidly after anaesthesia caused quite a stir. However, the course of events is difficult to interpret unless a great deal of information is available about the mental state and adaptation of the individual before the critical event held responsible.

Polak: Did you see any microglia, Dr Strich? Microglia are a good way to demonstrate the age of the plaque. In the early stages the cells are mainly microglial.

Strich: I haven't seen any in the very acute case but there were some in the others. How do you know that this is the beginning of the plaques?

Polak: We have studied senile plaques in Alzheimer's disease and in normal senility. When the plaque is an older one, the microglia disappear and we find astrocytes only in the periphery and not in the middle of the plaque. But at this time we don't find the other things that form the plaque.

Shelanski: How do you know you haven't cut the section ahead of or behind the plaque?

Polak: The argyrophilia is always seen at the beginning of the plaque, and there are mobilized microglia only around the substance that is correlated with the pathology of the senile plaque relating to capillaries in the brain, as Dr van Bogaert showed some years ago. We always look at the vessels and the plaques together. The age of the plaque is correlated with different types of cell structures.

Terry: Yes, but how do you know that is an early plaque?

Polak: We can find all transitions between an early and an old plaque.

Terry: How do you know what is an old one?

Polak: In the beginning we always find only this argyrophilic substance. If you use only one technique for senile plaques you will always find an argyrophilic effect. But in every plaque you will find different things according to whether you study it for vessels, or for reticulin or for glia or for oligodendroglia.

Roth: How do you date the plaque? Are you arguing from general pathological principles?

Polak: Yes.

McMenemey: Are those microglial creatures you have shown in a plaque coming or going? They could be taking material either to or from the plaque.

Polak: I cannot say. We can only demonstrate that at this time there are a lot of microglia in the plaque, whereas normally there are not many. Also, the microglia far from the plaque are always swollen and proliferating. In the plaques we always find about 15 cells in one section.

McMenemey: If you find thin ones, it could mean that they are going into the plaque to eat. Presumably the fat ones have eaten and are retiring from the plaque; on the other hand they could be carrying in amyloid in order to dump it.

Polak: It could be either.

McMenemey: I wish we knew if it was amyloid or fat they contain, or can it be both?

Jacob: Is amyloid in other organs argyrophilic, as it seems to be in the brain?

Polak: It is argyrophilic in other organs.

Terry: Amyloid is argyrophilic in the brain too. It stains variably, and it depends on how one differentiates the Congo red stain or the silver stain.

Jacob: With different silver techniques and methods one can get quite different results.

Hughes: Is all argyrophilic material in the brain amyloid?

Terry: No.

Polak: Not every argyrophilic substance is amyloid but amyloid is always argyrophilic. If one stains another section with a technique specific for amyloid and compares it with the silver stain, one can have a general point of view about argyrophilic material. If one stains with various techniques for amyloid in every part of the body, it always appears as argyrophilic.

Terry: Divry (1934) and von Braunmühl (1957) both pointed out that the earliest change in the neuropil is argyrophilia. Divry said that this *substance trichosique* is present and is argyrophilic before there is congophilia. This means to me that there is argyrophilic material before there is amyloid in the plaque, and we have evidence for this by electron microscopy as well.

van Bogaert: Divry also said that not every plaque has amyloid.

Tomlinson: Some cases of dementia with heavy plaque formation have little congophilia of blood vessels. Yet some non-demented old people without senile plaques have quite marked congophilia of blood vessels. On light microscopy, at any rate, some plaques do not contain congophilic material.

Roth: The electron microscopists have stated that the only morphological change which can be regarded as amyloid in character is identical in structure with proven amyloid in other tissues. This is present in some stages of plaque formation, or is it to be found in all plaques?

Terry: At high magnifications, say ×20 000, one will invariably see some amyloid and this may be invisible even at ×5000, let alone at light microscopic levels. The smallest lesion, however, is free of amyloid.

Roth: One occasionally sees patients of the kind Dr Strich has described, who appear to become demented in a step-like manner after anaesthesia, head injury, after bereavement or even after sudden removal to hospital. However, when a detailed history is available, it often becomes apparent that in many of these patients impairment of memory and deterioration of intellect and personality had been present before the accident, although often effectively concealed under a well-preserved social facade. These impairments may have developed slowly, may have been partly detected or unobserved. There is also a natural tendency to incriminate erroneously some dramatic event in the middle of a process. On the other hand, in a few patients, very limited brain damage may perhaps suffice to push the individual over the edge, as it were. We have observed (Roth, Tomlinson and Blessed, 1967; Blessed, Tomlinson and Roth, 1968) that there is a clear threshold effect in the relationship between plaques, which provide one measure of neuronal loss, and dementia. Most subjects with an average of more than 15 plaques per field have been indubitably demented during life. However, in subjects with mean counts of less than 12, dementia is very rare unless the damage is augmented by other changes such as infarction. There is some evidence that threshold levels also exist for other measures of cerebral degeneration in old age. If the plaque count is just below the threshold even minor cerebral change, such as that sustained during anoxia in anaesthesia or head injury or severe respiratory infection, might well push the individual beyond threshold point, so that he manifests dementia. However, the better the history the less inclined one feels to advance such explanations. Were the histories of your patients good enough for one to say that the affected persons were relatively normal before this event, Dr Strich?

Strich: As these patients were all perfectly well beforehand they had not been examined by a psychiatrist. We can only go by information from relatives.

Nevin: Is this gross dementia anatomically explained? In two or three days there must be very great damage to the brain to get this severe loss of function. What has caused this?

Strich: Perhaps one ought to think of these anatomical changes as symptoms of some general disturbance. Except in the patient who underwent cardiac surgery, there were very few infarcts and the neuronal loss generally was doubtful. I don't know in what way these vascular changes affect the functioning of the brain.

Nevin: The cardinal point in these cases is that there is a gross loss of mental function. Dr Terry, could the amyloid degeneration of the vessels occur within a few days?

Terry: I couldn't say at all.

Martin: One would have to test the patients very carefully indeed. The last patient with proven Alzheimer's disease we saw was a man who had been working normally until there was a slight irregularity in the hours of work. When we tested him, his IQ was only 48, but he still behaved quite normally and seemed apparently only depressed.

Dr Strich, the patient who underwent cardiac surgery had a mitral stenosis. Could blood clots dislodged by the surgeon during the operation produce infarcts, rather than the amyloid disease?

Strich: Yes, I think so. Most of the infarcts were recent. In some instances, though, the vessels were occluded by amyloid.

Hughes: Was there cerebral atrophy in the cases that went on for three or four years? Would you say they were cases of Alzheimer's disease?

Strich: How many plaques and tangles are needed to make it Alzheimer's disease? Some of the cases did not have Alzheimer's disease, two had no tangles and one had very few plaques. There wasn't much cerebral atrophy. All those who lived longer had ventricular dilatation, and brain weights of 1200 or 1150 g.

McMenemey: In the osteotomy case the question of fat embolism was raised shortly after the operation. The patient who had undergone cardiac surgery had been ill for some time with chronic heart disease and maybe that state had something to do with the brain condition. Had she not had the exacerbation of her cardiac condition and therefore had there been no operation, one wonders if she might not have shown a pathological disorder of the brain in later life.

Professor van Bogaert referred to the family Dr Worster-Drought,

Dr Greenfield and I described (1940, 1944). I remember Dr Worster-Drought when he brought in the brain for study saying that this was not Alzheimer's disease but something different, something he had not seen before. The two cases which came to autopsy turned out to have, rather like Dr Strich's case 6, these bizarre perivascular plaques in the cerebellum, demyelination of the white matter with not too many cortical plaques, and marked neurofibrillary degeneration in the hippocampus. Looking back, I think that pathologically it was primarily an instance of familial cerebrovascular amyloidosis, with Binswanger's disease superimposed because of the destruction of the white matter consequent upon the prevalence of the blood vessel disease there.

Dayan: In aged animals the only change described with any frequency is congophilic angiopathy, and plaques and tangles have rarely been described in their brains. This apparent difference between animals and man is striking and might throw some light on the possible mechanisms of senescence of the brain.

Terry: Have you looked at really aged animals like turtles, elephants and parrots, or are you talking about mice and rabbits and so on?

Dayan: Von Braunmühl (1956) described plaques in dogs aged 18 to 20 years. I have examined the brains of fifteen dogs older than 14 years and in none were there any demonstrable lesions. I have also studied the brain of a giraffe aged 26 years, which is much older than the expected lifespan, and it too showed no plaques or tangles. In a group of eight parrots aged up to about 30 years, which is as long as they usually live, there was congophilic angiopathy in four but no plaques or tangles. Of 40 pure line BALB/c mice living up to 30 months, two had congophilic angiopathy but no other cerebral lesions.

Roth: Until about six years ago Dr Heinrich Klüver had the oldest living monkeys I have ever seen in his laboratory in Chicago. Some of them were survivors from the early temporal lobectomy experiments. The appearance and behaviour of these animals resembled strongly that in human senile or arteriosclerotic dementia. I know that careful post-mortems were done on the animals that died and it would be of great interest to have the brain sections examined for plaques, neurofibrillary change and softening.

van Bogaert: Dahme and Deutschländer also wrote a very nice paper (1967) on congophilic modification in senile dogs.

Hirano: Dr Strich, did you examine the formalin-fixed congophilic material with the electron microscope?

Strich: No, not yet.

Hirano: Many fibrillary structures such as Alzheimer's neurofibrillary tangles, fibrin, glial fibrils, etc., may still be rather well preserved and identifiable even after prolonged formalin fixation. Electron microscopic examination of your material may therefore be useful.

Tomlinson: I saw a case some three years ago of a three-week survival in coma after rupture of an intracranial aneurysm in a woman of 42 years, in whom a considerable number of senile plaques were present. In cases of subarachnoid haemorrhage since that time I have not seen plaque formation. I have also examined a number of cases surviving weeks or months in deep coma after head injury. They show no tendency to have excessive numbers of plaques.

Hyalinosis in renal blood vessels has been shown electron microscopically to consist of two types of granules, one characteristic of ferritin, the other an unidentified protein moiety (Fisher, Perez-Stable and Pardo, 1969). In those same biopsies there were vessels showing light microscopic fibrinoid necrosis, but this also, in electron microscopy, consisted of ferritin and a protein moiety, and fibrin could not be identified. Such observations destroy faith in the specificity of light microscopic distinctions.

Dr Strich's examination of localized areas was in greater detail than we have ever done. The only thing that I could say about the distribution of plaques, in cases of Alzheimer's disease with a high mean plaque count, is that it is rare to find any area of the brain not involved to some extent; great variations even from field to field certainly occur. The commonest place to see heavy plaque formation, whether in Alzheimer's disease or in non-demented old people, is in the depths of the sulci, and that appeared to come out in Dr Strich's pictures. The depths of sulci often gave a false impression of plaque distribution over the rest of the cerebral cortex.

Roth: One possible implication of these findings for clinical practice should perhaps be mentioned. Heroic surgery is sometimes done on elderly patients without any examination of the mental state to ascertain whether there is evidence of memory defect or dementia. In the nature of the case, conclusive evidence is unobtainable and Dr Strich is, therefore, making a contribution of value in bringing these striking cases to our attention. It is possible that a prolonged anaesthetic for some heroic pieces of surgery may decide whether an elderly subject has to contend with some memory defect alone or whether she develops a more global intellectual and personality defect, having already had a certain amount of cerebral damage which could have been demonstrated, as Dr Martin said, by very careful testing. Other decisions might prevail in some such

cases if evidence of cerebral damage were brought to light. The differences in the mode of onset and course of Alzheimer's disease and senile dementia deserve closer attention.

Strich: We know nothing about the pathogenesis of Alzheimer's disease, so I do not think one should just dismiss these cases as having already had congophilic angiopathy, Alzheimer's disease or whatever, when the acute illness overtook them. You are probably right, but we need a change in outlook about these diseases: there may be generalized disease, there may be precipitating factors.

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ALZHEIMER'S DISEASE AND RELATED CONDITIONS
G. E. W. WOLSTENHOLME & MAEVE O'CONNOR

THE GENETICS OF ALZHEIMER'S DISEASE

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THERE are more than a dozen families (see Pratt, 1967) in which Alzheimer's disease, or a condition closely resembling it, is transmitted as a regularly manifested dominant trait. In some of these instances there are features peculiar to the family concerned, such as muscular twitching (Jacob, 1970), spastic paraplegia (van Bogaert, Maere and de Smedt, 1940), amyloidosis of the cerebral vessels (Worster-Drought, Greenfield and McMenemey, 1944; Corsellis and Brierley, 1954), or the absence of plaques and tangles on light microscopy in a family with early age at onset (Schaumburg and Suzuki, 1968). The regular Mendelian ratios and the close intrafamilial resemblance of these familial instances suggest that aetiologically they differ from the common form of Alzheimer's disease.

In sharp contrast, consecutive patients with Alzheimer's disease display a slight though definite tendency to familial aggregation of the disorder. There are three large and careful studies on the genetics of Alzheimer's disease and its relation to senile dementia, two of them from Sweden (Sjögren, Sjögren and Lindgren, 1952; Larsson, Sjögren and Jacobson, 1963) and the third from Switzerland (Constantinidis, Garrone and de Ajuriaguerra, 1962). Starting from patients with Alzheimer's disease as propositi, the findings from the two countries are in broad agreement. Of 72 parents in the Swedish series three had Alzheimer's disease and two senile dementia; of about 100 sibs aged 40 years or more there were two with Alzheimer's disease and two with senile dementia. In the Swiss series the proportions affected with Alzheimer's disease and senile dementia respectively were, for parents 1·4 per cent and 8·4 per cent, for sibs 3·3 per cent and 1·9 per cent, and for children 1·6 per cent and 1·6 per cent.

When patients with senile dementia were the propositi, the findings differ, for in the Swiss series the incidence of Alzheimer's disease was 0·4 per cent in sibs and 2·2 per cent in children, whereas in the Swedish series there was not a single instance of Alzheimer's disease in over 2000 first-degree relatives above the age of 50 years. The evidence from Sweden

was interpreted as indicating the determination of senile dementia by a single autosomal dominant gene carried by 12 per cent of the general population, and reaching 40 per cent manifestation at the age of 90 years. There are many difficulties (Carter, 1969) in deciding between polygenic inheritance with threshold effect on the one hand, and dominant inheritance with reduced manifestation on the other hand, in common diseases. I myself think that the above data, together with the finding in the Swiss series of both diseases occurring within the same sibship (both sibs with Alzheimer's disease on six occasions, both with senile dementia on 20 occasions, and both affected but one with Alzheimer's disease and one with senile dementia on four occasions), are more in keeping with polygenic inheritance with a shared predisposition both to Alzheimer's disease and senile dementia.

POSSIBLE GUIDES FOR FUTURE INVESTIGATIONS

Are there any findings in other fields of genetics that may offer some guide to future investigations on the aetiology of Alzheimer's disease? First, if a single-gene abnormality underlies Alzheimer's disease, then the risk "will be confined to carriers of the gene; and it is reasonable to undertake research to elucidate the nature of the specific defect" (Kay, 1964). Secondly, if the familial concentration is polygenic, it is not a hopeless quest; the success in tracing the factor of "muscularity" in infantile pyloric stenosis (Carter, 1961) and the influence of blood-group factors in peptic ulcer (McConnell, 1966) exemplify this. Thirdly, the evidence against recessive inheritance makes it unfruitful to look for an enzyme defect; it seems that in recessive disorders the presence of half the quantity of normal enzyme is a sufficient safety factor to protect heterozygotes from developing symptoms. A more probable defect would be in a structural protein, but if this were the case it might be difficult to detect the abnormal 50 per cent of a protein present possibly in very small quantities. Fourthly, the variety of amino-acid substitutions now known in the α and β chains of haemoglobin (Lehmann and Carrell, 1969) suggests that there may be many alleles at every gene-locus (Harris, 1969), and that the ultimate abnormality in the DNA may not be the same in every family affected by what appears to be an identical disorder; there may be greater heterogeneity within genetically-determined disorders than hitherto assumed. Finally, the role of somatic mutations in ageing of the cell may have great relevance in the degenerative disorders of later life.

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DISCUSSION

Roth: Dr Pratt has pointed out that according to what view one takes, one's research strategy is affected. On the one gene-one enzyme hypothesis one would look for a single specific enzyme deficiency or abnormality. If one thinks there is some polygenic abnormality the situation is rather different, but not necessarily entirely different, because of the rather important recent ideas about a threshold effect. The sex ratio in pyloric stenosis, and the difference in risk to the relatives of male and female index cases, are very interesting points.

Barondes: What about concordance rates in identical twins?

Pratt: The concordance rates in senile dementia are quite convincing. In Kallmann's (1956) series the figures were 43 per cent for monozygotic and 8 per cent for dizygotic twins. In Alzheimer's disease there is only the monozygotic pair that Dr McMenemey mentioned (Davidson and Robertson, 1955), with discordance after 20 years.

McMenemey: Two families have been described in which the disease appeared after a first-cousin marriage. In one family a male child of such a marriage and four of his five children all developed the disease in typical form in the fourth decade. There was only one autopsy, I believe (Lowenberg and Waggoner, 1934). In the other family three out of four children of a first-cousin marriage had the disease. I studied one of these three

and a child of one of them, and we too were only able to secure one brain for study (McMenemey *et al.*, 1939). Would it still be correct to call these the result of a recessive gene?

Pratt: There are two possibilities. The first is that the first-cousin marriages are fortuitous; the parental consanguinity rate in the general population at that time was about 1 in 100. The second possibility is that the affected parent of the affected offspring married a heterozygote; with a rare recessive (with an incidence of 1 in 1 000 000), the carrier rate in the general population would be about 1 in 500. I therefore think that these first-cousin marriages are not indicative of recessive inheritance, and that the disorder was dominantly inherited in the two families.

Terry: What genetic conclusion would you draw if the chemists and morphologists were able to demonstrate that there is but a single abnormality in this disease, which leads to all the other abnormalities? If this single abnormality were a single abnormal protein, would this distinctly mean a single gene, or could it still possibly be polygenic?

Pratt: It would mean that there was a single gene. But the fact that the disorder is not uniformly displayed in a Mendelian way would mean that some other contributory factor is required. If a child was born with galactosaemia and was never exposed to lactose or galactose, he would appear clinically quite normal; even something which is definitely determined by a single-gene abnormality may need environmental factors to bring it out. Of course everyone is exposed to lactose, but if it was something that only 10 per cent of the population happened to get exposed to, then the manifestation rate would be diminished.

Terry: Several environmental factors in an ageing population might have such an effect. Iron concentration is known to be elevated in the older brain, especially in Alzheimer's senile dementia. Poverty of antioxidant is another factor.

Pratt: Larsson, Sjögren and Jacobson (1963) paid attention to the possibility of environment playing a part. They concluded that the incidence of the disease was similar in rural and urban communities, although the hospital admission rate is higher in urban communities. If one is looking for an environmental cause, one is likely to miss the thing that turns out to be the answer. The kuru situation exemplifies this rather well; cannibalism is not something I would have thought of as the exogenous factor.

Roth: A question with a bearing on the kind of genetical factor likely to be operating is whether there is any qualitative difference, either structurally or biochemically, between normal persons and those with unequivocal Alzheimer's disease. The light microscopic appearances of plaques

and tangles are known to be similar in the two situations (Gellerstedt, 1933), and as far as I know, the only difference in the changes seen is a quantitative one. Dr Terry, have biopsies from mentally normal subjects (undergoing neurological investigation) been examined by modern electron microscopic techniques?

Terry: Senile plaques and Alzheimer's neurofibrillary changes have not been found in biopsies taken for other reasons, such as the study of lipidoses. So we can't compare plaques in the presence of normal mentation with plaques in dementia.

Roth: Is it known whether plaques and tangles from normal elderly subjects differ ultrastructurally from those in cases of Alzheimer's disease?

Shelanski: We could probably assume that wherever plaques and tangles are seen they are probably morphologically and biochemically the same as those in senile patients and those with Alzheimer's disease. The key question here is topography. One may see at autopsy a patient who has no history at all of multiple sclerosis, yet in some not very critical area there is one plaque or multiple plaques. He was just fortunate that his disease was in a "silent" area of the brain. Similarly, some patients probably could be classified pathologically as cases of senile dementia or Alzheimer's disease who had not yet developed the clinical picture, but who might have done so if they had lived long enough. My point is that topography would affect the clinical expression. If one really wanted to nail down the genetics one would have to do this microscopically and see whether the close relations who never express the disease clinically have substantial "Alzheimer changes" or not.

Pratt: This is very important. The closer one gets to the gene—and that means on the biochemical level—the more likely one is to get the right answer.

Roth: Are we not too lightly assuming that, genetically speaking, we deal with a homogeneous rather than a heterogeneous phenomenon? The families with many cases of Alzheimer's disease may well be different genetically from the other cases, and they are possibly illuminating from a biochemical point of view.

Taylor: Could the genetic data be used to investigate other variants of the hypothesis, such as the two genes to one protein factor? Besides the structural gene, some regulatory genes must be involved. Also, if the protein is being made in large amounts, the cell may have more than one copy of the gene.

Pratt: If Alzheimer's disease is a dominant and if a structural protein is

involved, 50 per cent of the protein concerned might occur in an electrophoretically abnormal form, or in a chemically abnormal form. To have much chance of finding this, one would have to study early states of the disease, rather than later stages when secondary changes may have supervened. It might be profitable to look chemically at those people who show the slightest, but nevertheless characteristic, histological changes without much in the way of secondary chemical abnormalities. I would suspect they are the people with mild memory disorder who if they lived longer would become demented.

Hughes: The sex distribution is usually about 3 : 1, females to males, in Alzheimer's disease, but I had two cases of men with collapsed vertebrae due to osteoporosis, which of that degree is unusual in men. Of course that could be cause or effect, since patients with Alzheimer's disease can develop malnutrition. What is the significance of finding something in males which is associated with females rather than males?

Pratt: On the analogy of congenital pyloric stenosis (Carter, 1961) one would expect that in a polygenically-determined disease the relatives of the more rarely affected sex, namely males in osteoporosis, would be more frequently affected than the relatives of the females. I don't know whether the data available are good enough though, because of the different hospitalization rate for men and women of similar degrees of illness: women are more likely to go into hospital than men.

Roth: And this is linked with the much shorter male life expectancy; the women much less often have a spouse to look after them at home.

Tomlinson: The suggestion that one should examine histologically the relatives of patients dying of Alzheimer's disease rather than make a clinical assessment is a good one, if it could be achieved. It would have to be done quantitatively against the background of what is present in the population in general. In the eighth decade, 70 to 80 per cent of people show some degree of plaque formation.

Barondes: The suggestion Dr Terry made of looking at other organs is probably very critical for genetic studies. If the abnormality were also present in other organs, one could do fairly large-scale screening.

Gonatas: Before we look to other cells and organs for microtubular abnormalities, we can ask whether other cells of the central nervous system richly endowed with microtubules, such as the oligodendroglial cells, show the same morphological changes (twisted tubules) as the neurons. The answer is no, and for this reason I doubt that we are dealing in Alzheimer's disease with a generalized abnormality of the microtubular protein.

Barondes: Another organ with a lot of microtubular protein is the testis. Is there in fact testicular atrophy in old age? What morphological correlates are there? Are there tangles in the testis? Has silver staining been used?

Kidd: If a dominant gene might be involved, how does the incidence of Alzheimer's disease in Down's syndrome work out?

Pratt: I wouldn't call it Alzheimer's disease in this condition.

Roth: Mongols are said to dement early and the brains of aged mongols contain plaques (Jarvis, 1948).

Dayan: In routine light microscopy of cases of Alzheimer's disease at autopsy I have seen nothing significant in other parts of the body.

Strich: The neurons in the rectum are easy to get at, but I don't know whether anyone has looked at them in Alzheimer's disease.

Tomlinson: I have looked at neurons in the appendix, in the rectum, in posterior root ganglia, and in the anterior horn cells of the spinal cord and have never seen tangles or plaques.

Pratt: Hunter, Ridley and Malleson (1969) biopsied the skin and found no difference in the number or morphology of the Meissner corpuscles under the light microscope.

Dayan: There is no evidence of an increased amount of deposition of amyloid-like material in the viscera specifically in cases of Alzheimer's disease. Regarding the point about neurofilaments, Levy and Poole (1966) have shown a difference in peripheral nerve conduction velocity between cases of senile dementia and the normal population. The cause of this change has not been determined but it could be due to damage to axons.

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ALZHEIMER'S DISEASE AND RELATED CONDITIONS
G. E. W. WOLSTENHOLME & MAEVE O'CONNOR

THE ULTRASTRUCTURE OF THE NEUROFIBRILLARY TANGLE AND THE SENILE PLAQUE*

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THE coarse fibrillar aggregates in the neurons of Alzheimer's disease are well known to all concerned microscopists. Thioflavine S (Schwartz, Kurucz and Kurucz, 1964) or Congo red preparations and birefringence (Divry, 1934) have led some to the conclusion that this intraneuronal substance is amyloid and is identical to the material in the core of the argyrophilic senile plaque. Electron microscopic studies (Terry, 1963; Terry, Gonatas and Weiss, 1964; Kidd, 1963, 1964), however, have shown significant differences among these elements, and it therefore seems useful to review the ultrastructure of the tangle and to compare it again with those fibres found in the plaque.

Several electron microscopic studies of the senile plaque have been published (Terry, Gonatas and Weiss, 1964; Luse and Smith, 1964; Kidd, 1964; Krigman, Feldman and Bensch, 1965; Gonatas, Anderson and Evangelista, 1967), most of them indicating an amyloid core surrounded by altered neurites and reactive cells. Although the various elements of the plaque have been thus identified, no correlation has been attempted between the electron microscopic findings and the several types of plaques recognizable by light microscopy. Modern microscopists have also avoided the questions concerning a nidus of formation as well as the order in which the elements of the plaque are formed. The relationship between tangles and plaques has received only cursory attention. Analysis of many plaques, in various apparent stages of formation, and of the intervening neuropil permits a reconsideration of the pathogenesis of these important lesions.

MATERIALS AND METHODS

A series of ten brain biopsies forms the basis of this work. The tissue was removed usually from the non-dominant second frontal gyrus, but occasionally we received tissue from the temporal area. The techniques of

* Supported by research grants NB-02255 and NB-03356 from the National Institute for Neurological Diseases and Stroke, United States Department of Health, Education, and Welfare.

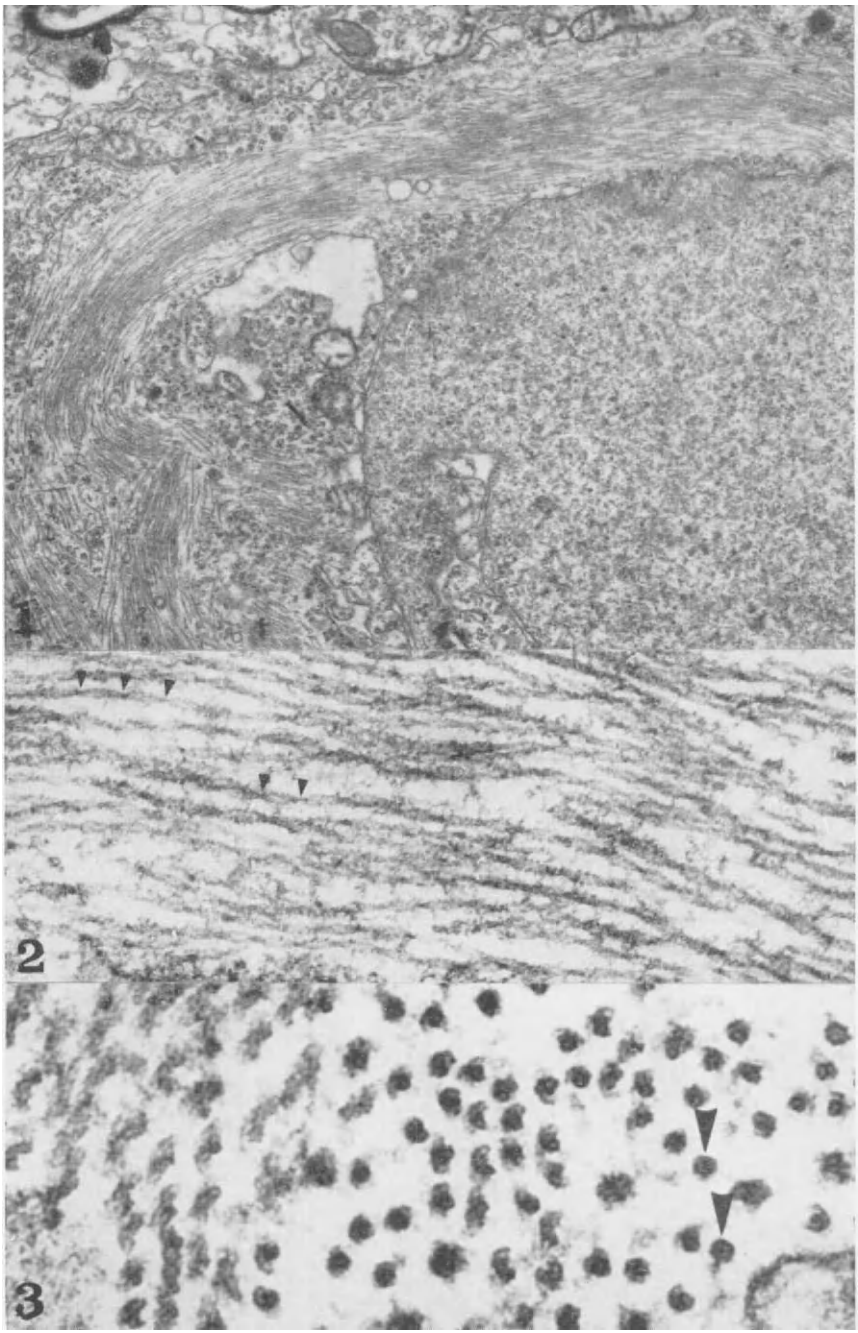

FIG. 1. A cortical neuron with a neurofibrillary tangle. The nucleus is at lower right, with the bundle of twisted tubules above and to the left displacing the normal cytoplasmic contents. × 12 000.

FIG. 2. Longitudinal section of twisted tubules displaying the periodic constrictions (arrows). × 77 000.

FIG. 3. Cross-sectioned twisted tubules have both circular and arcuate forms. Note the central filament (arrow) in the former. × 165 000.

fixation and embedding are standard, but it is worth noting that prefixation with glutaraldehyde was applied only to the latter half of the series. Plastic sections 1 to 2 μm thick and stained heavily with toluidine blue were found to be useful. They provide the basis for the light microscopic analysis, along with Bodian and Congo red preparations of paraffin sections.

RESULTS

Neurofibrillary tangles

The terms fibre, fibril, filament and tubule will be used here according to the following definitions:

> "Fibril" and "fibre" are synonymous, referring to the linear, argyrophilic structures seen with the light microscope.
>
> "Filaments" are in the ultrastructural domain, measuring about 10 nm (100 Å) in width with a poorly defined lumen and indefinite great length.
>
> "Tubules" or "neurotubules" or "microtubules" are also visible only with the electron microscope; they average 24 nm in width, and are straight, unbranching, and have a prominent lumen.

Neurons with tangles were readily apparent in the 1 μm toluidine blue sections, since the bundle of fibrils showed as a relatively clear zone with little stain among the deeply coloured normal organelles and lipofuscin. At the ultrastructural level (Fig. 1), the neuronal nucleus was sometimes slightly darker than normal because of closely packed chromatin granules. It was often eccentric with regard to the cytoplasm and was often more irregular in shape than usual, but the nuclear envelope was unremarkable. The cytoplasm of these neurons was also moderately dark, being rich in free ribosomes. All the expected organelles were present with notable mitochondria, lysosomes, Golgi apparatus, endoplasmic reticulum, and very sparse microtubules. Lipofuscin was often prominent, but may be entirely absent in a given section. These bodies usually had the typical bosselated outline and compound density. Much less common were lamellated bodies resembling myelin figures and varying up to 2 μm in diameter.

The tangle itself was also of variable size, sometimes almost filling the perikaryon. The lesion was made up of clusters of well-ordered tubules with a distinctive appearance suggestive of a twist about every 80 nm (Fig. 2). These elements are called twisted tubules to differentiate them from the normal neurotubule. In longitudinal sections they measured 20 to 22 nm

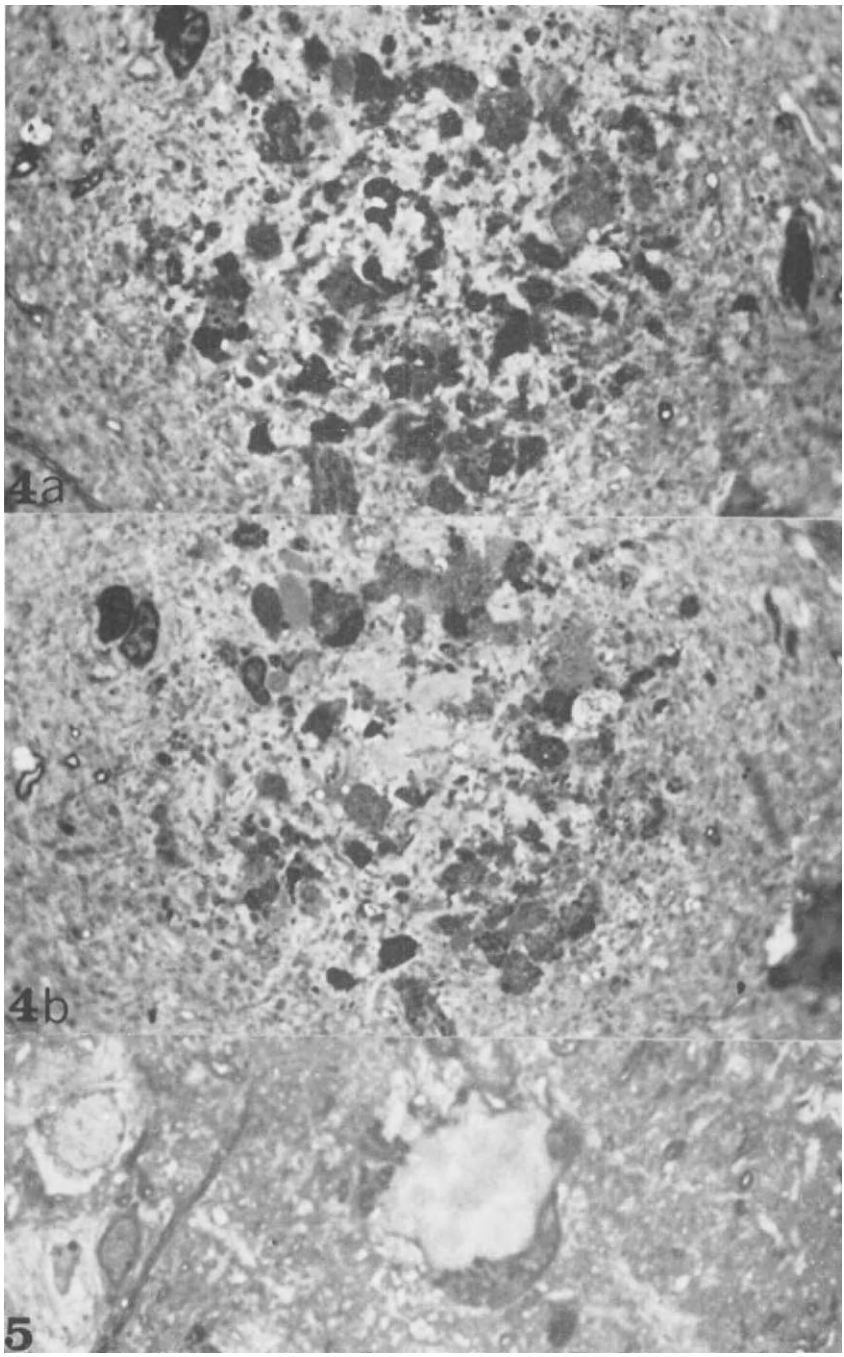

FIG. 4*a*, *b*. Serial 1 μm sections stained with toluidine blue passing through the same senile plaque. (*a*) is peripheral, showing little or no amyloid, which is prominent in the centre of (*b*). × 560.

FIG. 5. This senile plaque, seen in 1 μm section, is made up almost exclusively of amyloid with only a few neurites at its upper edge and a cell along the lower border. × 560.

at the widest point and about 10 nm in the narrow segments where they were presumed to twist. A circular profile, 15 to 20 nm wide, with prominent lumen and occasional central dot of 4 nm was sometimes apparent as the cross-section (Fig. 3). The wall of the tubule was seen here to be granular, 5 nm thick, and with ten to 13 elements forming the circle. Also seen in cross or tangential section was a very short arc, about 10 nm wide and 40 nm long, often with a nearby concentrically placed circle also 10 nm wide. The relationship between this latter compound profile and the larger simple circular cross-section is not clear, but the former might be a representation of the tubule as it twists. The bundles of these abnormal twisted tubules were not delimited from the cytoplasm by any sort of membrane. The individual twisted tubule was not found in any particular relationship with one or another type of organelle; specific continuity with or apposition to a normal membrane or granule was visibly lacking. The area of the tangle was usually free of other organelles, but an occasional lipofuscin body or small myelin figure might be found among the twisted tubules. These elements, singly or in aggregates, have not been found in the extracellular space.

Senile plaques

Three sorts of plaque have been seen with the light microscope, either by classical stains of ordinary sections or by toluidine blue stains of 1 μm sections from plastic-embedded material: (1) classical plaque with amorphous core; (2) plaque without amorphous material ("primitive" type); (3) plaque made up almost exclusively of amorphous material. Most plaques which at first seem to be free of amorphous material were found on serial 1 μm sections to have a central core (Figs. 4*a*, *b*). There remained, however, a few lesions which, even with serial sections, appeared to the light microscopist to be free of this substance. The third type was not uncommon (Fig. 5). It is well to point out that a toluidine blue stain of a 1 to 2 μm plastic section permits identification of each element of the plaque more readily than an individual Congo red or silver preparation of the usual thick section.

Electron microscopic study revealed many more plaques than expected by light microscopy. Small plaques were found even when the electron microscope samples were taken between serially cut 1 μm sections which seemed to be without lesions. The large aggregates were as complex as predicted by light microscopists, with the participation of several sorts of cellular and intercellular elements (Fig. 6). These were: (1) abnormal neurites; (2) amyloid; (3) cells.

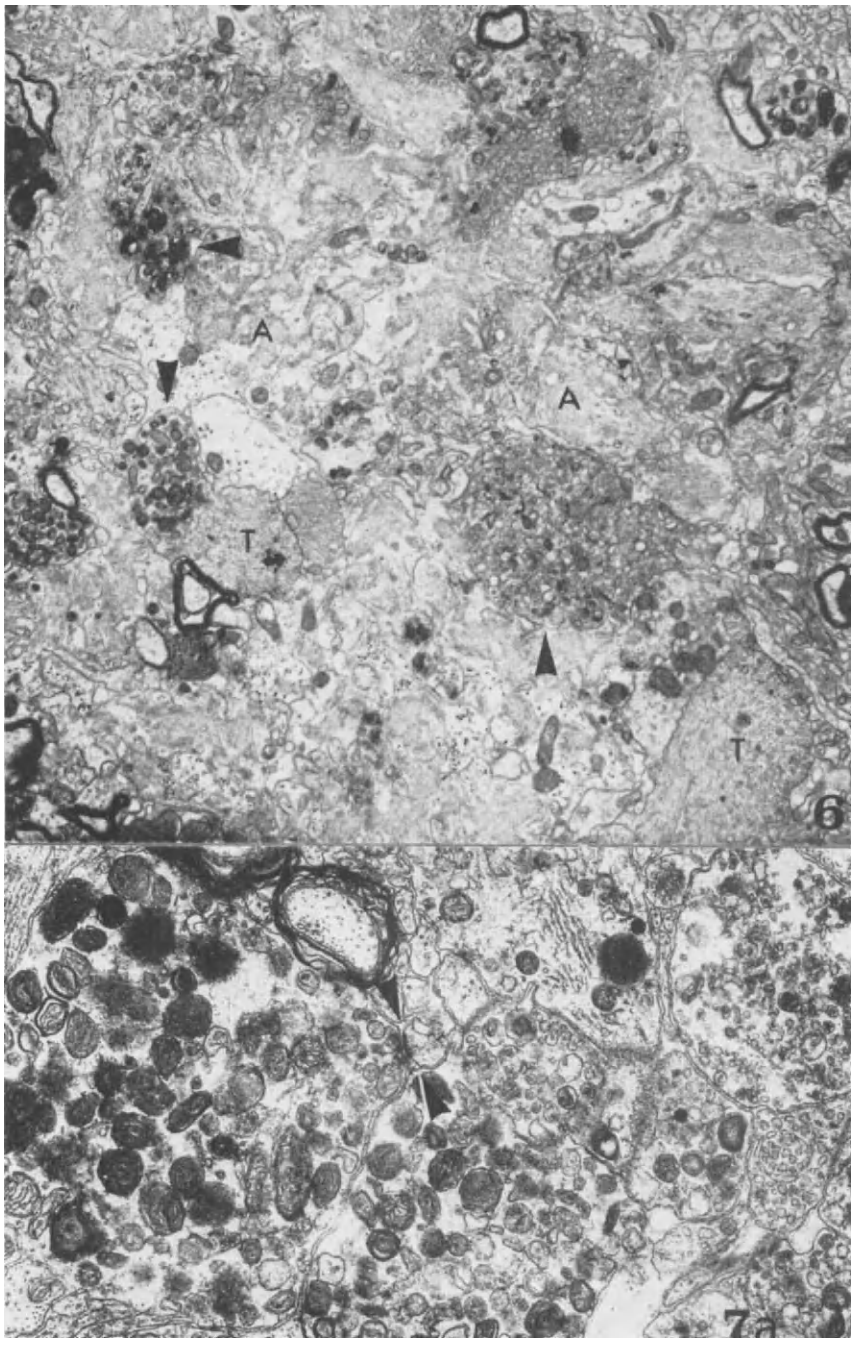

FIG. 6. A mature plaque with ample extracellular amyloid (A); and numerous abnormal neurites containing twisted tubules (T), mitochondria and dense bodies (arrows). × 5000.

FIG. 7*a*. An abnormal post-synaptic bouton participating in a senile plaque. The synaptic membrane is at the arrow, normal axon to the right. × 15 000.

Neurites. Unmyelinated neuronal processes were the most prominent feature of the senile plaque. Many of them could be recognized as dendritic terminals, that is as post-synaptic boutons (Fig. 7*a*). Occasionally they were demonstrably presynaptic, but often they were not terminals and could not positively be identified as axonal or dendritic. The abnormal often moderately distended structures formed a cluster easy to recognize at low electron magnifications. Myelinated processes were only very rarely involved in the process, but were rather pushed aside. Many of these neurites, of both types, contained large numbers of close-packed twisted tubules identical to those found in the neuronal soma (Fig. 7*b*). Just under the plasma membrane of these neurites there were often several mitochondria or dense bodies. In other such neurites, groups of these mitochondria were found which might be slightly denser than normal (Fig. 7*c*). The mitochondria were very often mixed with ovoid lamellar bodies similar in shape and size but of much greater density (Fig. 7*d*). Stages of transition between apparently normal mitochondria and these lamellar figures were readily found. A further sort of dense body with a relatively homogeneous, very dark core was surrounded by one or more unit membrane. Multivesicular bodies were present, but less common than a highly variable form of membrane-bounded vesicle filled with homogeneous material of low density. Many of the dense bodies revealed a reaction product when tested for acid phosphatase at the electron microscope level (Suzuki and Terry, 1967).

Amyloid. All plaques containing five or more abnormal neurites displayed at least some amyloid. This material was largely extracellular and was often arranged in oriented bundles of filaments. The individual elements averaged 10 nm (7·5 to 11 nm) in width with a well-defined lumen (Fig. 8). This last readily differentiated the amyloid fibres from glial and neuronal filaments. The protofilamentous subunits of the amyloid were variably clear, but the helical structure sometimes described in specially prepared material was not seen.

Cells. Occasional cells contained amyloid fibrils within their somas, and usually this material was surrounded by a unit membrane demarcating it from the cell sap (Fig. 9*a*). There were rare cells, however, which contained large amounts of the amyloid without any bounding membrane (Fig. 9*b*). Here the amyloid was in direct contact with the cytoplasm which was, in such cases, uncommonly rich in free ribosomes.

Aside from the amyloid-containing cells, there were at least two other non-neuronal elements of significance in regard to the plaque. One was the macrophage, rich in ribosomes and containing various sorts of lipid inclusions. Most of the latter resembled lipofuscin but some inclusions were

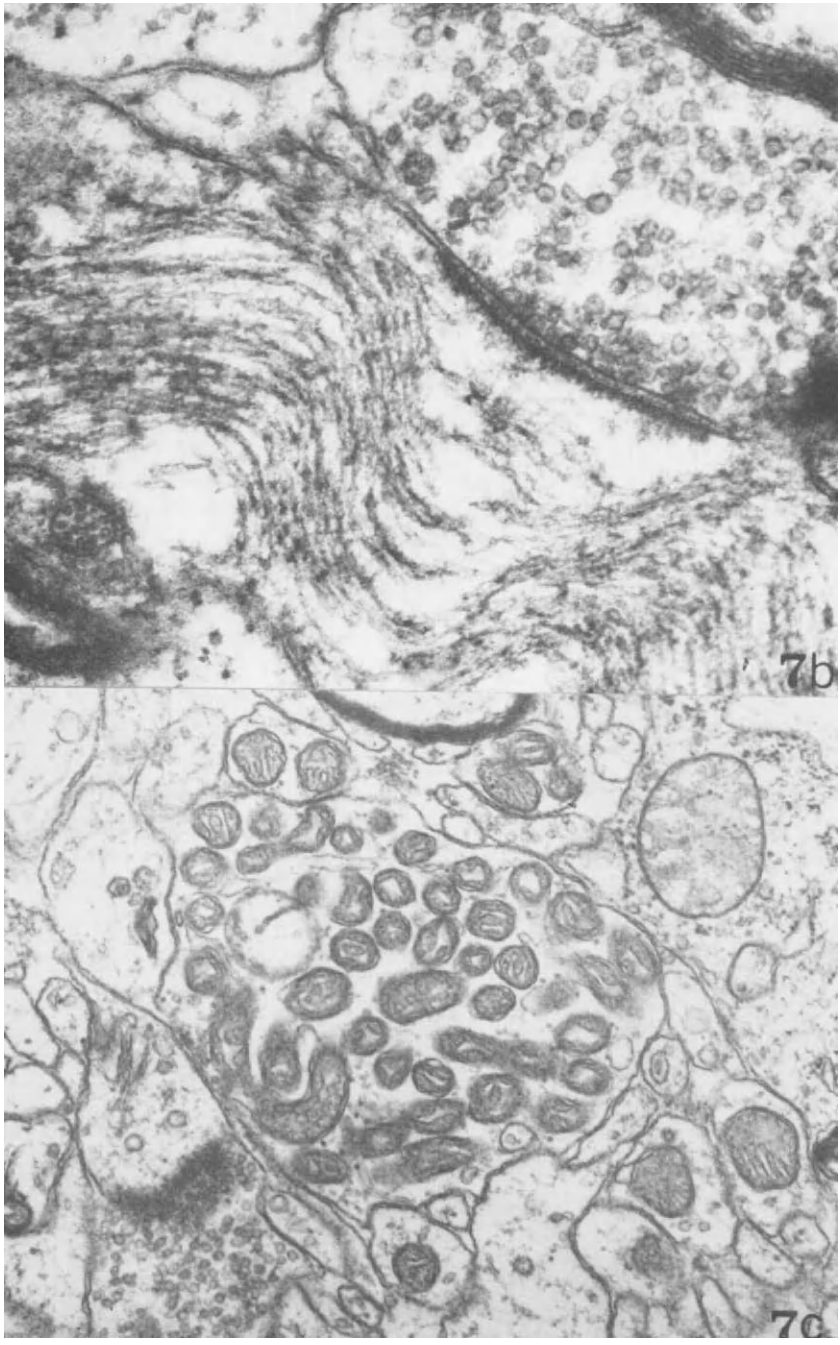

FIG. 7*b*. A second type of abnormal synapse in which the dendritic bouton contains twisted tubules. × 74 000.
FIG. 7*c*. An abnormal neurite of the early type contains numbers of mitochondria with coarsened membranes and cristae. × 28 000.

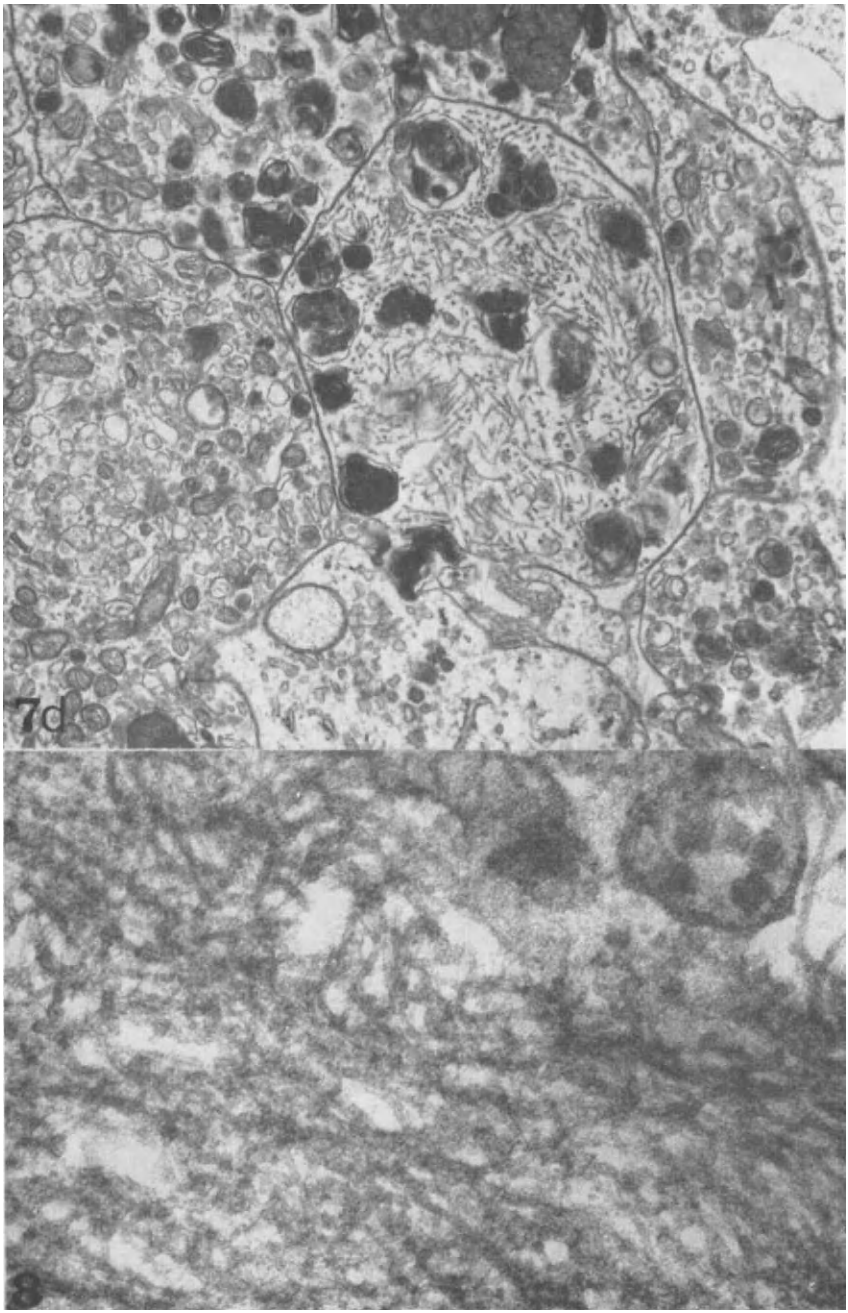

FIG. 7*d*. These neurites from a plaque are distended by a mixture of dense bodies and twisted tubules. Vacuoles and mitochondria fill the one at the left margin. × 18 000.

FIG. 8. Amyloid from the core of a plaque is made up of 10 nm tubules, the lumens of which are quite clear. × 103 000.

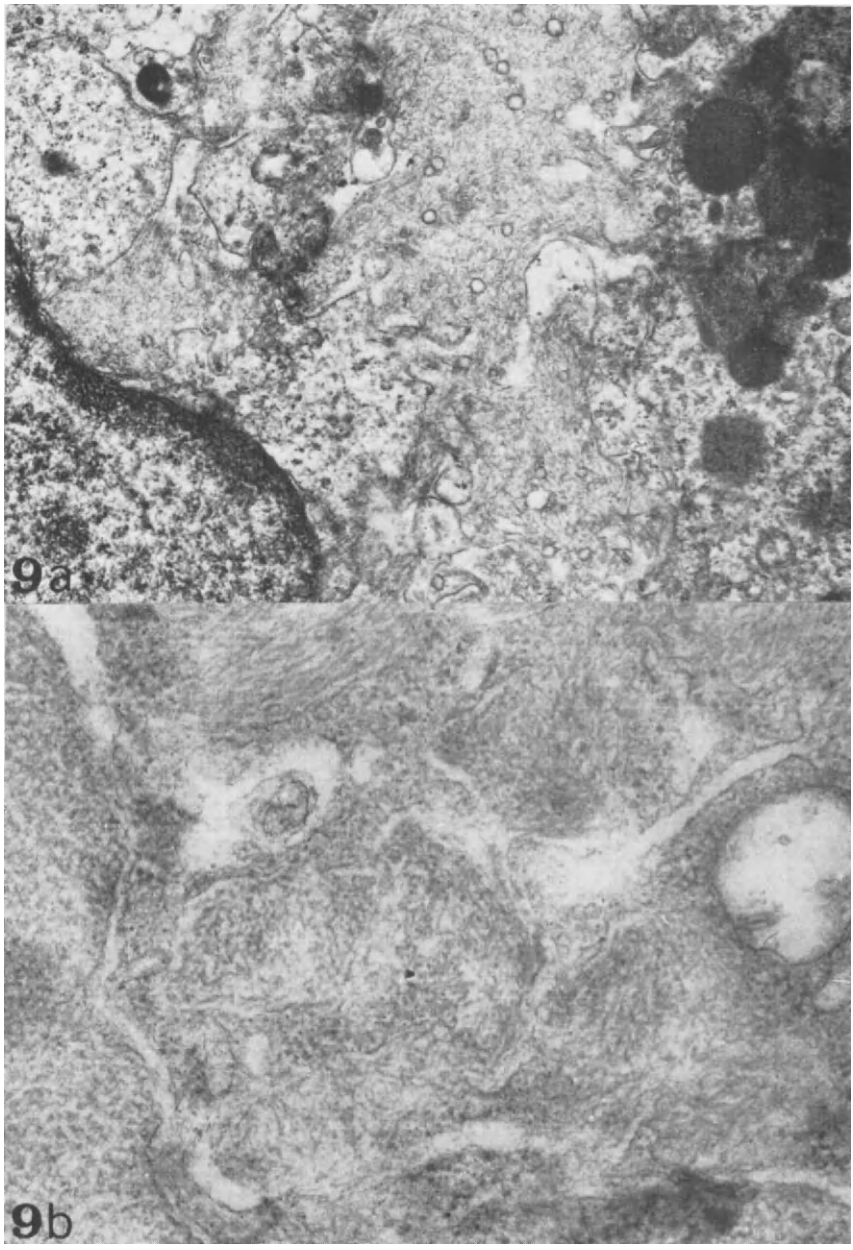

FIG. 9*a*. The intracellular amyloid here is isolated from the cytoplasm by a unit membrane. × 17 000.
FIG. 9*b*. The amyloid within this cell is free in the cytoplasm without a boundary membrane. The membranes of the endoplasmic reticulum lie between the masses of amyloid. × 59 000.

laminated, suggesting the presence of quantities of phospholipid. Similarly dense cells, but without abnormal inclusions, were present and were indistinguishable from cells which have been described as microglia (Schultz, Maynard and Pease, 1957), or as reactive oligodendroglia (Bunge, Bunge and Ris, 1961).

Astrocytic processes and their less common somas were prominent especially near the periphery of the plaque. These glial processes contained many filaments and also many glycogen granules. It is noteworthy that where glycogen was in particularly high concentration, there were few or no glial filaments.

The arrangement of two of the three major components of the senile plaque determines its type (Figs. 10, 11, 12). The most common form had a central core of amyloid surrounded by abnormal neurites. The sort called "primitive" by the light microscopist because it has no amyloid was found by the electron microscopist always to have at least wisps of this substance among the neurites. Occasionally a lesion made up almost exclusively of amyloid was found: even with the electron microscope, few or no neurites or cells were apparent.

It was indicated above that many lesions are apparent with the electron microscope which were wholly invisible with the light microscope. These were minute aggregates of abnormal neurites which were mildly distended by the clusters of mitochondria within them. Mixed with these organelles were occasional dense bodies. Rarely a lone, enlarged neurite was seen, containing only the dense bodies. Amyloid was always present if there were more than five such neurites in a field $\times$ 5000. The amyloid here may be so subtle as to be visible only at much higher magnification ($\times$ 20 000). When only one or two abnormal neurites were found in the field amyloid was almost always absent, even at high magnification. When amyloid was present in wisps with from none to two abnormal neurites, serial sections usually revealed an adjacent plaque.

The very smallest lesions were made up of only slightly enlarged neurites containing mitochondria almost exclusively. The other elements of the adjacent neuropil were intact. Larger plaques were composed of more numerous, somewhat larger neurites which contained more dense bodies in relation to the mitochondria. Usually the core of these larger plaques was compact amyloid, although sometimes this was not the case and the central region was loose and oedematous. As the amyloid increased in amount the number of neurites decreased, but each was large and apt to be surrounded by a less distinct plasma membrane. The neuropil here was by this stage replaced for the most part by amyloid and abnormal neurites.

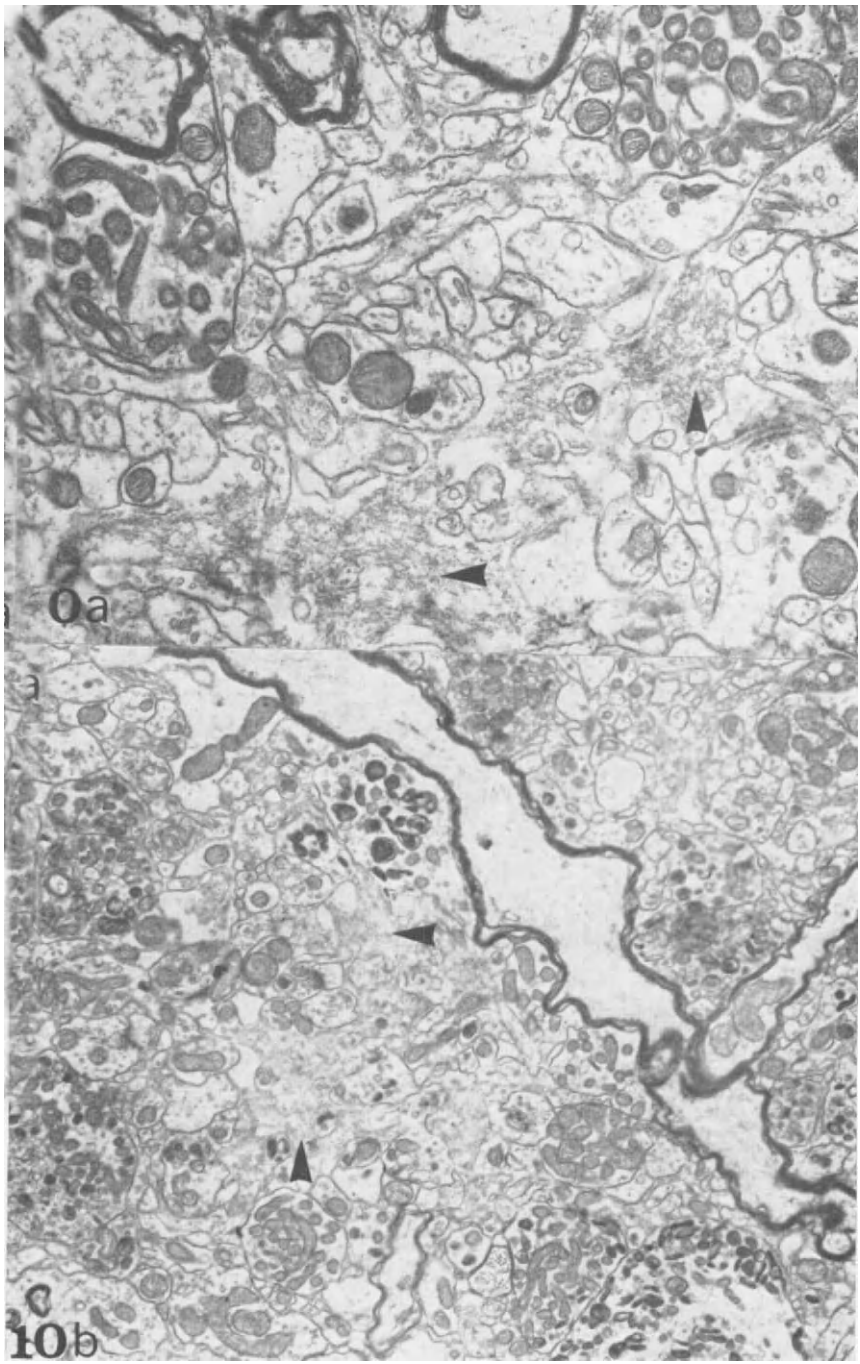

FIG. 10*a*. An early plaque with but a few abnormal neurites containing dense mitochondria and small wisps of amyloid (arrow) in the extracellular space. × 18 000.

FIG. 10*b*. A somewhat more mature plaque than in Fig. 10*a*, with more numerous affected neurites containing mitochondria and dense bodies. There is an increased amount of amyloid (arrow). × 5000.

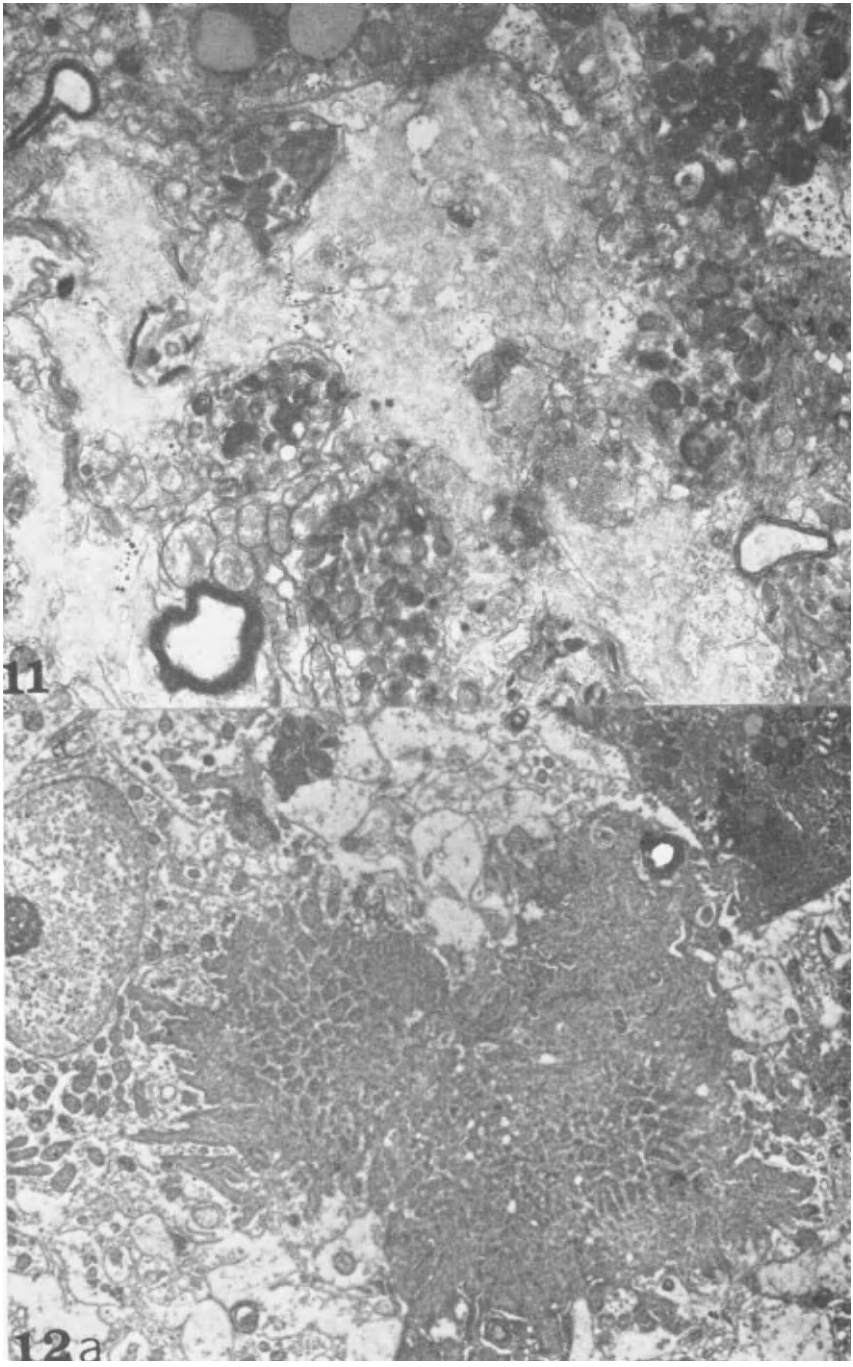

FIG. 11. This presumably old plaque has a dense core of amyloid surrounded by few but large neurites which are distended by dense bodies. These neurites have poorly defined plasma membranes and may have enlarged by fusion. × 10 000.

FIG. 12*a*. A burnt-out plaque comparable to that in Fig. 5. The very dense amyloid is surrounded by a few reactive cells but no affected neurites. There is a macrophage above and two cells involved with amyloid at left and below. (Reprinted from Terry, Gonatas and Weiss, 1964: *Am. J. Path.*, **44**, 284.) × 7600.

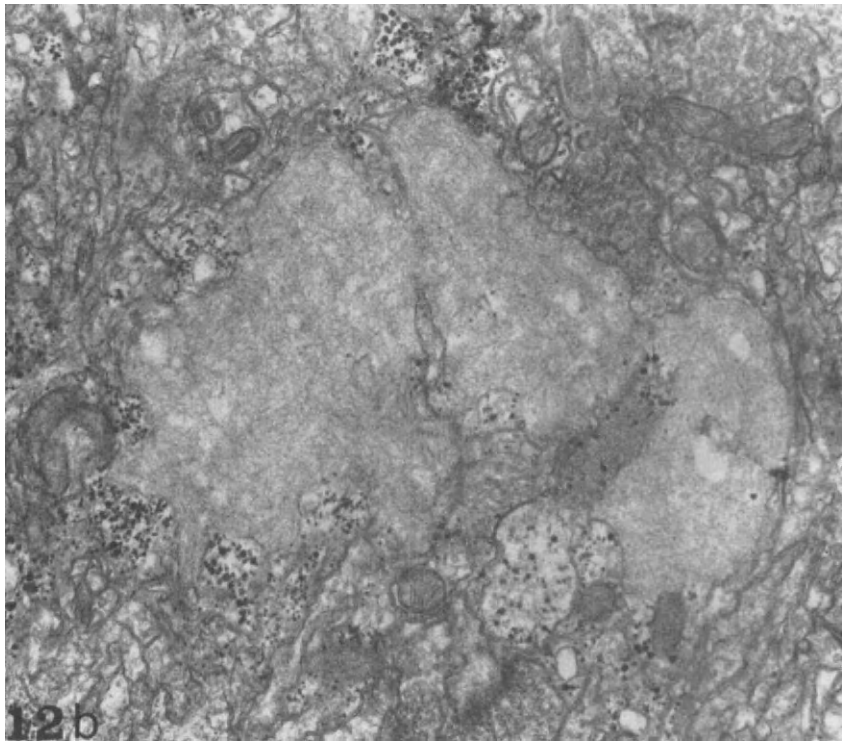

FIG. 12*b*. Another burnt-out plaque without any neurites or reactive cell soma. Astrocytic processes containing glycogen lie around the amyloid core. × 21 000.

Very rarely, one could find a mass of amyloid alone, surrounded by reactive glial processes, often with much glycogen, but without the abnormal neurites which characterize the other plaques. Thus four types of plaques are found by electron microscopy: (1) very small aggregates of abnormal neurites; (2) the type with wisps of amyloid; (3) the classical type, with a central core of amyloid; and (4) the type made of amyloid alone.

It is to be emphasized that the neuronal soma was only very rarely present in the plaque (Liss, 1960). The perikaryon may on occasion be adjacent to a cluster of abnormal neurites, but in the great majority of instances this was not the case. The soma may well play a role in causing the changes of the neurite, but the latter alone makes up the plaque lesion without any direct participation by the former.

Margolis (1959) has previously said that serial sections do not demonstrate any particular topographical relationship between vessels and plaques.

This has also been our experience with the much thinner sections afforded by the methods of plastic embedding. Occasionally one saw a capillary or venule adjacent to a plaque. These vessels were of normal appearance except for a slightly thickened basement membrane. Large vessels affected by amyloidosis as seen in paraffin sections have somehow not been found in our plastic embeddings.

A summary of the substructure of Alzheimer's disease is tabulated below:

Cortical atrophy: Neuron loss
White matter atrophy: Wallerian and primary demyelinization
Tangles: Aggregated twisted tubules
Plaques:
  Argyrophilia: Complex lipid and lipofuscin; tubule aggregates in neurites
  Congophilia: Amyloid—extracellular and intracellular; and twisted tubules
  Hydrolytic activity: Dense bodies in neurites
  Oxidative activity: Mitochondria in neurites
Granulovacuolar degeneration: Autophagic bodies
"Hirano bodies": Fibrillar material

DISCUSSION

Ultrastructural studies have revealed clear differences between the abnormal intraneuronal material and that in the core of the plaque. Since both neurons and core stain similarly with Congo red and thioflavine S, it must be concluded that the staining reactions are deceptive and non-specific. The fibrillar material in the core, in extracellular wisps and in microglia-like cells is identical to amyloid elsewhere in the human and in experimental animals. This core material may therefore be presumed to be true amyloid; but the intraneuronal elements are not amyloid, nor is there any deposit of amyloid on the surface of the neuron as Divry (1952) suggested.

Classification of plaques on the basis of light microscopic observations needs only slight revision. All plaques large enough to be visible to the light microscope contain amyloid, although it may be in such small quantity as to be visible only with the electron microscope, and then only at high magnification or with serial sections.

If one can assume that the smallest lesion is the same as the earliest lesion, then it follows that the first stage of plaque formation is an aggregate of two

or three abnormal neurites containing large numbers of mitochondria. Amyloid seems to appear soon afterwards, when there are three to five such abnormal neurites. The amyloid is at first in the form of wisps between the neurites, and may be invisible without magnification of about 20 000 diameters. As the plaque enlarges, the congophilic amyloid increases and condenses as a central core. Around this there is the halo of increasingly large argyrophilic neurites which contain fewer mitochondria and more dense bodies, as well as twisted tubules. As the amyloid increases, the number of neurites affected ultimately begins to decline, so that occasionally one finds a burnt-out plaque made up of a dense mass of amyloid with very few or no surrounding degenerative neurites.

This sequence fits well with the classical observations of von Braunmühl (1957) and Divry (1934), who both stressed that the primitive plaque was argyrophilic ("substance trichosique" of Divry) before it was congophilic. Despite this observation, von Braunmühl proposed that the earliest change was in the ground substance, which appears not to be the case since the first lesion seems clearly to be within the neurites while the extracellular material is still normal. That is, according to our electron microscopic observations, we must consider that the "nidus of formation" is the neurite.

The reasons are not yet known for the very early appearance of amyloid in relation to the abnormal neurites. As Schwartz, Kurucz and Kurucz (1964) rightly pointed out, amyloidosis of meningeal vessels is extremely common among the aged. It would seem possible that when amyloidosis is already present, the additional stimulus of the degenerative neurites might coincide to stimulate the formation of parenchymatous amyloid to complete the components of the typical plaque. The implication of this hypothesis and the observations on which it is based is that Alzheimer's disease is not a primary amyloidosis, although amyloid is a significant part of the lesion. It is worth pointing out here that in the Portuguese type of primary amyloidosis, where meningeal vessels and even the superficial cerebral parenchyma have extensive amyloid deposits, there are still no senile plaques. That is, amyloid in the tissue does not by itself cause the formation of senile plaques. On the other hand, in at least some of the pre-senile cases of Alzheimer's disease, amyloid is present only in plaques and cerebral vessels, not in extraneural organs. It is highly unlikely that this is simple coincidence.

The mechanism by which amyloid is formed in these circumstances is unknown to the same extent that it is unknown in all other human and animal situations. The morphological aspects in the brain are identical to

those in the spleen, liver or elsewhere. As always, the bulk of the material is extracellular, but two intracellular forms are met here as elsewhere. The more common is as bundles, each surrounded by a membrane. This might represent newly formed intracellular amyloid, but could just as well mean that the fibrils had been phagocytosed, or that they extended into the cell from the outside. Occasionally a cell with large amounts of unbounded amyloid is in intimate contact with the cytoplasm, and here it is difficult to escape the probability of intracellular formation. In a discussion of the first published description of the ultrastructure of the plaque (Terry, Gonatas and Weiss, 1964), it was proposed that these cells were representatives of the same system of monocytes and reticuloendothelial cells as is responsible for the synthesis of amyloid in non-neural organs. Others have suggested that these cells synthesize the protein which is usually polymerized in fibrillar form in the extracellular space. However, when the concentration of precursor is very high the fibrils may be precipitated within the cells. In any case, the sparsity of these amyloid-forming cells in relation to the plaques is of interest. Apparently a very few such cells can elaborate a good deal of the congophilic material, enough to form the core of a plaque.

It is beyond the scope of this presentation to explore thoroughly the source of the dense lamellar bodies in the abnormal neurites of the plaque. That they are of lysosomal nature was demonstrated by Suzuki and Terry (1967), who also pointed out that these bodies closely resemble the axonal bodies which characterize early Wallerian degeneration. Webster (1962), who first noted their presence in that type of axonal degeneration, suggested that they were derived from mitochondria, and intermediate forms have also been found in our cerebral material. Holtzman and Novikoff (1965), on the other hand, believe that such organelles in the Wallerian situation represent autophagic bodies. The change from mitochondrion to dense body, by whatever mechanism, is non-specific and is indicative only of degeneration of the neurites. It can be said that this type of alteration in neurites is usually related to a more proximal lesion in the neuron.

Before coming to the relationship between argyrophilic plaque and Alzheimer's cell change, the nature of the latter might be further clarified. In the affected neuron, all organelles except microtubules appear to be relatively normal. The latter are markedly reduced in number, and striking aggregates of twisted tubules are found. Although normal microtubules are lost by direct fixation with osmium tetroxide, the abnormal elements are well preserved by this procedure. The twisted tubules, then, are clearly different from normal microtubules as to solubility and structure. But this

is not the result of an encrustation since as individuals they are smaller than normal. The light microscopists, failing to consider the possibility of proliferation of fibres, mistook the aggregates of abnormal tubules for thickened normal fibres. Furthermore, consideration of the fine structure would indicate that precipitation of amyloid or some other protein on the normal tubules would not produce the twisted elements. These were the concepts of Divry (1934) as to amyloid, and von Braunmühl (1957) as to protein, but neither stands the test of electron microscopy. The former writer also suggested that part of the Alzheimer change was due to precipitation of amyloid on the surface of the neuron, but such deposits over the plasma membrane are also missing from the ultrastructural scene.

The twisted tubules, which are characteristic of the Alzheimer's cell change, are present in great numbers, such as to occupy significant portions of the neuronal perikaryon. Quite aside from any abnormal activity they might have themselves, their mere presence in such quantity must cause a reduction in the volume of normal organelles the same cell may hold. This reduction in its equipment might by itself cause the cellular metabolism to be inadequate to maintain its neurites.

Although there is at this time no *a priori* proof, the natural assumption is that the twisted tubules are a derivative or perhaps a replacement of the normal microtubules. If they are derivatives, one could postulate a mechanism such as oxidation of thiol groups in the protein of the tubule to account for the periodic contractions. In this regard, one might recall that King (1942) has pointed out the presence of large quantities of iron in the tissue in Alzheimer's disease. It would seem possible that this metal in the form of the ferric ion might be the oxidizing agent. If the twisted tubules differ from the normal neurotubules even more fundamentally, one must postulate a diverted protein synthesis, turned from the manufacture of 6S microtubule protein to the still unknown type composing the abnormal elements. A slow virus could be responsible for this sort of change. Von Economo's lethargic encephalitis and some cases of rabies (Achucarro, 1910) indeed present such examples.

One might, finally, consider the functions of the normal tubule in order to estimate the loss of function suffered by the diseased neuron. There are in this regard two major hypotheses, for both of which there is considerable support. Microtubules are distributed in many cells in such a fashion as to suggest that they are concerned with mechanical support of cellular asymmetry (Meves, 1911). Neurons are indeed highly asymmetrical and might well need such a skeleton, but there is no obvious way of relating this function to the alterations of Alzheimer's disease.

The second hypothetical function for normal microtubules has to do with their providing the energy and the guidelines for cytoplasmic movement (Ledbetter and Porter, 1963). In the neuron, this applies to axoplasmic flow. There is a great mass of data available about this, and the reader may refer to two recent reports of the Neurosciences Research Program (Barondes, 1967; Schmitt, 1968) for summaries of the evidence. If, as seems to be the case, neurotubules are responsible for cytoplasmic movement in the neuronal soma and for axoplasmic flow, then clearly these activities must be disrupted in Alzheimer's disease where the normal tubules are largely replaced by the twisted elements which not only block flow by their presence, but also may fairly be presumed to lack the normal energy capacity as they do the normal shape. The senile plaque is characterized by enlarged neurites containing degenerating mitochondria and dense bodies. These neurites might well represent the effects of diminished axoplasmic flow since similar changes are found when the cortex is underput (Guillery, 1965), or in the course of certain forms of the "dying back" phenomenon (Prineas, 1969).

In conclusion, our morphological evidence would indicate that the plaque is primarily a neuronal lesion although the soma is not directly involved topographically. The amyloid is an essential component of the developed lesion, but its location in the plaque is secondary to the changes in the neurites and may, in a sense, even be coincidental. Our work has by no means uncovered the ultimate aetiology of the disease. However, it does suggest two probabilities: a genetically determined change in metabolism of neuronal fibrous protein, or a similar change induced by virus infection. The altered tubule protein, that is the neurofibrillary tangle, and/or some other neuronal degenerative factor, would then give rise to the senile plaque by reducing axoplasmic flow to the extent of causing the neurites to degenerate.

SUMMARY

The neurofibrillary tangle has been seen in a series of biopsies to be made up of bundles of abnormal tubular structures, each measuring about 22 nm (220 Å) at their widest point and narrowing at intervals of 80 nm to a width of about 10 nm. In cross-section they are of two forms. The more common is a short arciform element. Also frequent is a circular cross-section with a lumen 12 nm wide and a wall made of about thirteen 5 nm granules. It is believed that these abnormal tubules are twisted, and that the arc is the result of this stress. The bundles of argyrophilic twisted tubules

displace the other organelles within the neuronal soma. Lipofuscin is common within these neurons, but does not bear any constant relationship to the neurofibrillary tangle.

The classical light microscopists have described several sorts of plaque, depending upon the distribution of amyloid, and correlates have been found by electron microscopy. The most common plaque has a central core of amyloid fibrils, surrounded by large numbers of neurites and synaptic boutons filled with dense bodies and/or bundles of twisted tubules. The dense bodies are often mixed with degenerating mitochondria. The outer cellular processes contain irregular lipid granules, some of which are so dense as to resemble lipofuscin. Some plaques do not have a central core of amyloid and may have only wisps of this material lying among a cluster of abnormal neurites. The burnt-out plaque is simply a mass of amyloid without surrounding abnormal neurites and without any twisted tubules. Argyrophilia of the plaque is related to the twisted tubules, the lipofuscin, the amyloid, and the dense bodies. These last account for acid phosphatase, while mitochondria are responsible for the oxidative activity.

Acknowledgements

The authors gratefully acknowledge the technical assistance of Lawrence Gonzales and Karen Berkman.

ADDENDUM

Very recently in biopsies from Alzheimer's disease we have found vessels with amyloid deposits in the basement membrane. The amyloid infiltrated the surrounding parenchyma but did not elicit any reaction on the part of the neurites. This supports the contention that the plaque is not a lesion of primary amyloidosis.

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DISCUSSION

Gonatas: Did you see twisted tubules either in myelinated axons or in presynaptic terminals? Were the ribosomes near the twisted tubules in any way unusual (dispersion, arrangement in large groups, etc)?

Terry: We have not seen twisted tubules in proven presynaptic terminals. Myelinated axons are almost invariably free of twisted tubules although they are obviously displaced and made abnormal by the presence of the plaque. I haven't really looked at the polysomes in a tangle-affected neuron. On the other hand an abnormal protein must obviously be manufactured here. Whether one sees the polysomes or not, those tubules have proteins. The inference is every bit as clear as the morphology of the polysomes in such cases.

Barondes: Do you feel that the pathological changes in neurons are consequences of a primary disturbance in the transport of axoplasm?

Terry: I think the single primary structural abnormality in this disease is the twisted tubule, which possibly gives rise to all the other changes, perhaps by changing axoplasmic flow.

Dayan: You are suggesting that at the ultrastructural level the tangle comes first. By light microscopy it is not uncommon in the normal senile brain to find plaques but no tangles.

Terry: I am puzzled by all sorts of imbalances here that I recognize and am not yet prepared to deal with. The hypothesis that filaments have regional differences in susceptibility to damage is a working one. We have seen this time and again in our experimental work with the animal models. There might well, therefore, also be differences in susceptibility to changes in axoplasmic flow; that is minor change in the neuronal soma could give rise to a plaque in one area or in one patient, whereas in another area it would take a massive change within a neuron. The reason why in some cases a lot of tangles and no plaques are seen could again be amplified by that sort of regional susceptibility, or pathoclisis. Why you see plaques without tangles could be similarly explained.

Sourander: Alzheimer's own second case (1911) of the disease named after him in fact was a case with numerous plaques but without tangles.

Terry: Twisted tubules are an essential component of the plaque, where they are found in the neurites. They are the same element that forms the tangle in the soma. Perhaps in some people twisted tubules form out in the neurites so that they get plaques but no tangles. Other people with this tendency, whether it is due to a gene or a slow virus or oxidation, may get their tangles within the neuronal soma and as a result get both perikaryon degeneration and plaques; these are perhaps the people who because of the neuronal disease develop symptoms.

Roth: From this one would anticipate that normal elderly subjects should have plaques alone whereas those with Alzheimer's disease or senile dementia should have tangles in the neuronal soma as well as plaques.

McMenemey: In patients with Alzheimer's disease with a short course who die of intercurrent conditions one usually finds more plaques than tangles. In other words plaques seem to appear ahead of tangles. Could this be an expression of the twisting process affecting the peripherally disposed neurites before the soma?

Terry: I don't know.

Kidd: A twisted object does not have constrictions; it only appears to have them when seen from the side. I am not denying the possibility of the cross-linking you suggest; for instance, twisting a tube requires some flattening of its profile, and this could be due to cross-linking.

Jacob: Why are plaques spherical?

Terry: The brain is gelatinous; if the elements are displaced in all directions equally it will build up in this spherical way.

Jacob: Do you think that von Braunmühl's hypothesis (1957) about the senile plaques in relation to the synergetic syndrome is acceptable?

Terry: The hysteresis analogy doesn't apply at all.

Barondes: How generalized is this disorder? What proportion of neurons are involved?

Terry: I cannot say. Ours is a biased population. The biopsies were from demented patients with Alzheimer's disease, from an area that was very much affected. Many neurons had tangles and there were many plaques. The critical question is, why do neurites degenerate in clusters? Does this imply that a single neuron with a tangle contributes all those neurites in a plaque, or is there something at that particular point which causes the neurites to degenerate, whether they are coming from a single neuron or from several? Golgi stains of normal human cortex are really lacking. We do not know where the neurites from this sort of neuron go. If many of the neurites from a single neuron go down into the same area, then it is possible that one neuron with a tangle could form a plaque.

Barondes: How many neurons do you think are involved in this disorder?

Terry: Vast numbers.

Barondes: Do you see many normal neurons?

Terry: We are looking at very thin sections. At one level a neuron may be normal, but 2 μm further on a tangle may appear in the same neuron.

Shelanski: What is known about dendritic flow of cytoplasm?

Barondes: Not much. To my mind axoplasmic transport exists to provide axons and nerve terminals with proteins which cannot be made in those parts of the cell. Since dendrites contain ribosomes they can presumably make the proteins they need. However these ribosomes must be replenished, as must messenger RNA and transfer RNA, and these are all made in the cell nucleus. Therefore transport of ribosomes and RNA undoubtedly occurs in dendrites and interference with such transport would result in impaired function.

Tomlinson: One light microscopic observation is relevant to what has been said. In the intellectually normal old person with senile plaques the intervening cortex between the plaques usually looks absolutely normal by light microscopy—apart, maybe, from some loss of neurons. In many cases of Alzheimer's disease, however, in between the definable plaques the cortex often looks grossly abnormal in terms of its neurofibrillary material. I am very interested to know that in between definable plaques there are microplaques which we don't see with light microscopy but which Dr Corsellis at least expected would be there.

Corsellis: Why is a plaque never larger than it is? One would expect that the core material might fuse into larger, confluent, masses at times.

In other words, the "amyloid core" of a plaque has a fairly standard size. Is this your observation, Dr Terry?

Terry: I agree with the observation but I have no explanation.

Roth: Is there not a range of variation in plaque size?

Terry: There is an upper limit.

Tomlinson: One sees quite a lot of light microscopic preparations in which plaques are formed in such close association with each other that they form aggregations. I would have expected them to look like continuous giant plaques in the electron microscope.

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ALZHEIMER'S DISEASE AND RELATED CONDITIONS
G. E. W. WOLSTENHOLME & MAEVE O'CONNOR

THE PATHOLOGY OF THE SYNAPSE IN ALZHEIMER'S DISEASE*

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MANY enlarged presynaptic terminals were observed in or near senile plaques from three patients with Alzheimer's presenile dementia. The enlarged terminals contained lamellated dense bodies or tubular and vesicular structures.

Table I summarizes clinical features and pathological findings at brain biopsy and autopsy from these three patients. Autopsy in Case 1 was performed by Dr L. Rorke and in Case 2 by the late Helena Riggs of the Philadelphia General Hospital. The autopsy material from Case 3 was studied in our laboratory by Dr K. Suzuki (Hospital of the University of Pennsylvania).

The clinical story in Case 3 was unusual; the patient, three years before she developed severe dementia, presented symptoms and clinical signs of Parkinsonism associated with a mild degree of short-term memory loss.

In the first two cases, diagnosis of Alzheimer's disease at brain biopsy was based on the electron microscope finding of senile plaques and neurofibrillary changes. In the light microscope, the diagnosis was consistent with Alzheimer's disease, since only thickened neurofibrils were seen but no classical plaques or tangles. In the third case, at brain biopsy numerous senile plaques and a few neurofibrillary tangles were observed on light microscopy. This observation was confirmed in the electron microscope. At autopsy, numerous senile plaques were found in all three cases. Neurofibrillary tangles were found in two cases. In the third case, loss of neurons, gliosis and Lewy bodies were observed in the substantia nigra.

The electron microscope findings in the synapses of the three cases were identical. We shall use previously unpublished micrographs obtained from Case 3 to illustrate them. The findings in the first two patients have already been reported (Gonatas, Anderson and Evangelista, 1967).

* Work supported by United States Public Health Service Grants NB-05572-06 and NB-08075-02.

TABLE I

CLINICAL AND PATHOLOGICAL FINDINGS IN THREE CASES OF ALZHEIMER'S DISEASE

Case	*Age at onset*	*Duration (years)*	*Main symptoms at onset*	*Biopsy*	*Autopsy*
1 T. M. male	47	4	Loss of memory, paranoid, combative behaviour	Thickened neurofibrils	Senile plaques in cerebral cortex
2* M. G. male	47	5	Loss of memory, dyspraxia, acalculia, dysgraphia, cog-wheel rigidity	Thickened neurofibrils	Senile plaques in cerebral cortex; neurofibrillary tangles in hippocampus
3 N. H. female	42	7	Parkinsonism; decreased memory	Senile plaques; neurofibrillary tangles	Neurofibrillary tangles and senile plaques in cerebral cortex; gliosis and Lewy bodies in substantia nigra

* Familial by history.

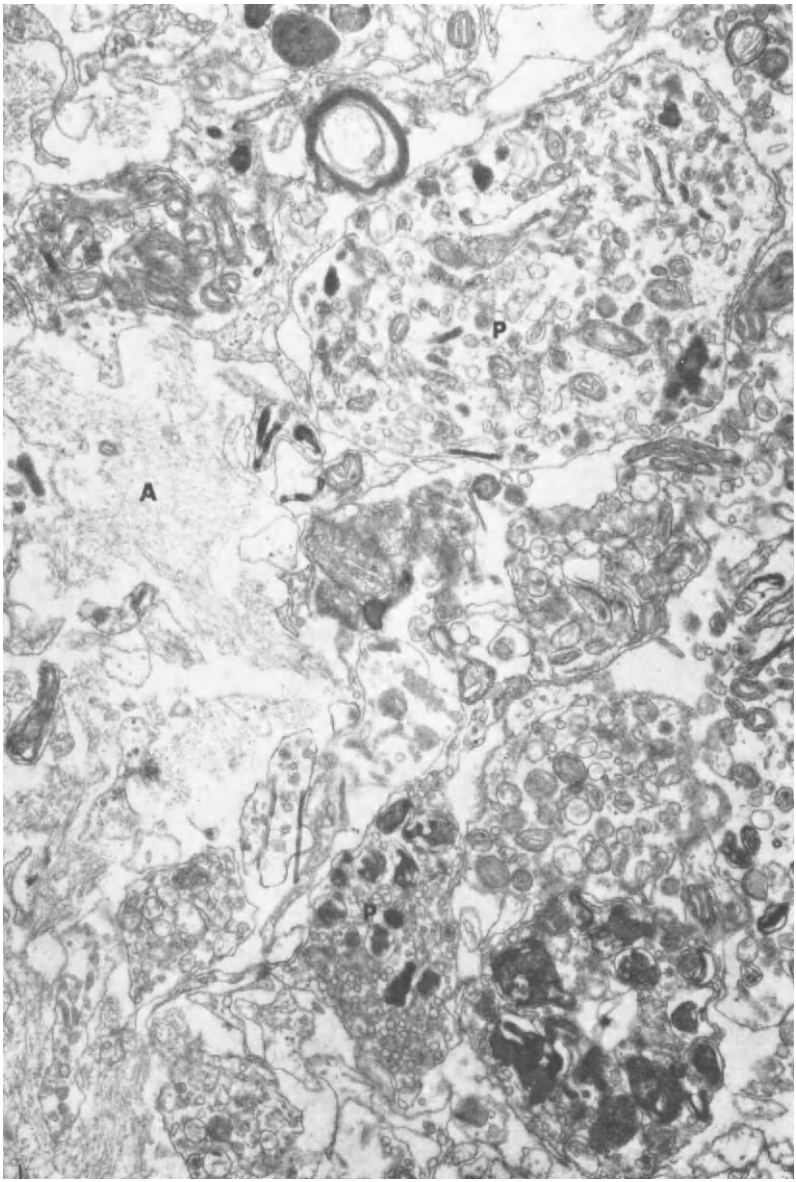

FIG. 1. Senile plaque. A: amyloid. P: neurites or probable presynaptic terminals. × 10 000.

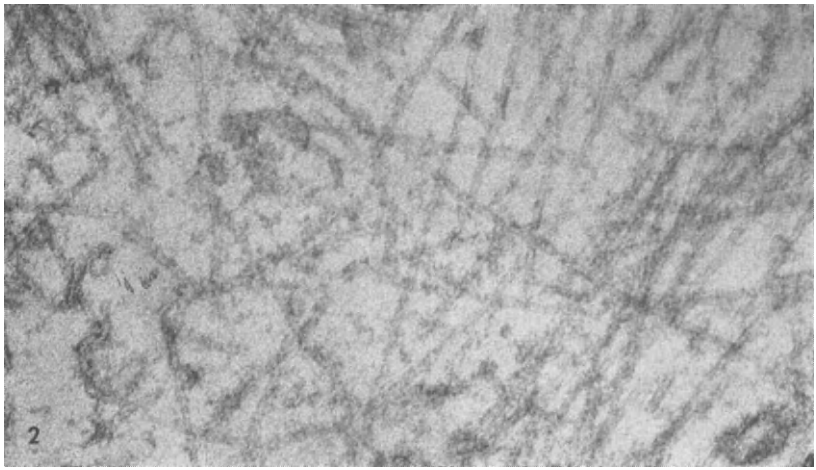

FIG. 2. Amyloid fibrils. × 150 000.

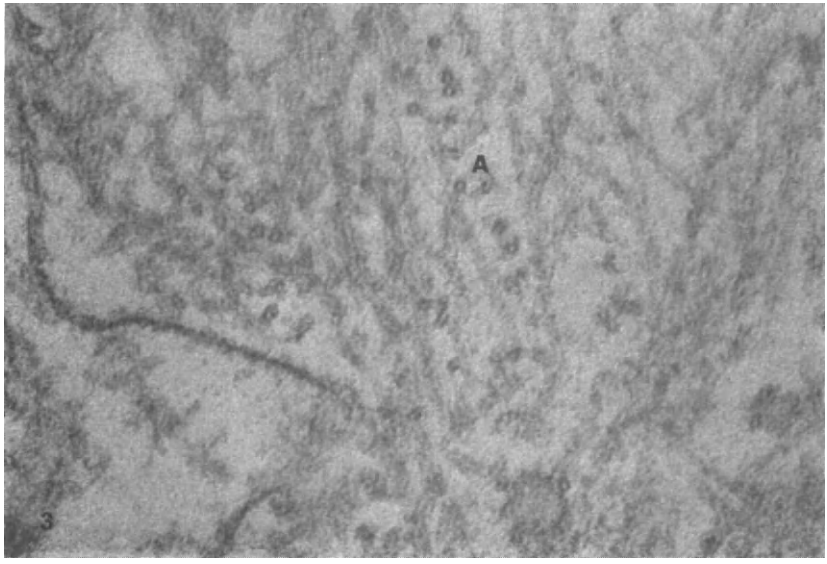

FIG. 3. Amyloid filaments. A: note circular profiles. × 130 000.

For electron microscopy, specimens obtained by open brain biopsy from the right middle frontal gyrus were fixed in 2 per cent osmium tetroxide in dichromate buffer (Dalton, 1955).

Senile plaques were identified from the central core of amyloid material which was surrounded by processes containing vesicles of varying size, dense bodies and increased numbers of neurofilaments or neurofibrillary material (Terry, Gonatas and Weiss, 1964) (Fig. 1). The amyloid filaments, extracellular in location and randomly oriented, had a triple-layered longitudinal profile and a circular cross-sectional profile; the thickness of the filament was 10 nm (Figs. 2, 3) (Bladen, Nylen and Glenner, 1966).

Examination of areas with senile plaques, preselected by semi-thin sections, showed that several of the cell processes with dense bodies and many processes with vesicular or tubular profiles were presynaptic terminals.

The presynaptic terminals with dense bodies were of normal size (about 1 μm in diameter) (Fig. 4). Synaptic vesicles were clustered near the synaptic cleft which contained the usual osmiophilic material. The dense bodies were made of several curved lines which were layered in a parallel fashion and frequently displayed a periodicity of about 5 nm.

The presynaptic processes with tubular or vesicular profiles were usually two to four times the normal diameter (2 to 6 μm) (Figs. 5, 6). The membranes forming the peculiar vesicular or tubular structures were 4·5 to 9·0 nm thick. The length of the tubulovesicular structures was indeterminate and on cross-section circular profiles 20 to 35 nm wide, suggesting a hollow structure, were observed. Trilaminar "unit-membrane" densities were never identified in the limiting membranes of the tubulovesicular structures. Tubulovesicular material was not observed in myelinated segments of axons; therefore this change occurs preferentially in terminal or in unmyelinated axons. A few transitional stages between the markedly enlarged axon terminals and normal presynaptic terminals were observed; on a rare occasion a presynaptic terminal contained, in addition to synaptic vesicles, elongated vesicles or a few tubules which could conceivably represent the beginning of the accumulation of the tubulovesicular material (Fig. 7).

A rare but probably significant change of axons, presynaptic axon terminals and dendrites, observed only in Case 3, consisted of accumulation of oval vesicles, 60–160 nm in diameter, in clusters and usually near the plasma membrane (Fig. 8). These vesicles resemble very strikingly those noted in the growth cones of the cerebellum of the kitten (De Cerro and Snider, 1968).

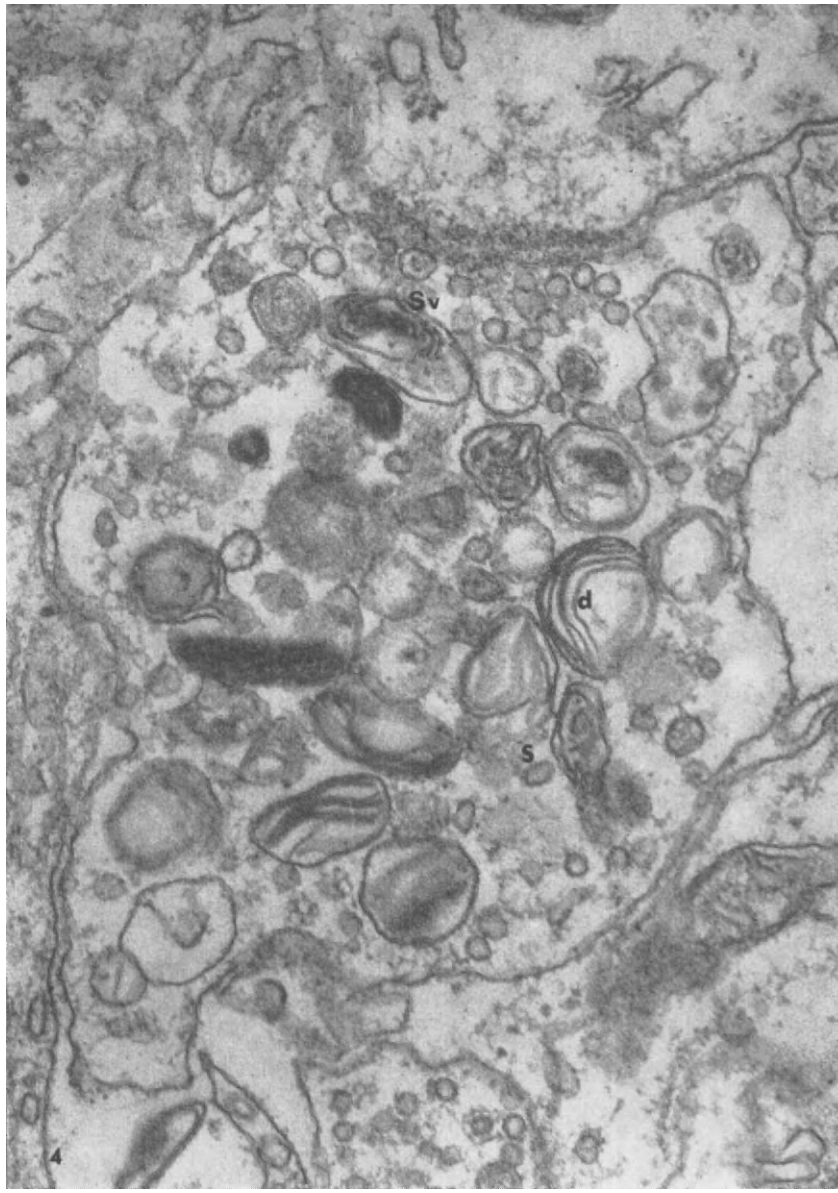

FIG. 4. S: abnormal presynaptic terminal with dense bodies (lysosomes ?). Sv: synaptic vesicles. d: dense bodies. × 68 000.

The pronounced heterogeneity of ultrastructural changes in the senile plaque defies a unitary explanation. We shall limit our comments to the synaptic lesions.

The dense bodies in presynaptic terminals and in neurites probably represent lysosomes. The intense acid phosphatase reaction in the senile

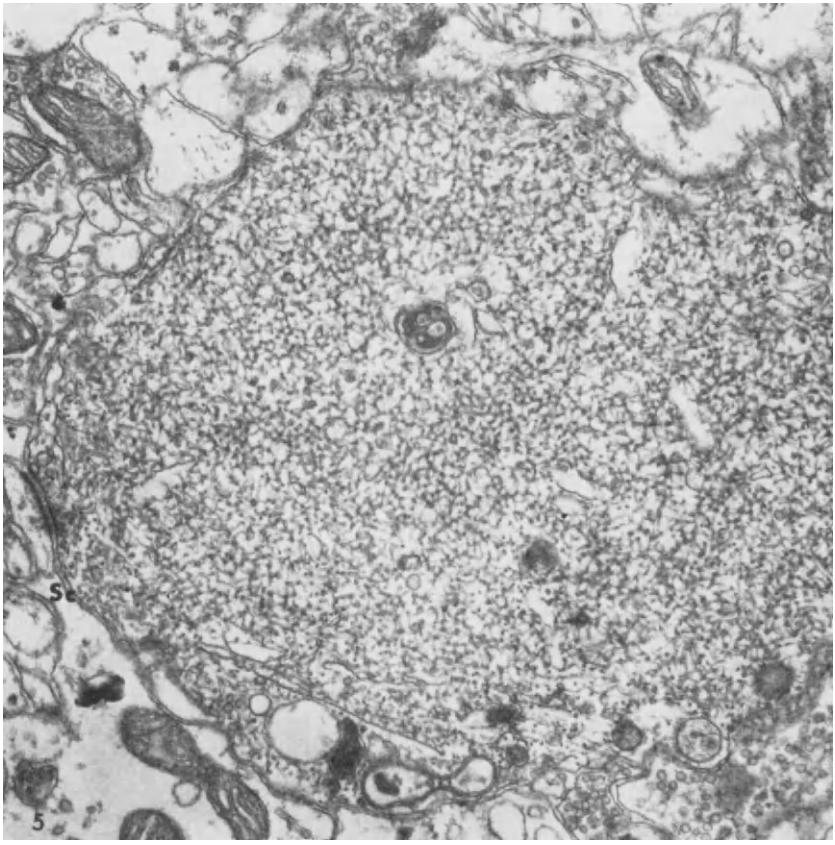

FIG. 5. Abnormal presynaptic terminal. Sc: synaptic cleft. × 25 000.

plaque and in the dense bodies indicates that degradative activity of acid hydrolases probably occurs in neurites and presynaptic terminals with dense bodies (Friede, 1965; Krigman, Feldman and Bensch, 1965; Gonatas, Anderson and Evangelista, 1967; Suzuki and Terry, 1967).

The enlarged presynaptic terminals with tubulovesicular material probably are pathologically altered. Similar tubulovesicular material

has been observed in injured nervous system in a variety of conditions, such as tetanus intoxication (Peracchia, 1967), early Wallerian degeneration (Laatsch, 1969), degeneration in the cerebellum (Smith, Hudgens and O'Leary, 1966), *in vitro* maintenance of superior cervical ganglion (Forssmann and Rouiller, 1966), near brain tumours (Ramsey, 1967) and

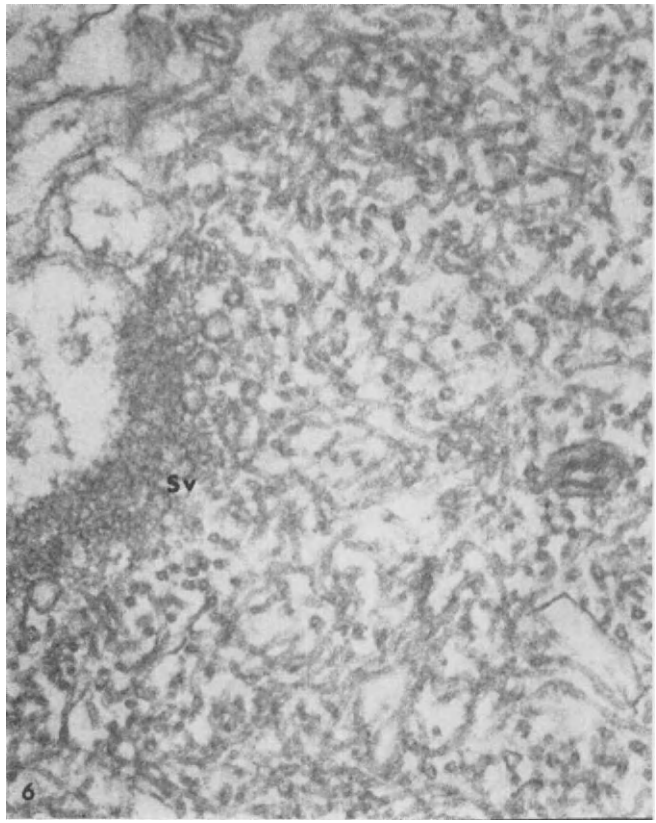

FIG. 6. Tubulovesicular material filling enlarged terminal. Sv: synaptic vesicles. × 66 000.

degeneration of serotonin pathways (Aghajanian, Bloom and Sheard, 1969); also, similar profiles have been observed in normal nerve endings in frog myoneural junction (Birks, 1966), and in human chronic degenerative diseases of the central nervous system (Hedley-Whyte, Gilles and Uzman, 1968; Herman, Huttenlocher and Bensch, 1969; Gonatas, Evangelista and Walsh, 1967; Gonatas and Goldensohn, 1965).

The elucidation of the chemical nature and of the pathogenic mechanisms leading to the production of the tubulovesicular material must be of great interest in view of its widespread occurrence in injured nervous tissue. This material is clearly distinct from the neurofibrillary change of Alzheimer's disease, normal neurotubules, neurofilaments or amyloid fibrils. Smooth

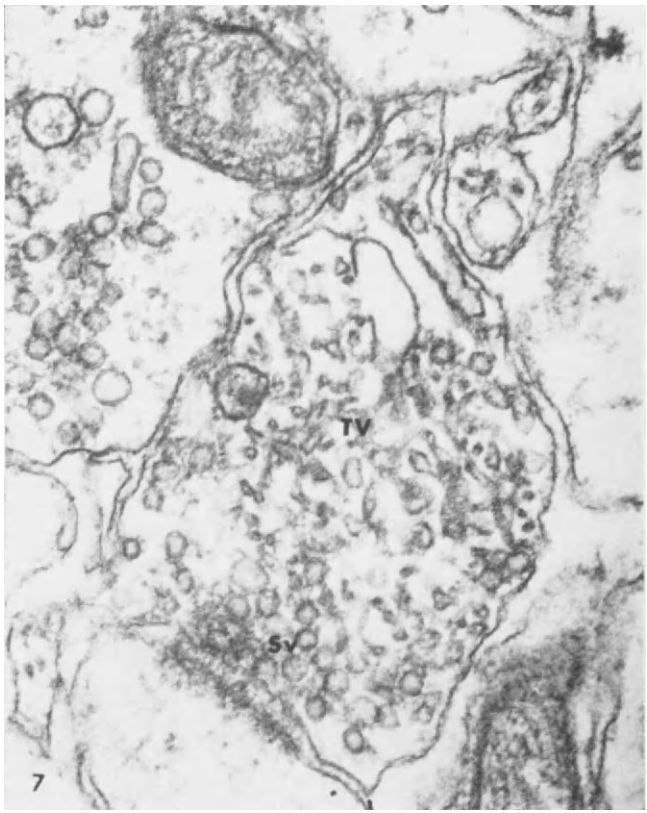

FIG. 7. Presynaptic terminal with a few tubular or vesicular profiles. Sv: synaptic vesicle. T.V.: Tubulovesicular profiles. × 90 000.

membranous structures resembling the tubulovesicular material have been described in liver cells of hamsters treated with phenobarbitone or of rats poisoned with dichlorodiphenyltrichloroethane (DDT) (Fawcett, 1966; Ortega, 1966). It has been suggested that the vesicular or tubular material in the liver cell represents proliferation of the smooth endoplasmic reticulum. A similar origin of the tubulovesicular material in presynaptic terminals is likely.

Another probable source of the tubulovesicular material is the neurotubules; this is certainly a possibility in view of the morphological resemblance, the diameter (20–35 nm compared with 24–27 nm for neurotubules), and the lack of a triple-layered substructure in the limiting walls of the tubulovesicular profiles. Cross-sections of neurotubules do not show a triple-layer membrane substructure, whereas most cytomembranes,

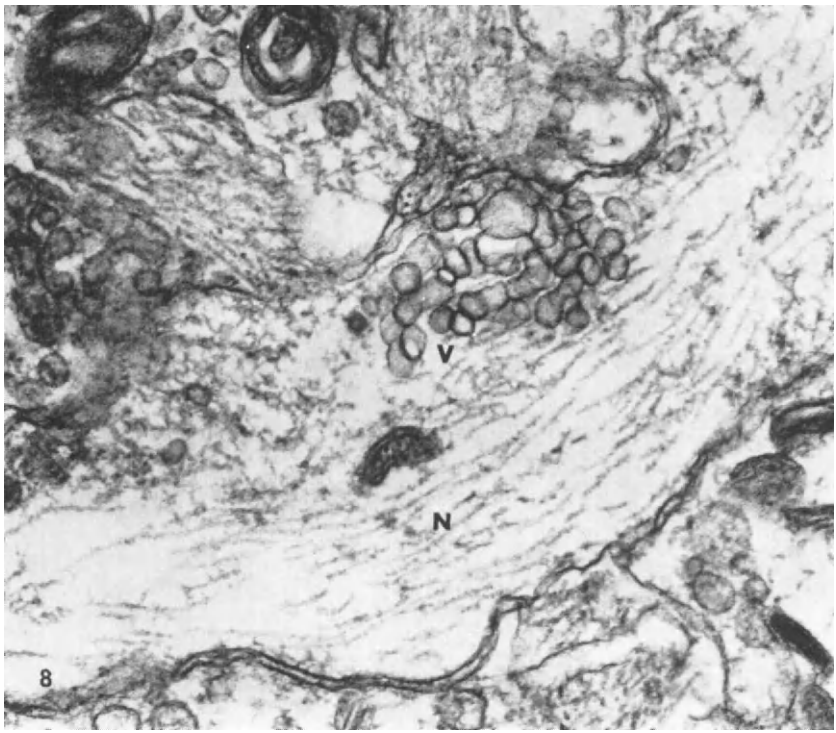

FIG. 8. Axon in senile plaque. N: neurofilament. V: cluster of large vesicles, 60–160 nm in diameter. × 50 000.

including those of synaptic vesicles and plasma membrane, show the so-called triple "unit membrane" substructure.

A third possible source of the tubulovesicular material, suggested from micrographs like Fig. 7, is the synaptic vesicles.

The changes in *boutons terminaux* in Alzheimer's disease are either secondary to malfunctioning neuronal perikarya, or primary lesions of axons and terminals produced within the senile plaques; the synaptic lesions were seen only in the area of plaques, and they should not be dismissed as secondary to the widespread neuronal degeneration; similar lesions of the

boutons terminaux have not been seen in other human diseases characterized by widespread neuronal degenerations (Gonatas, Baird and Evangelista, 1968; Gonatas, Gambetti and Baird, 1968). For these reasons, the synaptic changes in Alzheimer's disease may be significant, particularly in view of the probable importance of the synapse in memory and learning, and the occurrence of similar synaptic lesions in other human degenerative conditions of the central nervous system associated with mental retardation or dementia (Gonatas, 1967).

Finally, the recognition in neurites and dendrites of vesicular aggregates identical to those found in growth cones (Fig. 8) suggests that regenerative activity may be going on in senile plaques.

SUMMARY

Altered presynaptic axon terminals were seen in brain biopsies from the right frontal lobe from three patients with Alzheimer's presenile dementia. The altered presynaptic terminals found in or near senile plaques contained either lamellar dense bodies or tubulovesicular material.

Similar changes in presynaptic terminals have not been observed in other human disorders of the central nervous system accompanied by extensive degeneration of neuronal perikarya, or in secondary experimental degeneration.

It is suggested that the synaptic changes in Alzheimer's disease may be related to the disturbance of memory.

Finally, in one case, aggregation of large vesicles in axons, dendrites and presynaptic terminals, similar to those found in growth cones in the cerebellum, suggests that regenerative activity may be present in the senile plaque.

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DISCUSSION

Terry: How often do you find each of these changes? Is the dense body change commoner than the tubulovesicular change?

Gonatas: We saw these in three out of the five cases of Alzheimer's disease we examined. The dense body change is five or ten times more frequent than the tubulovesicular change.

Sourander: These changes in synapses and presynaptic terminals lead to the question of the transmission of catecholamines. As I mentioned (p. 15), there are some indications that dopamine metabolism is affected in presenile and senile dementias. Usually when one thinks of catecholamines one thinks of motor functions, but it has been suggested that they might also have a significance for mental function (Gottfries, Gottfries and Roos, 1969).

Polak: With the light microscope we have always seen fibrillary degeneration in astrocytes and in ependymal cells, which are the only two cells in the neuroglia connected with the blood vessels. Have you seen any changes in this type of glial element in the electron microscope? Do you think these two types of cells have some relation with the blood vessels?

Terry: Astrocytic processes are found on the periphery of the plaque and contribute to some extent to the plaque. I feel that it is a simple non-specific reactive sort of change. There is no evidence that they are a primary part of the plaque.

Polak: This type of glial degeneration is found only in the ageing process. Also, del Rio Hortega (1929) described an unusual lesion, fibrillary degeneration of the ependymal cells in senility.

Terry: I saw nothing specific in glia. They do scar a little; they develop more filaments than usual and contain glycogen as they do in any such scar process.

Dayan: The changes that Dr Gonatas has shown us are similar to those described recently by Sung (1966) and Blakemore and Cavanagh (1969), amongst others, in the gracile nucleus in aged rats and mice and in rats poisoned with a *p*-bromophenyl urea derivative. It has been clearly shown that this type of tubulovesicular structure is derived from endoplasmic reticulum, and is a feature of degeneration.

Dr Terry has mentioned the possibility of disordered axoplasmic flow. Experimentally one can show damming back of synaptic vesicles proximal to a block and a deficiency in their numbers distal to it (Dahlström, 1967). Can the presynaptic changes be correlated with the post-synaptic changes?

Gonatas: No, the presynaptic changes did not correlate with any post-synaptic alterations.

Terry: When one side of the synapse is abnormal the other is normal.

Kidd: In normal adrenal cortex Fawcett (personal communication) showed pictures like your pictures of tubulovesicular structures, and suggested that this type of membrane structure might be rich in cholesterol.

Shelanski: A damming up of vesicles is an index only for that particular component of axoplasmic flow.

Terry: Another situation which might be relevant to that seen with tubulovesicular material is that seen when one treats cells with spindle inhibitors. One effect of such inhibitors is to depolymerize microtubules, but they affect the smooth endoplasmic reticulum in just this way.

Gonatas: In other words the tubulovesicular material might be of tubular origin?

Terry: No, it is not of tubular origin. The same thing which causes tubules to degenerate happens to cause change in other cytoplasmic organelles. I am talking about our colchicine treatment of tissue cultures, with the change not in the synapses but in the perikaryon.

Taylor: Granting that the tubules decrease in number, what happens to the neurofilaments?

Terry: The 10 nm (100 Å) filaments in the human material are unchanged, as best as I can tell. The filaments as opposed to the tubules are very sparse and do not play a part in the plaque.

Roth: The changes we see as tangles and plaques may perhaps originate in certain specific neurons and not in others. Hillarp, Fuxe and Dahlström (1966), using fluorescent microscopy, claimed that there were three types

of neuron in the diencephalon, corresponding to the three possible transmitters—dopamine, adrenaline and 5-hydroxytryptamine. Would these techniques be of value in identifying the kinds of neuron from which changes originate in certain parts of the brain?

Dayan: Cortical neurons are not cholinergic.

Roth: Nor are there catecholamines to be found in the cortex.

Sourander: In the rat dopaminergic terminals have been demonstrated in limbic structures (Fuxe, 1965).

Gonatas: Much of our difficulty in interpreting these findings is due to the lack of knowledge of the more basic facts of the structural and chemical organization of the neocortex.

Barondes: Do you feel that the major morphologically identifiable abnormality is in the nerve terminals?

Gonatas: In a senile plaque one finds lots of unidentifiable processes without a myelin sheath. They contain either laminated dense bodies, and these are probably lysosomes, or the tubulovesicular material.

Barondes: The lamellar bodies are abnormal?

Gonatas: They are not normally found in presynaptic terminals.

Barondes: Are they lysosomal or what?

Gonatas: They show an acid phosphatase reaction and are lysosomal in nature, as Dr Terry described (Suzuki and Terry, 1967).

Barondes: But their morphology is not typically lysosomal.

Terry: They are typical of autophagic bodies, which are a form of lysosome. The mode of formation is different for this sort of body as opposed to the usual more granular lysosome with a unit membrane, but there are no pertinent differences.

Tomlinson: Do you ever find normal neurites within a senile plaque?

Terry: There are many normal processes in the small early plaques (granting the hypothesis that small is equal to early).

Tomlinson: Would it be unusual to find normal elements in a plaque of a size recognizable by light microscopy?

Terry: No.

Tomlinson: Normal neurites may in fact pass through plaques, so that the functional interruption, if you like, by a plaque may not be as complete as it would appear.

Terry: I showed one plaque with a myelinated process running right through the centre.

Dayan: Do pre- and post-synaptic changes occur with equal frequency? As plaques are more common than tangles, if they are due to a "dying-back" type of process affecting the terminal extensions of neurons, they

might reasonably be expected to occur without any visible perikaryal changes.

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ALZHEIMER'S DISEASE AND RELATED CONDITIONS
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NEUROFIBRILLARY CHANGES IN CONDITIONS RELATED TO ALZHEIMER'S DISEASE

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SINCE their first description by Alzheimer in 1907, and until relatively recently, intraneuronal fibrillary tangles were the subject of much more polemic speculation than meaningful analysis. This, for the most part, was due to the lack of technological capabilities until the middle of the 1950s.

Alzheimer first described neurofibrillary tangles in a case of presenile dementia (Alzheimer, 1907). Neurons containing the tangles were observed in great numbers in the cerebral cortex, associated with abundant senile plaques and characterized by their affinity for silver. Since that time, these changes have been seen in a wide variety of conditions in various parts of the brain by numerous classical investigators (see von Braunmühl, 1957; Greenfield, 1958; Hirano and Zimmerman, 1962; McMenemey, 1963). Most prominent among these conditions are senile dementia and postencephalitic Parkinsonism. The large number of other states associated with neurofibrillary changes (Malamud, Hirano and Kurland, 1961; Weinmann, 1967; Hirano, Arumugasamy and Zimmerman, 1967; McMenemey, 1968; Yase *et al.*, 1968; Blumenthal and Miller, 1969) include some such as mongolism (changes seen at 35 years and older) (Malamud, 1964), various long-standing post-infectious conditions (changes at 12–23 years of age) (see Hirano and Zimmerman, 1962) and tuberous sclerosis (at 13 years) (Hirano, Tuazon and Zimmerman, 1968)—which affect relatively younger patients.

While Alzheimer's disease and senile dementia on the one hand and postencephalitic Parkinsonism on the other both display neurofibrillary changes, a dramatic distinction can be made between the pathological findings in Parkinsonism and those in senile, or presenile, dementia solely on the basis of the distribution of the neurofibrillary tangles (see Hirano and Zimmerman, 1962). While some overlap may be discerned (Hirano

and Zimmerman, 1962; Ishii, 1966; Torvik and Meen, 1966), the overwhelming majority of the neurofibrillary tangles in senile dementia are found in all parts of the cerebral cortex, while the basal nuclei and brain stem, especially the pigmented neurons of the substantia nigra and locus ceruleus, are only mildly affected, if at all. In Parkinsonism, however, especially the postencephalitic variety, the picture is almost precisely the opposite. That is, the cerebral cortex is virtually spared whereas the brain stem and basal nuclei are heavily involved. This is especially prominent in the substantia nigra and locus ceruleus which are apparently special targets in Parkinsonism (Greenfield and Bosanquet, 1953; van Bogaert, 1957; Scott and Netzky, 1961; Alvord, 1968; Earle, 1968).

In a more recently elucidated condition the neurofibrillary tangles are seen throughout the central nervous system. This is the amyotrophic lateral sclerosis-Parkinsonism dementia (ALS–PD) complex as it appears among the indigenous Chamorro population on the island of Guam in the Marianas (Hirano, Malamud and Kurland, 1961; Hirano *et al.*, 1966, 1968*b*). The condition is characterized by the presence of widespread neurofibrillary tangles, often in great numbers, in the cerebral cortex, basal nuclei, brain stem and sometimes even in the spinal cord. As a matter of fact, all the areas affected in either senile dementia or Parkinsonism are likewise affected in the ALS–PD complex.

The topographical distributions of neurofibrillary tangles in senile dementia, Parkinsonism and ALS–PD have several points in common. Certain areas of the nervous system are invariably spared, regardless of the disease. These areas include, amongst others, the Purkinje cells of the cerebellum, the primary sensory nuclei such as the mesencephalic nucleus of the trigeminal nerve, the Gasserian and the spinal dorsal ganglia, and the lateral geniculate body. Other nuclei are only rarely affected; these include the inferior olive, the dentate nucleus of the cerebellum, anterior horn cells and Betz cells. The pontine nuclei also exhibit neurofibrillary tangles only rarely, but recently Steele, Richardson and Olszewski (1964) have described a condition known as progressive supranuclear palsy in which the pontine nucleus is one of the sites of predilection for neurofibrillary changes. The areas which are relatively constantly affected in one disease or another are the hippocampus, especially the pyramidal cells in Sommer's sector, and the glomerular formation of the hippocampal gyrus; the frontotemperal cerebral cortex, especially the orbital gyri and the temporal pole; various hypothalamic nuclei; the substantia innominata; the amygdaloid nucleus; the periaqueductal grey matter including the oculomotor nuclei; the median raphe; the substantia nigra; the locus ceruleus; the reticular

formation of the brain stem; the dorsal efferent nucleus of the vagus; and the intermediate grey matter of the spinal cord. The reasons for certain areas being selectively affected in particular diseases must, in some mysterious way, be related to the presumably different aetiological agents.

Among the earlier workers dealing with neurofibrillary changes the view arose that the changes occurring in the cortex in either Alzheimer's disease or senile dementia were different from those appearing in the basal nuclei and the brain stem in Parkinsonism, especially postencephalitic Parkinsonism. Certainly at the optical level the shape differs considerably between the two areas. In the cortex, the tangle assumes a triangular configuration filling the cytoplasm of the perikaryon. It extends into the apical dendrite, which gives it its characteristic pyramidal or flame shape, but it does not penetrate into the axoplasm. In the basal nuclei and brain stem, on the other hand, the tangles usually assume a spherical or "spool-like" form within the perikaryon.

The staining characteristics of the two kinds of neurofibrillary changes, on the other hand, are identical. They are both haematoxylinophilic and argyrophilic to a greater or lesser degree, and are equally birefringent in polarized light. Furthermore, it is possible to account for the apparent difference in shape on the basis of the configuration and orientation of the particular neurons involved. The cortical neurons are most often examined in vertical section so that the triangular shape of the cone-like apical dendrite is apparent. In fact, when such neurons are sectioned at right angles to that plane the neurofibrillary tangles appear round, and although usually smaller they are otherwise quite similar to those of the brain stem.

Regardless of whether one considers the neurofibrillary tangles in the cortex and those in the brain stem as identical or not, similar questions may be raised concerning their origin. Two views seem possible. First, the neurofibrillary tangles merely represent an increased number of the pre-existing, normally occurring neurofibrils. Second, it is possible that the neurofibrillary tangles are new structures arising as a result of the aetiological agent and not necessarily directly related to normally occurring neurofibrils.

With the advent of the electron microscope and superior methods of preservation, the structure of the neuron under both normal and pathological conditions became progressively clearer. The optically observed neurofibrils, it was found, may represent one or both of two kinds of intracellular fibrils discernible at the fine structural level (Fig. 1). First are the smaller neurofilaments, 6–10 nm in diameter. These are straight filaments of indeterminate length. Second are the neurotubules which

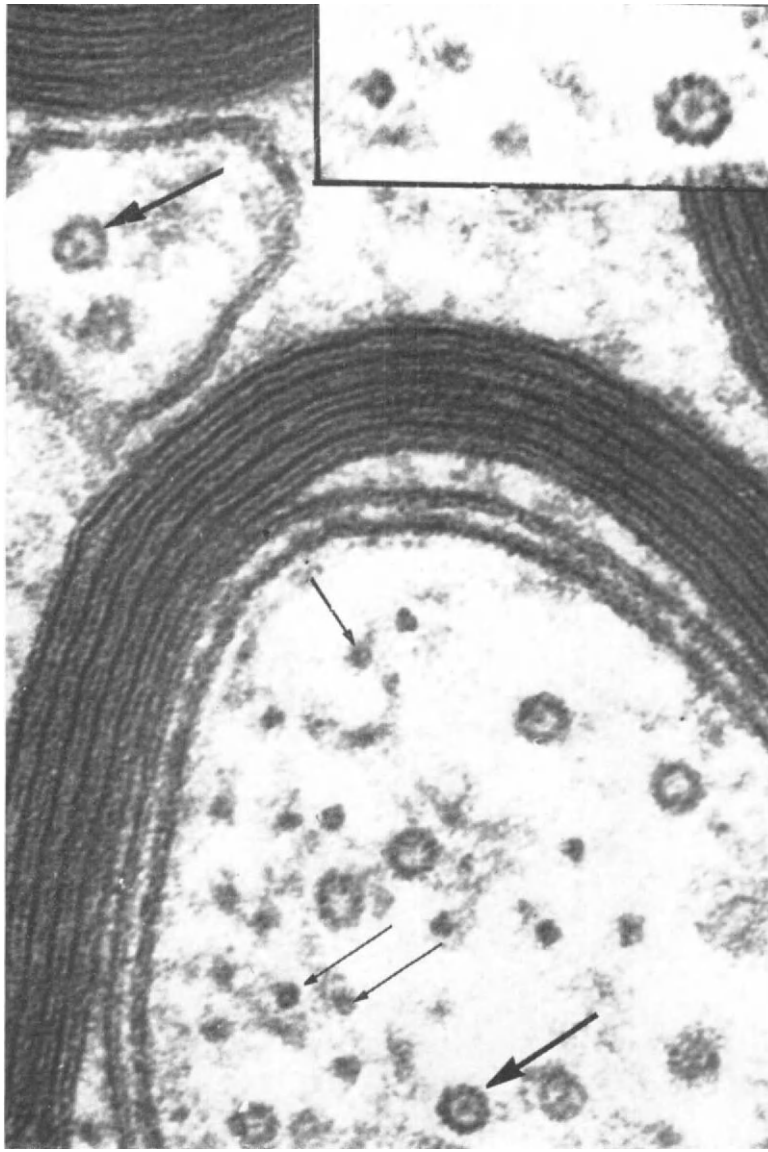
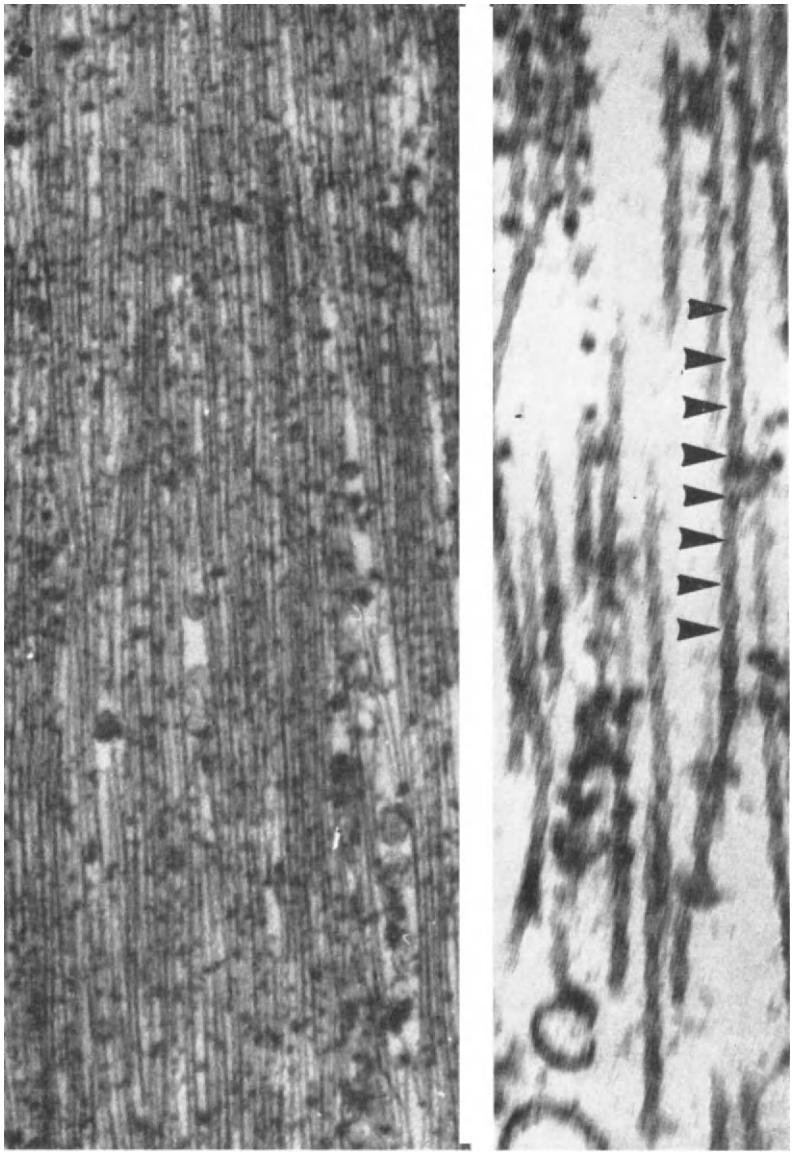


FIG. 1. Cross-section through a myelinated axon and an unmyelinated cell process in the cerebral white matter of a rat. Microtubules apparently consisting of several subunits and containing central dense spots are clearly visible at the large arrows. Neurofilaments, some with lucent central cores (small arrows), may also be seen. × 260 000. Inset: higher magnification of a microtubule and neurofilaments. × 400 000.



a) (*b*)

FIG. 2*a*. Longitudinal sections through a neurofibrillary tangle in a pyramidal neuron in Sommer's sector of a patient who died of the amyotrophic lateral sclerosis-Parkinsonism dementia (ALS–PD) complex on Guam. Numerous filaments are evident. ×40 000.
FIG. 2*b*. Higher magnification of a similar area to that in Fig. 2*a*. Regular constrictions about 80 nm apart are indicated at the arrows. ×96 000.

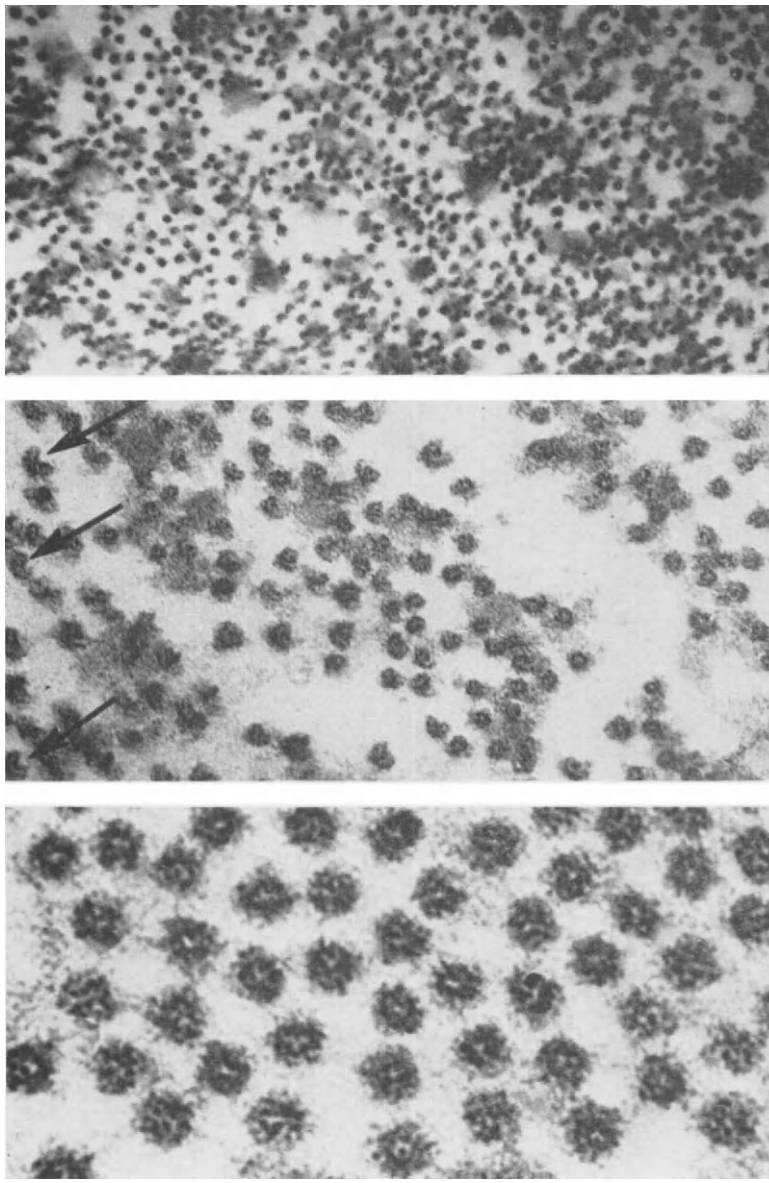


FIG. 3*a*. Cross-section through a neurofibrillary tangle in a pyramidal neuron in Sommer's sector of an ALS–PD patient. × 51 000.
FIG. 3*b*. Higher magnification of a similar area to that in Fig. 3*a*. Circular as well as arciform profiles (arrows) are evident. × 112 000.
FIG. 3*c*. Higher magnification of a similar area to that in Figs. 3*a*, *b*, showing the tubular nature of the fibrils. × 400 000.

are indistinguishable from the microtubules seen in a wide variety of cells. These are also straight structures of indeterminate length but much wider than the neurofilaments. They measure between 20 and 25 nm in width and they contain a prominent central electron-lucent core, about 10 nm

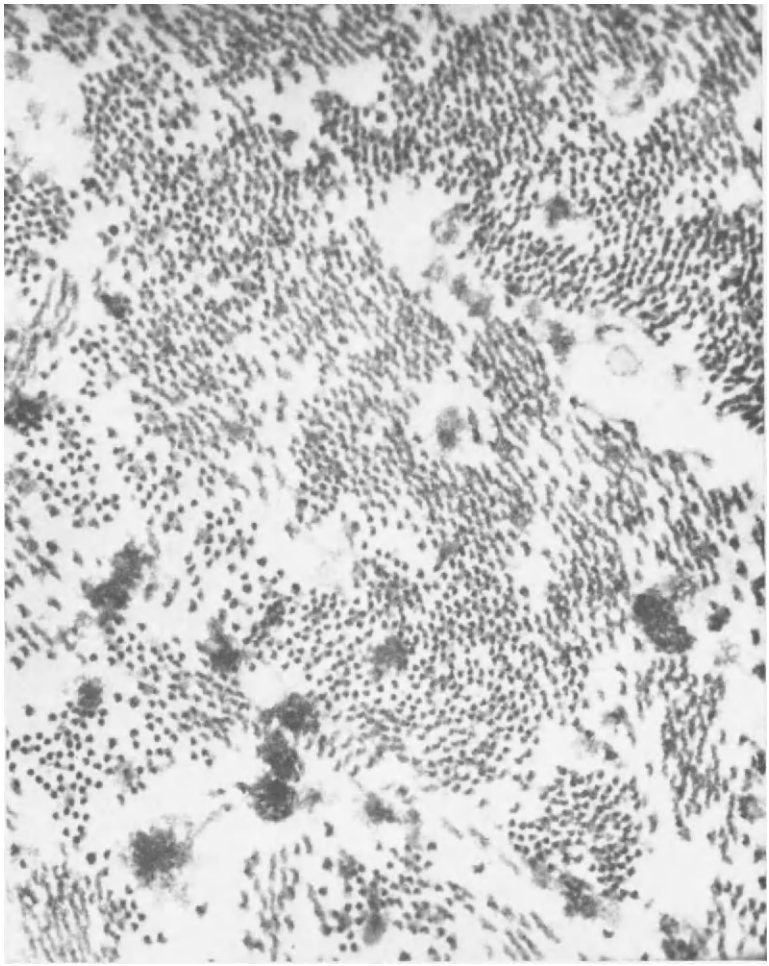


FIG. 4. Cross-section through neurofibrillary tangles in a pigmented neuron of the locus ceruleus of a patient with postencephalitic Parkinsonism. × 51 000.

wide, which lends the structure a tube-like appearance. Often small dense spots about 5 nm in diameter may be found within the central lumen (Gonatas and Robbins, 1964/1965).

The question of the origin of the neurofibrillary tangles is even more complicated. Are they really filaments, tubules, a combination of both, or

neither? When neurofibrillary tangles were first observed in the electron microscope by Terry (1963), Terry, Gonatas and Weiss (1964), and Kidd (1963, 1964) in cortical biopsies of patients with Alzheimer's disease, it was soon learned that the fibrils making up the tangles had characteristic regular constrictions about 80 nm apart (Fig. 2*a*, *b*). Cross-sections of the fibrils show both arciform and tubular profiles (Fig. 3*a*, *b*, *c*). Later studies indicated that the neurofibrillary tangles seen in postencephalitic Parkinsonism (Fig. 4) and in the ALS–PD complex are both identical to those observed in Alzheimer's disease. Such constrictions are *not* normally seen among either neurofilaments or microtubules in any area of the brain. The fibrils found in the tangles are, therefore, something quite different from normally occurring organelles (Hirano *et al.*, 1968*a*). (Compare, for example, Fig. 1, inset, with Fig. 3*c*.) On the other hand, we cannot rule out the possibility that the constricted fibrils of the neurofibrillary tangles arose from a distortion of some previously occurring normal constituent of the neuron. The question still remains: What is their origin?

As one means of studying neurofibrillary changes, several investigators have turned to their experimental production in laboratory animals (see Terry, 1968). Among the earliest of such studies in which fine structural analyses were performed were those of Ule (1961, 1962) and Chou and Hartmann (1964, 1965), who administered β, β′-iminodiproprionitrile to experimental animals. The effects of the drug were seen in the proximal portion of the axons of the anterior horn cells in which ballooning occurred due to the enormous accumulation of neurofilaments. Similar filaments were also observed by Terry and Peña (1965) in perikarya of anterior horn cells and large neurons of the brain stem after the intrathecal application of aluminium phosphate (Klatzo, Wiśniewski and Streicher, 1965; Klatzo, 1968). Similar effects of aluminium salts have since been reported by others (Mori, 1968; Seil, Lampert and Klatzo, 1969).

More recently, certain alkaloids which are known to be mitotic spindle inhibitors have been applied to nervous tissue. These include colchicine (Wiśniewski and Terry, 1967; Wiśniewski, Shelanski and Terry, 1968), vinblastine and vincristine (Schochet, Lampert and Earle, 1968; Wiśniewski, Shelanski and Terry, 1968; Schochet, Lampert and Earle, 1969; Shelanski and Wiśniewski, 1969; Schlaepfer, 1969). These agents first result in the disappearance of the microtubules normally found in neurons. There is a concomitant appearance of crystalloid structures composed of hexagonal subunits. Soon afterwards a large accumulation of filaments may be seen. Colchicine too gives rise to filament accumulation but the crystalloid structure has not been illustrated. Still later, according to Wiśniewski,

Shelanski and Terry (1968), regardless of the alkaloid used, the filaments are replaced by microtubules once more.

In our own laboratory, we have observed the appearance of the crystalloid structure (Fig. 5) in both axons and oligodendroglia and the

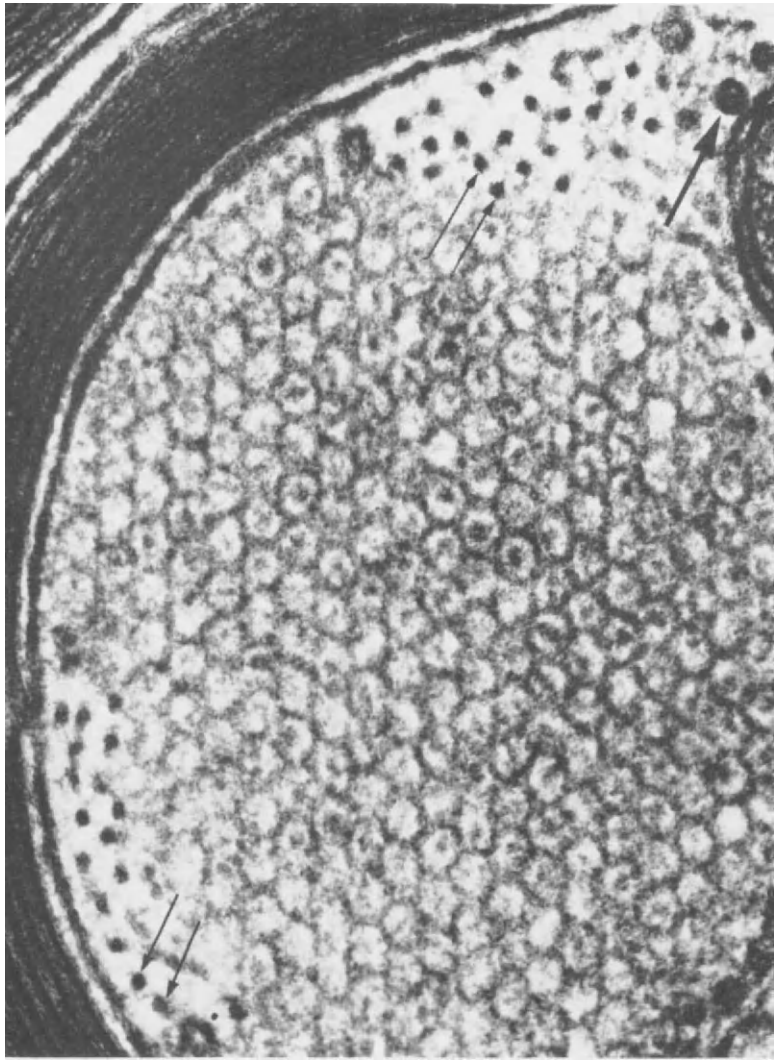


FIG. 5. A portion of a nerve cell process in the white matter of a rat four days after implantation of vinblastine sulphate. A crystalloid structure occupies most of the area in the cell process. It is composed of hexagonal subunits, many of which contain central dense spots. Rare microtubules (large arrows) and neurofilaments (small arrows) are seen on the periphery of the crystalloid. × 170 000.

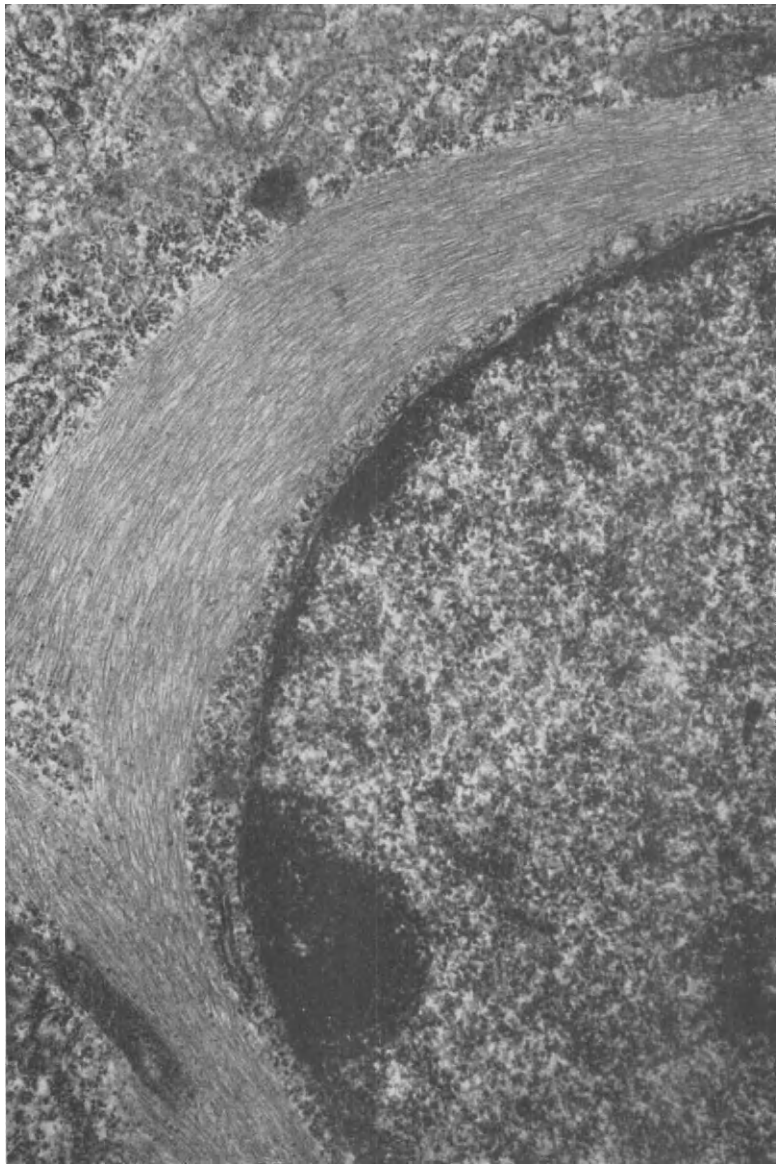


FIG. 6. Portion of a neuronal perikaryon in the cerebral cortex of an animal treated as in Fig. 5. A bundle of filaments is evident in the perinuclear region. × 25 000.

concomitant disappearance of almost all the microtubules within 30 minutes after cerebral implantation of vinblastine sulphate. Later changes included the production of filaments (Fig. 6) in both neurons and glial cells. These changes are simultaneous with the appearance of parallel linear structures in regions crowded with ribosomes in the neuronal perikayron (Fig. 7). Apparently identical structures have been reported in cultured leucocytes (Bensch and Malawista, 1969).

Interesting as the results of these experiments may be, in a sense they represent a failure of the original purpose. In no case, so far, have the experimentally produced filaments shown the characteristic regular constrictions exhibited by the fibrils making up the naturally occurring Alzheimer neurofibrillary tangles. Nor, indeed, have they been reported in any animal, normal or otherwise, other than the human.

Under certain conditions, some neurons can reflect physiological alterations by the formation of various other unusual structures generally associated with neurofibrillary tangles. These include granulovacuolar bodies and eosinophilic rod-like inclusions, both of which have recently been subjected to fine structural analysis in our laboratory (Hirano *et al.* 1968*a*).

Granulovacuolar bodies were studied in pyramidal cells of Sommer's sector in brains of patients who died of the ALS–PD complex. These structures, as indicated by early workers using the light microscope, are surprisingly enough both granular and vacuolated. They consist of a membrane-bounded collection of granular material. We have been unable to observe any fibrillary component and there is no obvious relationship between them and what seem to be normal neurofilaments and microtubules. If anything, they bear some resemblance to lipofuscin granules, another normal constituent of neurons. Granulovacuolar bodies seem to be confined, for the most part, to Sommer's sector although they have occasionally been observed in other areas (Hirano, Tuazon and Zimmerman, 1968). They are encountered in aged brain, in Alzheimer's disease and in other, more uncommon, conditions. In general, one might say that granulovacuolar bodies are found in those conditions in which neurofibrillary tangles are found in the cerebral cortex.

Eosinophilic rod-like inclusions were first described in Sommer's sector in patients with the ALS–PD complex (Hirano, 1965). On fine structural analysis, these bodies were found to contain a filamentous element about 6 to 10 nm in diameter as well as a sheet-like material about 10 to 15 nm thick and composed of a trilaminar structure in which two 4 nm dense lamina are separated by a clear space (Figs. 8, 9). Since their first description,

closely similar or identical structures have been reported in various conditions and areas. Ramsay (1967) reported somewhat similar structures, "lamellar whorls", within altered synaptic terminals in the cerebral cortex near gliomas. They were also reported in Sommer's sector in two cases of Pick's disease (Rewcastle and Ball, 1968; Schochet, Lampert and Lindenberg, 1968). More recently, similar bodies were observed in the

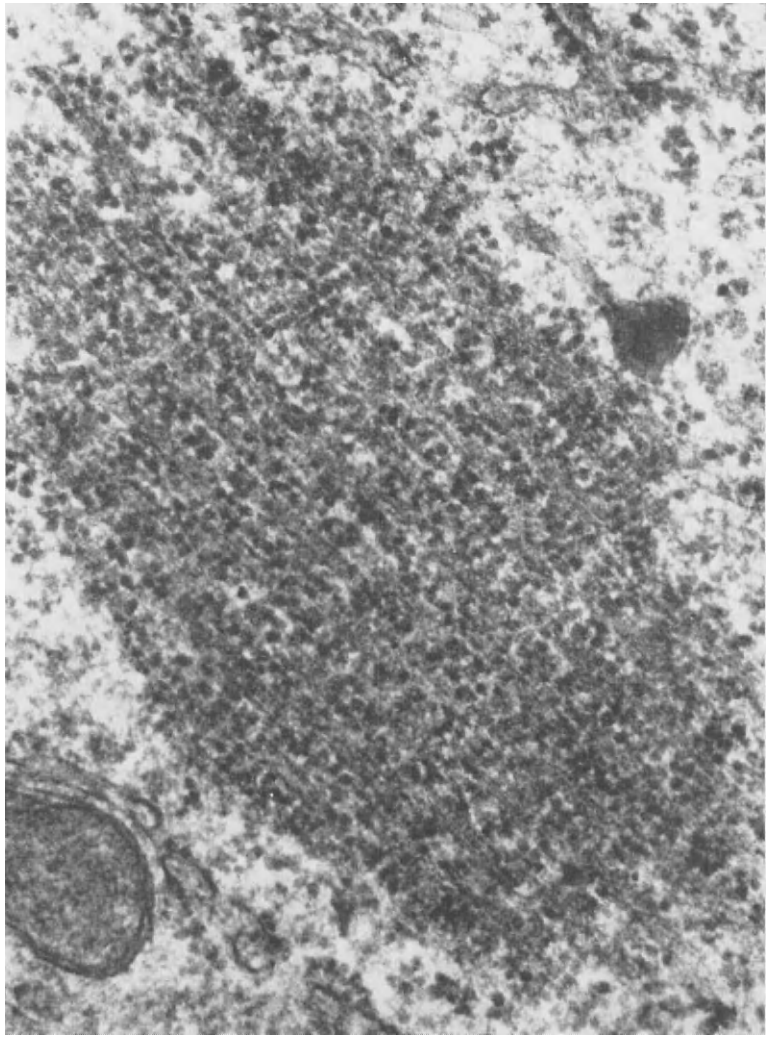


FIG. 7. A portion of a neuronal perikaryon similar to that in Fig. 6. An orderly array of parallel linear structures along which numerous ribosomes are distributed is evident. × 25 000.

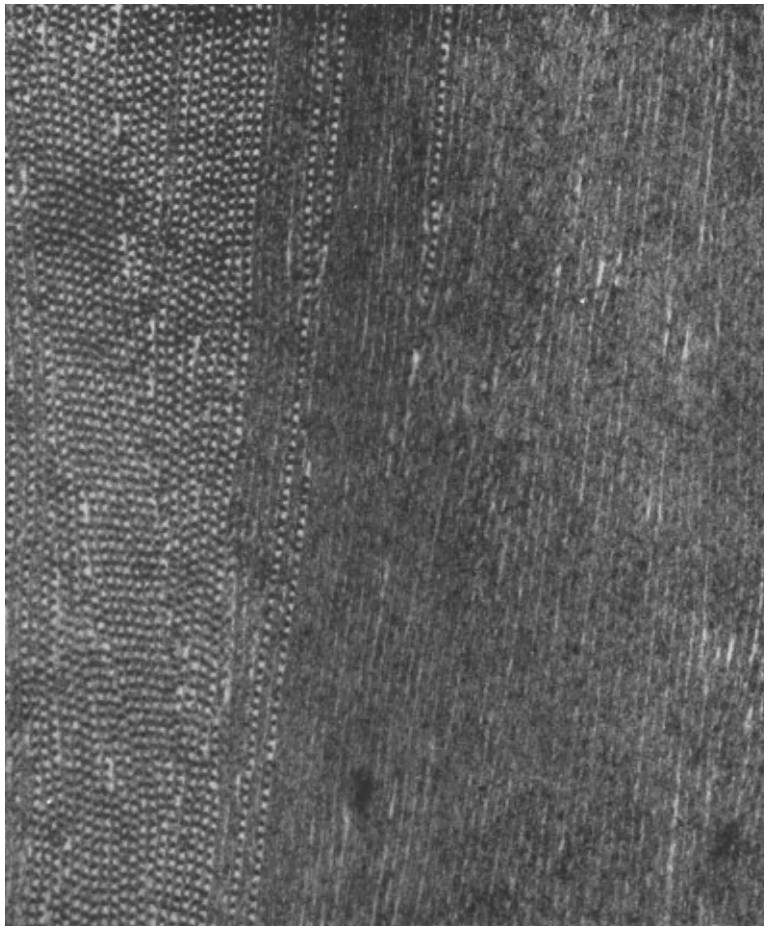


FIG. 8. Electron micrograph of an eosinophilic rod-like structure in Sommer's sector of an ALS–PD patient. Cross-sections through rows of the dense 6–10 nm filaments, alternating with layers of the less dense sheet-like material seen on the left. × 100 000.

dendrites of granular cells as well as in other cell processes of the cerebellar cortex of kuru-infected humans and chimpanzees (Field, Mathews and Raine, 1969) and in unidentified cells or cell processes in the cerebral cortex of scrapie-infected mice (David-Ferreira *et al.*, 1968). They have also been seen recently in anterior horn cells in a case of human motor neuron disease (Schochet *et al.*, 1969). The most recent report describes them in anterior horn cells in a case of amyotrophic lateral sclerosis (Chou *et al.*, 1969). The relationship, if any, between the eosinophilic rod-like inclusion

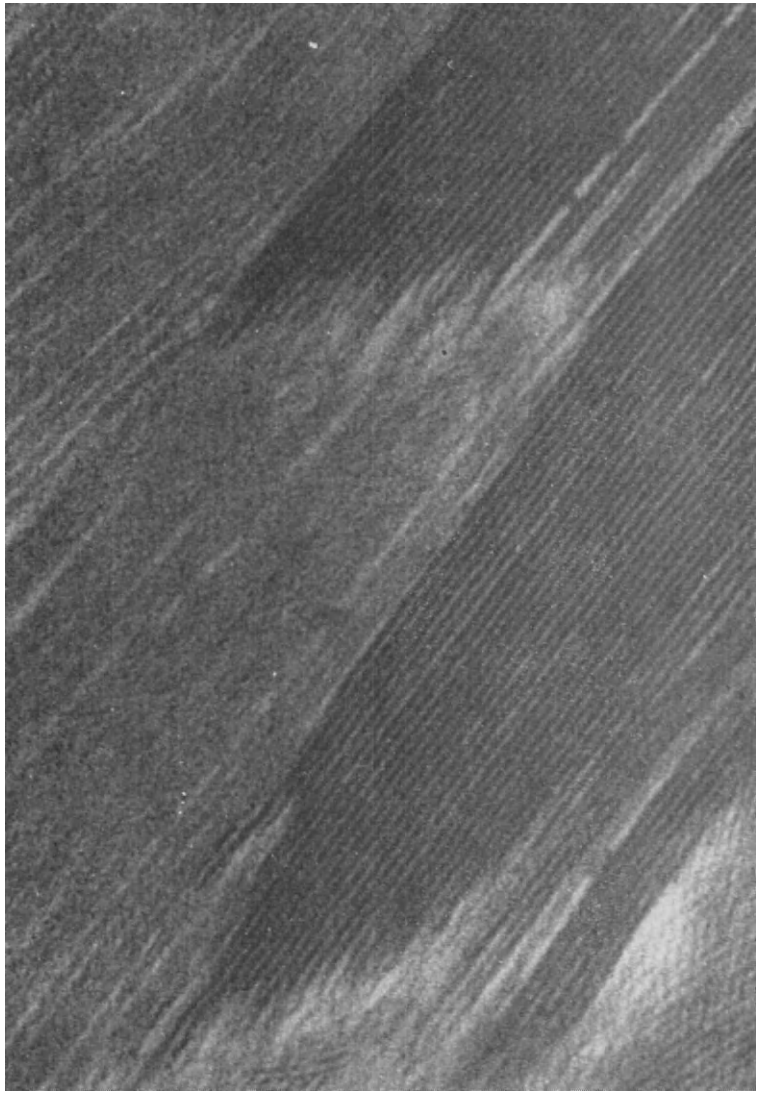


FIG. 9. A section of an eosinophilic rod-like structure cut in a plane essentially parallel to the dense filaments. × 95 000.

and Alzheimer's neurofibrillary tangles or normally occurring neurofibrils is obscure.

In conclusion, we may say that, based on their staining reactions and fine structural features, the Alzheimer's neurofibrillary tangles which appear in various parts of the brain under different disease conditions are all

essentially identical. On reflection, this fact should not be surprising since it is understandable that a single cell type may have only a limited number of ways in which to respond to a noxious stimulus. More interesting, it seems to us, might be an enquiry into the rather remarkable specificity of some aetiological agents for only certain varieties of central nervous system neurons. How is it that we can in most cases so clearly distinguish between Parkinsonism and Alzheimer's disease purely on the basis of the topographical distribution of neurons showing neurofibrillary tangles? In other words, how does one neuron vary from another so that the aetiological agent, whatever that may be, is able to distinguish between them? Similar questions arise regarding the almost total absence of such changes in certain neurons, as enumerated above. In order to approach the answer to such questions, much work is still needed regarding that perennial riddle: the origin of the neurofibrillary tangles.

SUMMARY

Since first described by Alzheimer in the cerebral cortex of a patient with presenile dementia, neurofibrillary tangles have been observed in a number of other human diseases. Best known among these is senile dementia and postencephalitic Parkinsonism. Pathologically, senile and presenile dementia differ from Parkinsonism in the topographical distribution of affected neurons, especially with regard to those displaying neurofibrillary tangles. In senile and presenile dementia, the tangles are mainly found in the cerebral cortex, while in cases of Parkinsonism they are confined mostly to the brain stem. More recently, neurofibrillary tangles have been observed as a principal pathological feature of the amyotrophic lateral sclerosis-Parkinsonism dementia (ALS–PD) complex as it appears among the native Chamorros on Guam. In this condition, both the cerebral cortex and the brain stem contain numerous neurons with neurofibrillary tangles. On the basis of their staining reactions and fine structural characteristics, neurofibrillary tangles in senile and presenile dementia, in Parkinsonism and in ALS–PD, all seem to be identical regardless of their location. When seen in the electron microscope, they appear to consist of fibrils displaying characteristic regular constrictions at about 80 nm. So far, no clear relationship has been demonstrated between Alzheimer's neurofibrillary tangles and any other pathological or normally-occurring structures.

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DISCUSSION

Terry: Not all tangles in other diseases are made up of twisted tubules. In some situations one sees what looks in the electron microscope like an Alzheimer tangle but isn't. In patients with leukaemia and treated with vinblastine Dr Shelanski and Dr Wiśniewski saw in the dorsal root ganglia neurofibrillary tangles made up of 10 nm filaments.

Roth: With light microscopy such tangles would presumably look identical.

Strich: Is there any difference in the electron microscope between the tangles that were argyrophilic and those which were not?

Hirano: The difference seems to consist only in the concentration of the "twisted tubules".

Shelanski: What about the tangles in sporadic motor neuron disease?

Terry: They don't look quite identical to those in Guam-Parkinson dementia.

Hirano: They are distinctly different and I think such changes properly belong to an entirely different class.

Gonatas: This is a point which should be clarified. Many neuropathologists think that the so-called neurofibrillary change, produced experimentally with colchicine and so on, is the identical change of Alzheimer's disease.

Roth: Are you suggesting that these should be described simply as neurofibrillary tangles as distinct from Alzheimer's neurofibrillary tangles?

Gonatas: No. I think we can use the term "twisted tubule" provided we do not imply that this is necessarily a twisted normal neurotubule, or we can use the term "Alzheimer neurofibrillary change", referring only to the twisted tubules. The colchicine-produced change could be called a neurofibrillary or neurofilamentous change caused by colchicine.

Terry: In the dementias, in the Guam-Parkinsonism Dr Hirano demonstrated, in post-encephalitic Parkinsonism and in the centre of the Pick bodies one gets twisted tubules. As far as we know they appear nowhere else. In sporadic motor neuron disease, vincristine neuropathy and all the other experimental tangles, one gets bundles of 10 nm filaments. The difference in appearance will be demonstrated later (pp. 223–240).

Kidd: When I suggested that the abnormal structures in the electron microscopy of this disease were twisted, I was aware that they could be twisted tubes, but I felt that they were more like twisted pairs of filaments (Kidd, 1963). Now I am not sure which they are. One thing that makes me doubtful is their appearance when negatively stained. I crushed up some brain from a case which had been in formalin for two years, and managed to obtain a few electron micrographs (see Fig. 1). In some places the structure could be a twisted flattened tube, and in others it could be a pair of filaments. This line could be followed better by people more experienced in these techniques—one gets a suggestion of subunit structure in places.

Shelanski: The microtubule has a well-defined wall structure which means it can be distinguished in negative contrast preparations from any other tubular structures.

Hirano: Our model in the Alzheimer neurofibrillary tangles (Hirano *et al.*, 1968) consists of a tubule twisted over part of its length. The untwisted part retains its circular cross-section while the twisted portion is distorted so that sections through it would result in curved, arciform profiles.

Terry: To call it a hexagonal pattern with the central unit out is a matter

of semantics, but the elasticity of the microtubule *in vivo* might be such that it relaxes between twists and is cylindrical in these areas, while it flattens as it twists so that there is an arciform cross-section.

Gonatas: There should be a belly between the two constrictions, but the arciform profile will be lost then.

Terry: Another sort of plastic could twist intermittently, leaving

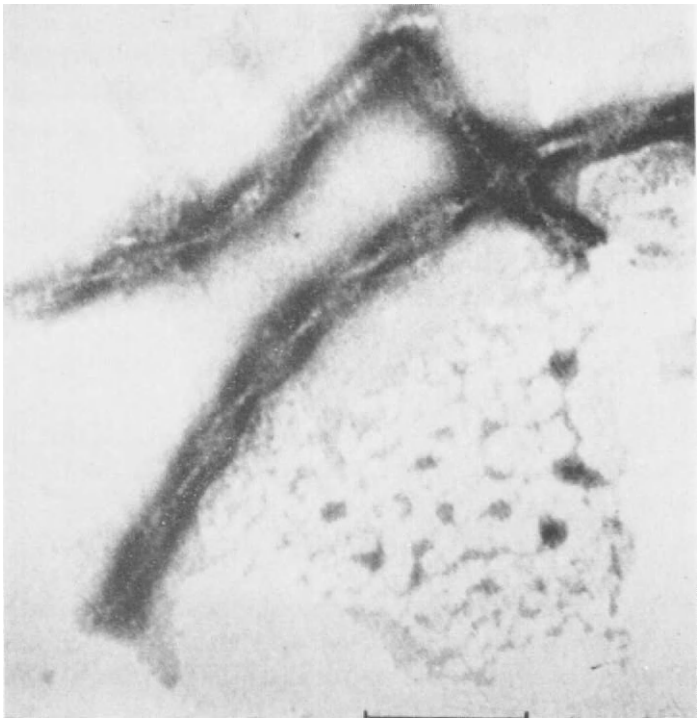

FIG. 1 (Kidd). Twisted filaments from old formalin material from a case of Alzheimer's disease. 1% potassium phosphotungstate negative stain. The bar represents 100 nm.

circular profiles between the twists. It depends on the physical properties of the model you happen to use.

Kidd: Dr Hirano (Hirano *et al.*, 1968) seemed to be saying that a tubule was twisted for only part of its length. In the neocortex in Alzheimer's disease I found only one picture of filaments with what might be a variation of this type. Otherwise the twisting always seemed to occur at consistent intervals.

Taylor: Do you find a consistent 80 nm period, or regions which do not appear to show any twisting, Dr Terry?

Terry: Our material seems to indicate that it is quite consistent. I haven't seen the mixed straight and twisted tubules.

Gonatas: Wasn't your original figure 160 nm, Dr Kidd?

Kidd: It was 80 nm (160 nm for a complete turn, which is twice the period) but I have seen some which were all consistently up to 100 nm, so maybe the twisting is not very regular.

McMenemey: Dr Hirano, what do you think is the relationship between granulovacuolar degeneration and neurofibrillary degeneration in the cells of the hippocampus in Alzheimer's disease? In the Parkinson-dementia syndrome in Guam did you see granulovacuolar degeneration in any cells apart from the hippocampus?

Hirano: I don't know the relationship but granulovacuolar bodies are often associated with cells which show tangles in Sommer's sector and in adjacent areas in the hippocampus. Neurofibrillary changes and granulovacuolar bodies may sometimes also be observed in certain other areas of the central nervous system but much less frequently and much less consistently.

McMenemey: Do you think one condition leads to the other, as Morel and Wildi (1952) suggested?

Hirano: I don't know. There is no fine structural evidence to suggest such a relationship. Granulovacuolar bodies remind me of lysosomes. Tangles do not.

Polak: Are the granules in the vacuoles Congo-red positive?

Hirano: Yes.

Terry: In an autopsy specimen from the hippocampus of a senile dement, we have seen a vacuole bounded by a formalin-disrupted membrane. The centre contained the dense material which made up the granule of the so-called granulovacuolar change. We have seen changes like this in certain experimental conditions, suggesting that this is an autophagic phenomenon involving a type of lysosomes.

McMenemey: Would one find the same if one could have a biopsy of the hippocampus?

Terry: No, in life the membrane would be a complete one, and the central granule would probably be somewhat more compact.

Roth: Why is it almost entirely localized to the hippocampus?

Terry: Neurons, although they look alike to microscopists, are very different from one another. They have varying metabolic rates and they are susceptible to different sorts of stimuli. The poliovirus kills motor neurons and not sensory neurons. Aluminium affects neurons of the spinal

cord but far fewer neurons of the cerebral cortex. The neurons of the hippocampus are susceptible to whatever causes granulovacuolar change; those of the neocortex are not.

Roth: So neuron specificity is the explanation of some specific vulnerability to some unknown metabolic or toxic influence.

Pratt: Is there any correlation between granulovacuolar changes and dementia, Professor Roth?

Roth: Yes. The high correlation between the proportion of cells in the hippocampus exhibiting granulovacuolar degeneration and dementia reported by Woodard (1962, 1966) has been confirmed by our own findings (Tomlinson, Blessed and Roth, 1968). When the brain shows more than 10 per cent of change at this site the other pathological changes associated with Alzheimer's disease will also be found.

McMenemey: But such a change is found in some normal people.

Roth: Yes, but in them the proportion of hippocampal cells affected is almost invariably less than 10 per cent.

McMenemey: Why did Woodard think that this kind of change was specific for Alzheimer's disease?

Roth: He said it was specific when seen in more than a certain percentage of hippocampal cells.

Did I understand you correctly to say that 10 per cent of the adult population of Guam is affected by the Guam form of Parkinsonism? Is anything known about the underlying genetical factors?

Hirano: I don't know.

Terry: Wasn't it 10 per cent of the adult deaths, not 10 per cent of the living population with this disease?

Roth: If 10 per cent of adult and late adult deaths in a population such as that of Guam are due to this disorder, the total morbid risk from the disease cannot be far from 10 per cent. I was thinking in terms of a corrected life expectation of approximately 10 per cent. Even half of this figure would be a high morbid risk for a genetically determined disorder.

Hirano: The incidence of amyotrophic lateral sclerosis among the Chamorros on Guam has been reported to be 50–100 times higher than the incidence of classical amyotrophic lateral sclerosis elsewhere. If the adult deaths from both amyotrophic lateral sclerosis and Parkinsonism dementia are combined, they account for approximately 20 per cent of all the Chamorro adult deaths on Guam (see Brody and Chen, 1968).

Roth: One wonders how a condition in which hereditary factors appear to play some part has managed to resist being weeded out by natural selection and remained so common. Although it does not begin until

adult life, it can disfigure and disable people from the age of about 30, so that it presumably impairs fertility.

Shelanski: It is not a population which delays its fertility artificially and they may have had all their children by that age.

Roth: A five per cent reduction in fertility would be sufficient to render the gene rare in the population unless modified or attenuated forms of the disorder (perhaps exhibited by heterozygotes) carried some selective advantage.

Hirano: Patients with amyotrophic lateral sclerosis can, in most instances, be delivered of normal infants (Huston *et al.*, 1956).

McMenemey: Is this seen only in the Chamorros?

Hirano: Yes, in Guam that is so. Recently amyotrophic lateral sclerosis with tangles was reported among several Japanese patients (Nakai, 1969; Shiraki, 1968; Yase *et al.*, 1968).

Pratt: I have always felt that the Parkinsonism-dementia complex is not a genetically-determined condition, but analogous to the kuru situation. What is the current incidence in the small group of Chamorros who live in the United States? The figures reported earlier (Lessell *et al.*, 1962) showed that the incidence was about the same as in Guam. The critical evidence should be available by now.

Hirano: Recently Dr Brody's group reported that the incidence was still the same as on Guam (Eldridge *et al.*, 1969).

Roth: Most of the diseases that have been proved to be due to major genes have been rare. The only exceptions, as far as I am aware, have been conditions in which the heterozygote carried some selective advantage.

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ALZHEIMER'S DISEASE AND RELATED CONDITIONS
G. E. W. WOLSTENHOLME & MAEVE O'CONNOR

DETERMINATION OF NEUROFILAMENT AND MICROTUBULE DENSITY IN NERVE FIBRES

(What Factors Control Axon Calibre?)

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A brief statement may be in order to explain the choice of this subject for my presentation. My interest in Alzheimer's disease has been focused for some time on the histochemistry and enzyme histochemistry of senile plaques. These plaques are characterized by the accumulation of high molecular weight polysaccharides (Schiffer, 1956, 1957; Ishii, 1958; Margolis, 1959; Diezel and Vogel, 1965). Increased activities of several oxidative and hydrolytic enzymes were demonstrated histochemically (Josephy, 1949; Friede and Magee, 1962; Friede, 1965) and confirmed in several laboratories (Krigman, Feldman and Bensch, 1965; De Giacomo, 1966; Thorack, 1966; Thomas, 1968). Acid phosphatase activity has been localized in plaques at the fine structural level (Suzuki and Terry, 1967). Little has been added to this body of information during the past few years; what is known seems to create more questions, rather than open new avenues towards understanding the mechanism of plaque formation. Accordingly, I appreciate the opportunity to discuss recent findings from our laboratory on neurofilament densities in nerve fibres. Although not directly related to Alzheimer's disease, they provide an insight into the possible functional significance of microtubules and neurofilaments in nerve fibres. Our findings will show that neurofilaments, and perhaps also microtubules, are in some way involved in controlling the calibre of axons. However, if we want to drive this point home, I have to ask you to follow me into what may seem a rather circuitous journey into the problem of "what factors control the calibre of an axon."

The neuronal perikaryon is generally considered the main site of synthesis of cytoplasmic protein and of formation of cytoplasmic organelles, which are distributed from there into and along the dendrites and axons. The nerve fibre is generally considered the equivalent of a pipe within which axoplasm is propelled and redistributed by a still unknown force. The concept of a "pipe" is enforced by the structure of the myelinated nerve

fibre, with its axis cylinder encompassed by a thick myelin sheath. Determinations from electron micrographs of axon circumference and the number of turns of myelin lamellae in the sheath show that sheath thickness increases with axon size according to the formula:

$$\text{Number of myelin lamellae} = \frac{\text{axon circumference in } \mu\text{m} - 2{\cdot}32}{0{\cdot}24}$$

In other words, there is an average of one full turn of myelin lamella for every 0·24 μm increase in axon circumference above the value of 2·32 μm, or mean circumference of a non-myelinated fibre* (Friede and Samorajski, 1967).

A curious aspect of sheath thickness was revealed by quantitative analysis of axon-sheath relations during the period of myelination, when axons grow from very thin foetal fibres to their adult calibre. The number of turns of myelin lamellae in the sheaths increased in proportion to the increase in axon calibre, in both peripheral and central fibres (Friede and Samorajski, 1968; Samorajski and Friede, 1968*a*). This means that as a new turn of myelin lamella is laid down, each of the previous turns has to elongate equally to accommodate the larger axon. Since the lamellae form a continuous spiral from inner to outer mesaxon, the process may involve growth of all portions of the lamella, or growth at mesaxons with slippage of the lamellae.

It was of interest, therefore, to investigate the effects of experimental manipulations of axon calibre on sheath structure. On transection, axons invariably swell at and near the stumps, a phenomenon known as reactive axon swelling, the causes of which are beyond the scope of this presentation. The effect of acute axonal swelling on the sheath may be rupture of the sheath, or stretching, or slippage of its lamellae. Quantitative analysis of the changes in axon calibre and in the number of myelin lamellae in the sheath of swollen fibres demonstrated conclusively that the myelin sheath unwinds like a spring by outward slippage of its myelin lamellae—that is, the number of turns of myelin lamellae decreases as axon circumference increases; the distance between two lamellae remains unchanged (Martinez and Friede, 1970*b*). There is reason to believe that excessively swollen axons can become denuded by this process.

If an increase in axon calibre unwinds the sheath, what would be the changes in sheath structure with shrinkage of the axon? A parallel study for the first week of Wallerian degeneration demonstrated that the axis cylinder collapses and the sheath increases in thickness because of inward

* The values are for non-fixed tissue (Friede and Samorajski, 1967); their magnitude may change with variation in preparatory technique without affecting the validity of the formula.

slippage of the myelin lamellae; this phenomenon was more marked for fibres with thin sheaths than for fibres with thick sheaths (Friede and Martinez, 1970).

Let us return to our model comparing the nerve fibre with a pipe. The myelin sheath evidently cannot be equated with the wall of a "pipe". The sheath forms an investment of the axis cylinder which adjusts passively to experimental changes in axon calibre. It cannot act as a restraint for axoplasm under ambient pressure. Hence the axon has to be viewed as a "self-containing system", and the control of its calibre becomes more and more intriguing. The most logical next step was to search for an interrelation between axon size and the neuronal perikaryon.

It is generally assumed that a relation exists between the size of the cell body and the size of its axon. Among various species, the size of the neuronal perikaryon increases with body size (Hardesty, 1902). The volume of Purkinje cells increases with the logarithm of body weight, probably in direct relation to the average length of their axons (Friede, 1963). To discover whether a relation exists between cell size and axon length in a homologous population of neurons in the same species we determined the segmental variation in cell size, nuclear size and nucleolar size in rat spinal cord. The underlying assumption was that the motor cells have longer and thicker axons in the segments from which the nerve plexus of the extremities originate than those supplying the neck and trunk. The largest perikarya corresponded, indeed, to the segments of origin of the cervical and lumbosacral plexus (Koya and Friede, 1969). Do neurons maintaining longer and larger axons have a higher protein synthesis than those with short axons? A segmental study of leucine incorporation, using scintillation counts in cord segments and grain counts in radioautograms, demonstrated that the uptake of leucine per volume of cytoplasm of motor neurons was constant along the entire cord and did not vary with cell size (Koya and Friede, 1969). This demonstrated that neurons having longer and larger axons did indeed synthesize more protein than neurons with short axons; however, they did so because they had larger cell bodies, larger nuclei and larger nucleoli, and *not* because they had a higher rate of protein synthesis per volume of cytoplasm. Our findings imply that there is some balance between the size of a neuronal perikaryon, the volume of protein synthesized per perikaryon and the size or length of its axon.

Much better insight into this coordination was obtained by comparing, in growing rats, the increase in the volume of axoplasm with the increase in the volume of cytoplasm in the perikaryon (Martinez and Friede,

1970*a*). The changes in the volume of axoplasm were estimated from changes in the length of the limb and nerve and from changes in the fibre spectrum, which in turn were determined from electron micrographs at 12 phases of development. Cell sizes, nuclear sizes and nucleolar sizes of the anterior horn motor neurons and spinal ganglion cells of the corresponding segments were determined by planimetry and measurements of diameters. The growth curves for the volume of axoplasm in the fibres were nearly

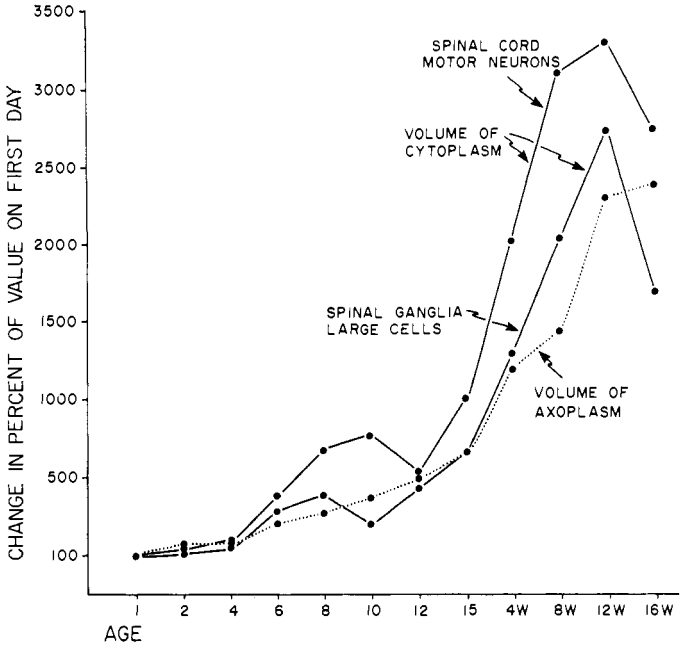

FIG. 1. Growth curves for the volume of cytoplasm in the anterior horn motor neurons and in the large cells of the spinal ganglia, and for the volume of axoplasm in rat sciatic nerve fibres (Martinez and Friede, 1970*a*). See text. W: weeks.

identical with those for the volumes of cytoplasm in the perikarya of the respective nerve cells (Fig. 1). These findings show that an amazingly precise correlation exists between the rates of changes in the volumes of axoplasm and of perikaryal cytoplasm. Such a correlation strongly suggests the existence of a system of controls and feedbacks between cell body and axon.

With these observations as a basis, I wish to propose a concept of "neuronal economy" which postulates that a rather precise coordination must exist between the rate of protein synthesis, that of cytoplasmic

redistribution within the cells and that of protein decay. In the perikaryon, a steady state must exist between:

$$\frac{\text{Protein decay within perikaryon} + \text{net protein export into cell processes}}{\text{Protein synthesis per perikaryon}} = 1{\cdot}0$$

(assuming no protein is leaking through the cell membrane). In the axon, a steady state must exist for:

$$\frac{\text{Protein decay} + \text{loss of protein through cell membrane}}{\text{Net protein import (fast and slow flows)} + \text{local protein synthesis}} = 1{\cdot}0$$

(loss of protein may occur at the synaptic end of the fibre, as shown by Rahmann, 1968). The process of *growth*, or *hypertrophy*, is characterized by a change in any factor or combination of factors producing $> 1{\cdot}0$; atrophy is characterized by a change producing $< 1{\cdot}0$.

Such a system of neuronal economy relating widely removed portions of the cell evidently necessitates some system of communication and correlation. Neither the myelin sheath nor axonal flow *per se* nor the rate of neuronal protein synthesis can explain the formation, existence and sustenance of an axis cylinder of uniform calibre extending for distances of the order of the second to third power of the cell's diameter.

At this point, our attention was drawn to the neurofilaments and microtubules in the axons. Recent studies in invertebrates indicate that microtubules may be responsible for maintaining or altering the shape of the cells (Porter, 1966; Tilney, 1965). Moreover, microtubules and neurofilaments are being considered as organelles possibly involved in the convection of axoplasm (Weiss and Holland, 1967; Ochs, Sabri and Johnson, 1969; Sjöstrand and Karlsson, 1969).

Electron micrographs of peripheral nerves show an extremely regular density of neurofilaments: the number of microtubules varies with axon calibre, as there are fewer tubules per area of axoplasm in the thick myelinated axons than in non-myelinated axons (Peters and Vaughn, 1967). Counts in rat and mouse sciatic nerves showed that the ratio of filaments to microtubules is approximately five times greater in non-myelinated than in myelinated fibres; the average ratio was 1·0:1·38 per non-myelinated axon and 1·0:0·29 per myelinated axon (Friede and Samorajski, 1970). We next counted the number of neurofilaments and microtubules in relation to planimetric determinations of axon calibre. Needless to say, this is rather tiresome as a large fibre has several thousand units. Myelinated and non-myelinated fibres were studied in sciatic nerves of three rats and

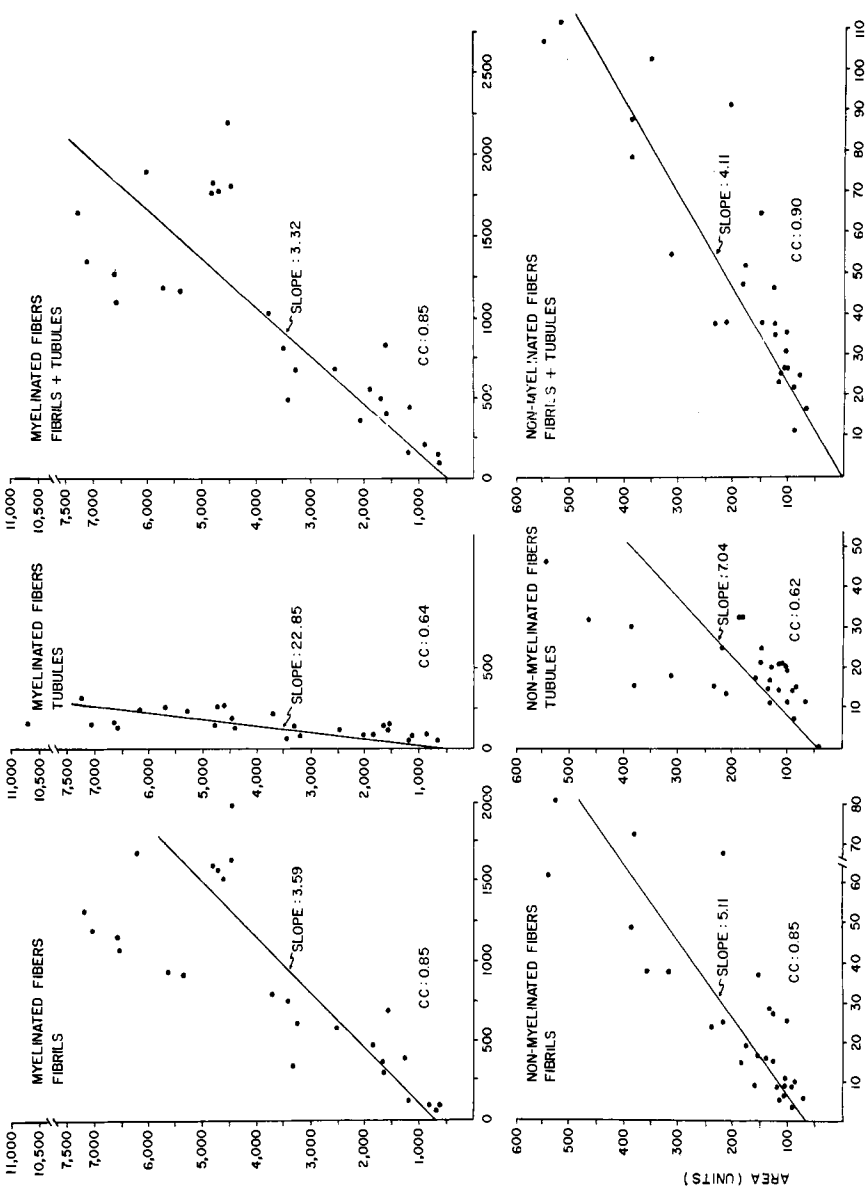

FIG. 2. Densities of neurofilaments (fibrils) and microtubules in relation to the cross-sectional area of axoplasm in fibres of a mouse sciatic nerve (Friede and Samorajski, 1970). See text. Above: myelinated fibres; below: non-myelinated fibres; the latter are plotted on a much larger scale. CC: correlation coefficients.

three mice and the data were subjected to statistical regression analyses by computer (Friede and Samorajski, 1970). The area of axoplasm showed linear correlations, statistically significant, with the number of filaments, the number of microtubules and the sum of the numbers of filaments and microtubules (Fig. 2). However, the correlation coefficients between axoplasmic area and number of microtubules obtained from three mice and three rats were always considerably lower than those between axoplasmic area and number of filaments. The correlation coefficients for axoplasmic area and the sum of filaments plus microtubules were only slightly better than those for axoplasmic area and filaments. Hence we concluded that the calibre of an axon is correlated either with the sum of the numbers of its filaments and microtubules, or with the number of its filaments, the former being more likely. No other structures in the axoplasm show such a correlation: the density of axoplasmic mitochondria (Samorajski and Friede, 1968*b*) and that of combined volumes of mitochondria and vesiculotubular organelles (Martinez and Friede, 1970*b*) decreased with increasing axon calibre. The myelin sheath is the only other structure that shows an extremely high degree of correlation with axon calibre, but the preceding data indicate that the sheath thickness adjusts to axon calibre, and not *vice versa*.

The slopes of the regression curves and the correlation coefficients for the relation between axon calibre and neurofilament-microtubule material were identical in myelinated and non-myelinated axons (Friede and Samorajski, 1970). This shows that the relation between axon calibre and number of filaments and microtubules is independent of the presence of a myelin sheath. The same conclusion was reached in a recent quantitative analysis of peripheral and central fibres in the quaking mutant of mouse, showing that the relation between axon calibre and neurofilament-microtubule material was independent of the thickness of the myelin sheath (Samorajski, Friede and Reimer, 1970). The quaking mutant is characterized by a disturbance of myelin formation, probably due to disturbed cerebroside synthesis (Bowen and Radin, 1969*a*, *b*). Quantitative analysis of electron micrographs showed that approximately half the normal number of turns of myelin lamellae were formed around the axons. Axon growth was not disturbed and the neurofilament-microtubule density in these axons was the same as in normal control animals. Occasionally there were huge non-myelinated axons showing the same filament-tubule density as normal myelinated fibres of the same calibre.

These observations indicate that the number of filaments, probably in conjunction with the number of microtubules, is involved in some way

in the processes that determine the calibre of an axon, whether myelinated or not. It is also apparent that the correlation between neurofilament-microtubule density and axon calibre can exist only if the number of neurofilament-microtubule units normally maintains a fairly constant relation with the volume of liquid axoplasm dispersed between them. A relationship to axonal flow may also be implied because the maintenance of the size and shape of a neuron is inseparable from the redistribution of cytoplasm within it. The conclusions drawn from these findings are in consensus with invertebrate studies and suggest that the neurofilament-microtubule system represents a cytoskeleton along which the motive force for cytoplasmic streaming is generated (Porter, 1966).

At this point it is tempting to hypothesize that the perikaryon of a nerve cell controls the calibre of its axon by the number of neurofilament-microtubule units projecting from the cell body; a tapering axon could be explained by variable lengths of these units. However, two observations cast serious doubt on this simple model. First, electron microscope observations of Purkinje cells show an initial thin segment of the axon emerging from the hillock (O'Leary *et al.*, 1968). The illustrations do not indicate any unusual increase in filament density, suggesting that most of the more distal filaments are not continuous with the cell body.

Secondly, more doubts are being created by an analysis of the structure of optic fibres. This line of work was stimulated by the incidental observation that the cholesterol content of human optic fibres increases by 110 per cent between the segment of the nerve nearest to the eyeball and that of the tract nearest to the lateral geniculate nucleus (Friede and Hu, 1967). In cat optic fibres we have seen a gradual proximo-distal increase of approximately 50 per cent in cholesterol content. Correlated morphological studies demonstrated that both sheath thickness and axon calibre increase along the fibres by approximately the same value. In other words, the axis cylinders of cat optic fibres are nearly 50 per cent thicker in the proximity of the lateral geniculate nucleus than near the eyeball; sheath thickness increases proportionately. Neurofilament-microtubule density in these fibres, now being determined, suggests there is equal density in all parts of the fibres. Accordingly, the number of neurofilament-microtubule units increases along the fibre in strict proportion to the increase in axon calibre. This peculiar structure of cat optic fibres demonstrates that the constant proportion between axon calibre and neurofilament-microtubule elements does not depend on continuity of the latter with the perikaryon.

A better understanding of the normal functional significance of neurofilaments will be of help in interpreting pathological data and also in the

formulation of research strategy. For the sake of academic argument, let me play with a few metaphors on the "cytoskeleton". Cachexia would certainly not be called "skeletal hypertrophy", adiposity "skeletal atrophy", or progressive emaciation "skeletal proliferation". Yet in electron microscopy, where the field of observation is minute in relation to the size of the cell, one is especially prone to conclude that there is "proliferation" or "loss" of units when all that is observed is a change in their density. Too often, change in density of filaments (or of smooth endoplasmic reticulum) is accepted as evidence of proliferation. This point may be of particular significance in studies on pathological alterations in the neurofilament-microtubule material, because we find that these components correlate precisely with axon size. It is of supreme interest, therefore, to find and study models which would allow us to evaluate changes in density in relation to changes in cell volume.

We are just now beginning to scratch the surface of the problem of the functional significance of the neurofilament-microtubule apparatus of nerve cells. The main point of my presentation, therefore, is to stress options in the interpretation of pathological changes in filament density. Increases in filament material may reflect changes in the structure or chemistry of the filaments, or an actual proliferation, or a reduction in the volume or density of cytoplasm dispersed between the filaments. The process may therefore be dystrophic, hypertrophic or atrophic in nature. It may be impossible to distinguish between these possibilities without careful analysis of the whole system. Let me also stress the concept of "neuronal economy" for understanding atrophy as well as hypertrophy of the cells. We cannot hope for a satisfactory analysis of a pathological state of a neuron without getting involved in the balances that apparently exist between the perikaryon and its extensions.

This report could have been more pertinent to Alzheimer's disease if I had described in detail the enzyme histochemical features of senile plaques. I hope that you will find that it was more exciting to discuss the perspectives for future work on neurofilaments and microtubules, uncertain as they are at present.

SUMMARY

The density of neurofilament-microtubule material in axons was studied as part of an extensive series of experiments on factors that may control the calibre of an axon. There was no indication that the myelin sheath can control axon size; the sheath was found to adjust to swelling or collapse of the axon by outward slippage or inward slippage, respectively, of the

myelin lamellae; constant readjustment of the sheath was also associated with myelin formation. That axon size is related to the size of the perikaryon can be shown by comparison of various species, and by segmental variation in cell size in the spinal cord. Radioautographic studies indicate that nerve cells with long axons synthesize more protein only by virtue of having larger cell bodies, and not by having a higher rate of protein synthesis. A study of sciatic fibres in developing rats shows that the volume of axoplasm increases strictly *pari passu* with the volume of cytoplasm in the perikarya of the corresponding nerve cells in the cord and spinal ganglia.

These findings indicate that the calibre of axons is normally controlled by an intrinsic mechanism which is correlated in some way with the perikaryon of the cell. A precise balance must exist between local rates of protein synthesis, redistribution of proteins and local rates of loss or decay of proteins. This concept is called "economy of the neuron".

Counts show that the number of units of the neurofilament-microtubule material in the axoplasm of sciatic nerve fibres is in extremely precise correlation with axon calibre, regardless of whether the fibre has a myelin sheath or not. Additional evidence from optic fibres and in pathological material supports these observations. The data are interpreted as indicating a cytoskeletal function for the neurofilament-microtubule material, which may also be involved in the redistribution of cytoplasm and cytoplasmic organelles between remote portions of the cell.

Acknowledgement

This study was supported by United States Public Health Service Grant NB 06239 from the National Institute of Neurological Diseases and Stroke.

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DISCUSSION

Gonatas: What is the significance of this difference in ratio between neurofilaments and microtubules in large myelinated axons as compared with that in small non-myelinated axons? What do you think happens? If the neurofilament is a precursor of the microtubule why do filaments in small axons polymerize into tubules while in larger axons they don't?

Friede: When we planned these studies, I hoped we would obtain some information on whether neurofilaments change to microtubules and *vice versa*. However, our findings appear to be more consistent with static differences in fibre organization than with a steady state in the rates of transmutation between these organelles. The only clue to the significance of this difference in axon organization was that the density of mitochondria in axoplasm (Samorajski and Friede, 1968) decreases *pari passu* with the density of microtubules. This may suggest that microtubules are related to the transport of particulate material along the fibre.

Barondes: In the optic nerve the ratio of microtubules and neurofilaments changes during development. Have you looked at the developing sciatic nerve?

Friede: Yes. We used three adult mice, one rat aged six days, one rat of 12 days, and one adult rat. Myelination of rat sciatic nerve begins on day 1; by day 6 the nerve is growing and in active myelination. There was no difference in the ratio of neurofilaments and microtubules between the growing fibres and the adult fibres. In other words the decrease in the number of microtubules is not related to age, but rather to the calibre of the fibre.

Strich: Is the ratio between microtubules and neurofilaments the same in the central nervous system as in the peripheral nervous system? If it is just a matter of stability, the peripheral nerve may have different requirements to keep the nerve fibre physically stable.

Friede: The ratios appear to be similar in the central nervous system but I did not count.

Shelanski: Many rather asymmetrical cells, the mammalian red blood cell for one, have no microtubules. The form of platelets is also not strictly dependent on the marginal band of microtubules. Therefore, it is not necessary in all cases for microtubules to serve cytoskeletal functions.

Friede: No, but the axon cylinder is an extreme case. I can only emphasize the extremely high correlation coefficients obtained between fibre calibre and the sum of neurofilaments and microtubules; no other axoplasmic organelle shows a similar correlation. If another factor is involved in controlling axon calibre, it is one that cannot be visualized by any of the available methods.

Terry: What do you think of the rather vague branches running from one filament to the next? We pointed these out in some experimentally-induced tangles (Terry and Peña, 1965) but they were denied as valid structures by other investigators.

Friede: These "extensions" are a source of error in counting filaments, especially when the latter are not cut precisely perpendicular to their axis. One wonders whether some material, or force, is keeping the filaments apart, as they are quite uniformly spaced and do not coalesce in osmotically shrunken fibres. But I do not believe that this indistinct material adhering to the filaments forms bridges between them.

Shelanski: This question was partly answered at the University of Colorado symposium on *Control of Form in Biological Systems* (September, 1969). Dr L. Tilney, studying *Actinosphaerium*, and Dr R. McIntosh, studying mitotic spindles, found cross-bridges occurring randomly between microtubules. A whole area might have only one or two. Their impression, and the impression of others working on the nervous

system, was that the microtubules were spaced out by these cross-bridges; furthermore Dr McIntosh's impression was that the microtubules might be moved in relation to each other by some sort of motile system in these bridges.

Friede: I have not seen intertubular bridges in axons, but if such bridges exist, their length would have to vary in inverse relation to fibre calibre. The spacing of microtubules is much less regular than that of neurofilaments.

Shelanski: Those findings were strictly with microtubule systems. The results didn't seem clear. The interesting thing is that not only could Dr Tilney generate the spacings with two distinct sizes of bridges but the angles at which these bridges came are those that one would expect to be generated by the 12- or 13-fold symmetry of the microtubule.

Barondes: What happens to microtubules and neurofilaments with branching? Have you followed any axons that give off branches? If the ratio changes as a function of diameter one would expect to wind up with more microtubules in the derivative axonal pieces than in the main trunk axon. This would argue very much against the single continuity of microtubules throughout a whole nerve fibre. In other words the branches would be of smaller diameter and should therefore have relatively more microtubules, if the relationship held throughout the axon.

Friede: All the counts of sciatic fibres were taken at the same level; however the findings on optic nerve which I mentioned briefly suggest that neurofilaments and microtubules are not always continuous with the cell body.

Shelanski: At the Boulder conference in 1969, Dr Paul Weiss presented results on counts of main trunks and branches of microtubules only (Weiss, 1970). His impression from a great number of tubules was that the microtubules were in continuity with the cell body, or that the numbers split proportionately among the branches. If this were true, it would have to be the neurofilaments which were the variable element and not the microtubules themselves.

Friede: Many neurons have a rather thin initial axon segment; for example Purkinje cells have an initial, thin non-myelinated axon segment of approximately 0·5 μm diameter (O'Leary *et al.*, 1968). No counts of filaments or microtubules were made for this segment, but it is difficult to believe that all the filaments and microtubules found distally in the axon would squeeze through this narrow initial portion. It is likely that many of them do not connect with the perikaryon.

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ALZHEIMER'S DISEASE AND RELATED CONDITIONS
G. E. W. WOLSTENHOLME & MAEVE O'CONNOR

AN EXPERIMENTAL APPROACH TO THE MORPHOGENESIS OF NEUROFIBRILLARY DEGENERATION AND THE ARGYROPHILIC PLAQUE*

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PREVIOUS concepts of the origin and development of Alzheimer's tangle and senile plaque were based on the light and electron microscopic study of human autopsy material. Without these studies, we would know little or nothing about the morphology of the disorder, but proof of the morphogenetic hypothesis must be based on experimental models which duplicate the human lesions under controlled conditions. It is our purpose to report experiments which partially fulfill this need.

Two major hypotheses have been offered in the past: one that the lesions represent a form of primary amyloidosis (Divry, 1952), and the other based on ageing of colloid inside and outside the cells (von Braunmühl, 1957). The resolving power of the electron microscope utilizing human tissue has demonstrated the limited but important role of amyloid in this area (Terry and Wiśniewski, 1970). The same instrument applied to experimental models permits study of the earliest changes, revealing clearly the significant similarities with and differences from the human disease. The models also afford an opportunity to study the intriguing relationships between neurofibrillary tangle and argyrophilic plaque.

MATERIALS AND METHODS

Experiments were carried out on mice, rats, rabbits, cats and monkeys. The following aluminium compounds were used: the phosphate, prepared as Holt's adjuvant; the chloride; and the hydroxide paste. The spindle inhibitors colchicine, vinblastine, vincristine, and podophyllotoxin were also investigated.

Mice and rats were very resistant to the desired effects of aluminium, and rabbits, cats and monkeys proved more useful. For acute experiments the

* Supported by Research Grants NB-02255 and NB-03356, from the National Institute of Neurological Diseases and Stroke, United States Department of Health, Education and Welfare.

aluminium compounds were injected directly into the cerebral tissue through a burr hole, or more commonly into the cerebrospinal fluid by puncture of the cisterna magna. The amount of each compound is given in Table I. The chronic lesions were induced by very slow infusions of the

TABLE I

DOSES (ml) OF ALUMINIUM

	$AlCl_3$ (1%)	$AlPO_4$ (*adjuvant*)	$Al(OH)_3$ (*paste*)
Rabbit	0·1	0·1	0·3–0·5
Cat	0·5–1·0	0·5–1·0	0·3–0·5
Monkey	0·5–1·0	0·5–1·0	0·3–0·5

aluminium chloride or phosphate through a fine cannula in the subarachnoid space over the cerebral convexity or into the spinal canal. The rate of infusions was such that 0·1 ml of solution entered in ten to 15 minutes. When the spinal route was used, the animal's head was held 15 to 20 cm above the level of infusion; the head was lowered during intracranial infusions. Aluminium paste was injected into the frontal subcortical white matter. The animals with chronic lesions were observed for periods up to 12 months.

The spindle inhibitors were used in amounts shown in Table II. They were injected intracerebrally in mice and rats, and into the cerebrospinal

TABLE II

DOSES (μg) OF SPINDLE INHIBITORS

	Colchicine	*Vinblastine*	*Vincristine*	*Podophyllotoxin*
Mice	1– 10			
Rat	1– 20			
Rabbit	30–1000	100–300	100–300	400
Cat	30– 100	100–300	100–300	400

fluid over the cerebrum, in the cisterna magna, or in the lumbar spinal canal of rabbits and cats. The drugs were all dissolved in saline, but podophyllotoxin needed first to be taken up in 50 per cent ethanol.

The cortical slab technique was used, following Szentágothai (1965). A curved wire was carried along under the cortex to undercut areas of varying thickness and width. Frontal lobotomies were performed in the cat 3 cm posterior to the pole.

The perfusion technique of fixation was used throughout by the method previously reported (Wiśniewski and Terry, 1967). Mice and rats were perfused with glutaraldehyde, while the larger animals were started with paraformaldehyde followed by glutaraldehyde. Dalton's chrome-osmium

was the post-fixative, followed by embedding in Epon. All electron micrographs were taken with the Siemens Elmiskop.

RESULTS

Clinical observations

Animals injected intracerebrally or into the cisterna magna with Holt's adjuvant or aluminium chloride developed quadriparesis and generalized epileptic seizures within ten to 20 days after an incubation period during which they seemed completely normal except for the electroencephalogram. Most animals died after a few days of seizures, by the 15th day of injection. When they were treated with the same compound by a slow infusion through a fine cannula, or when aluminium paste was injected subcortically, 60 to 70 per cent of the animals did not develop neurological symptoms except for sporadic *grand mal* or Jacksonian seizures. The remaining third developed symptoms as in the first group, and followed a similarly brief course. Slow infusion into the lumbar subarachnoid space resulted in a similar acute episode in about a third of the animals, and these did not survive for chronic observation. The remainder developed over two to four weeks slight paresis of the hind legs and very sporadic generalized seizures. It was this group which was useful for long-term study. The cat is hardier than the rabbit and has been found preferable for the work on chronic lesions.

Quadriparesis and respiratory insufficiency were seen within 24 hours when mice and rats were injected intracerebrally, or when rabbits and cats were injected with spindle inhibitors into the cisterna magna. Death followed within two to five days. Treatment of the larger animals via the lumbar spinal canal led to incontinence and severe paraparesis or paraplegia within 24 hours. When proper clinical care was given the animals in this latter group could survive for months. After slow infusion of these drugs into the cerebrospinal fluid over the cerebral convexity, clinical signs were not found.

Frontal lobotomy of the cat caused contralateral hemiplegia which soon diminished to hemiparesis. Clinical manifestations were not apparent after small cortical areas were undercut.

Gross morphological observations

Gross changes in the brain were not observable during the first few weeks after treatment with aluminium in the cerebrospinal fluid. Mild hydrocephalus appeared a few months after similar treatment (Fig. 1). Subcortical injection of aluminium paste produced a small cyst.

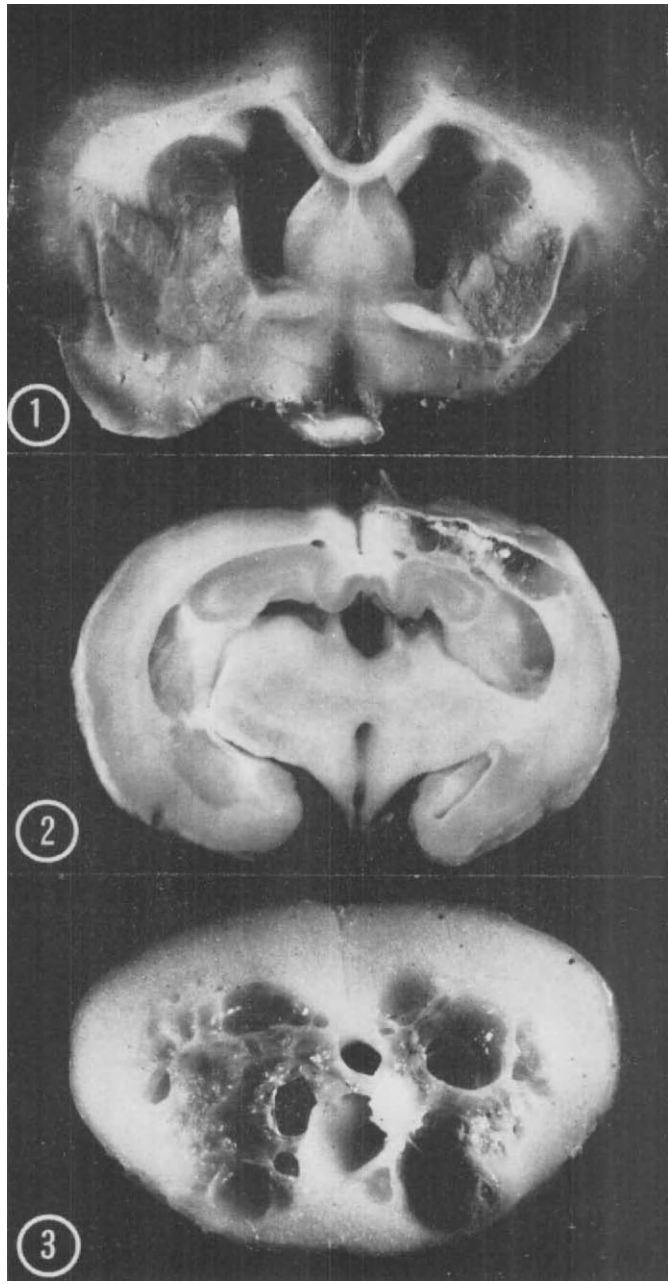

FIG. 1. Rabbit brain, six months after infusion of $AlCl_3$ over the cerebrum. The ventricles are dilated. Many neurofibrillary tangles were present in the cortex.

FIG. 2. Rabbit brain, three months after infusion of 50 μg of colchicine into the cerebral cortex. A local cyst is apparent at the site of injection.

FIG. 3. Rabbit spinal cord, two months after infusion of 1000 μg of colchicine into lumbar subarachnoid space. Cystic myelomalacia was present up to the lower thoracic level.

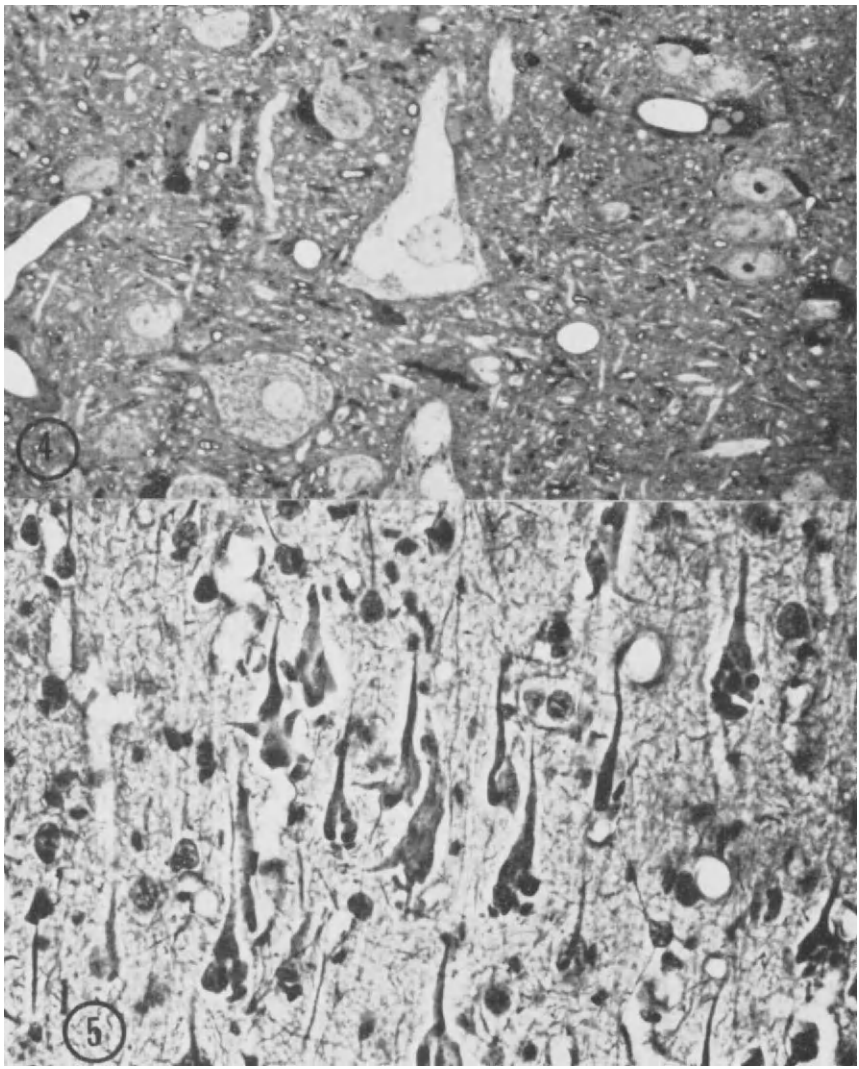

FIG. 4. Toluidine blue stain of 1 μm section of rabbit cortex treated with aluminium. The central neuron displays clear zones in the cytoplasm which correspond to the filamentous aggregates. × 560.

FIG. 5. Bodian preparation of paraffin section of rabbit cortex treated four months previously with aluminium reveals prominent neurofibrillary tangles. × 560.

Spindle inhibitors did not cause gross lesions to develop until after about one week. Then the cortex showed either cyst (Fig. 2) or scar, depending on the dose, while spongy necrosis was found in the cord (Fig. 3) when more than 100 μg of colchicine or more than 200 μg of vinblastine or vincristine was used.

Microscopic findings

(*a*) *Aluminium.* Neurons in particular locations were clearly more susceptible than others. Those most likely to be altered by the aluminium were the large and medium-sized neurons in the basis pontis and spinal anterior horns. The limbic cortex and basal ganglia were of second-order sensitivity. Some neurons in any area could be affected if the local concentration of the drug was maintained at a high level.

During the first week the affected neurons had clear, faintly pink zones in their somatic cytoplasm when stained with haematoxylin and eosin, and colourless zones with toluidine blue (Fig. 4). The corresponding silver (Bodian) preparation revealed these vacuole-like lesions to be strongly argyrophilic (Fig. 5). Slight congophilia was also noted. During the second week this silver-staining material came to fill most of the soma and its proximal neurites, resembling the neurofibrillary degeneration seen in Alzheimer's disease.

The earliest ultrastructural change was one or more island of 10 nm filaments, most often near the neuronal nucleus or in a dendritic hillock (Fig. 6). From the beginning there was no topographical relationship between these close-packed filaments and any of the normal cellular organelles; they seemed to arise *de novo* from the cytoplasmic matrix. As time passed the cell enlarged to a certain extent, while the masses of filaments increased enormously (Fig. 7). The filaments were organized as bundles, and at no stage were they separated from the rest of the cytoplasm by a membrane. The outline of the filamentous bundle, which was sharply defined despite the lack of a membrane, was apparently modified by the shape of the cell, being rounded in round cells and elongated in multipolar cells. The other cellular organelles including the nucleus were remarkably well preserved without any evidence of degeneration or autophagia. Even when the cytoplasm was almost entirely occupied by the filaments the nucleus had a normal appearance. It is interesting to note that neurotubules were preserved in the cytoplasm outside the filamentous region. The most proximal portions of the neurites were usually moderately distended by bundles of filaments. In the neuropil, however, only a few distal processes were

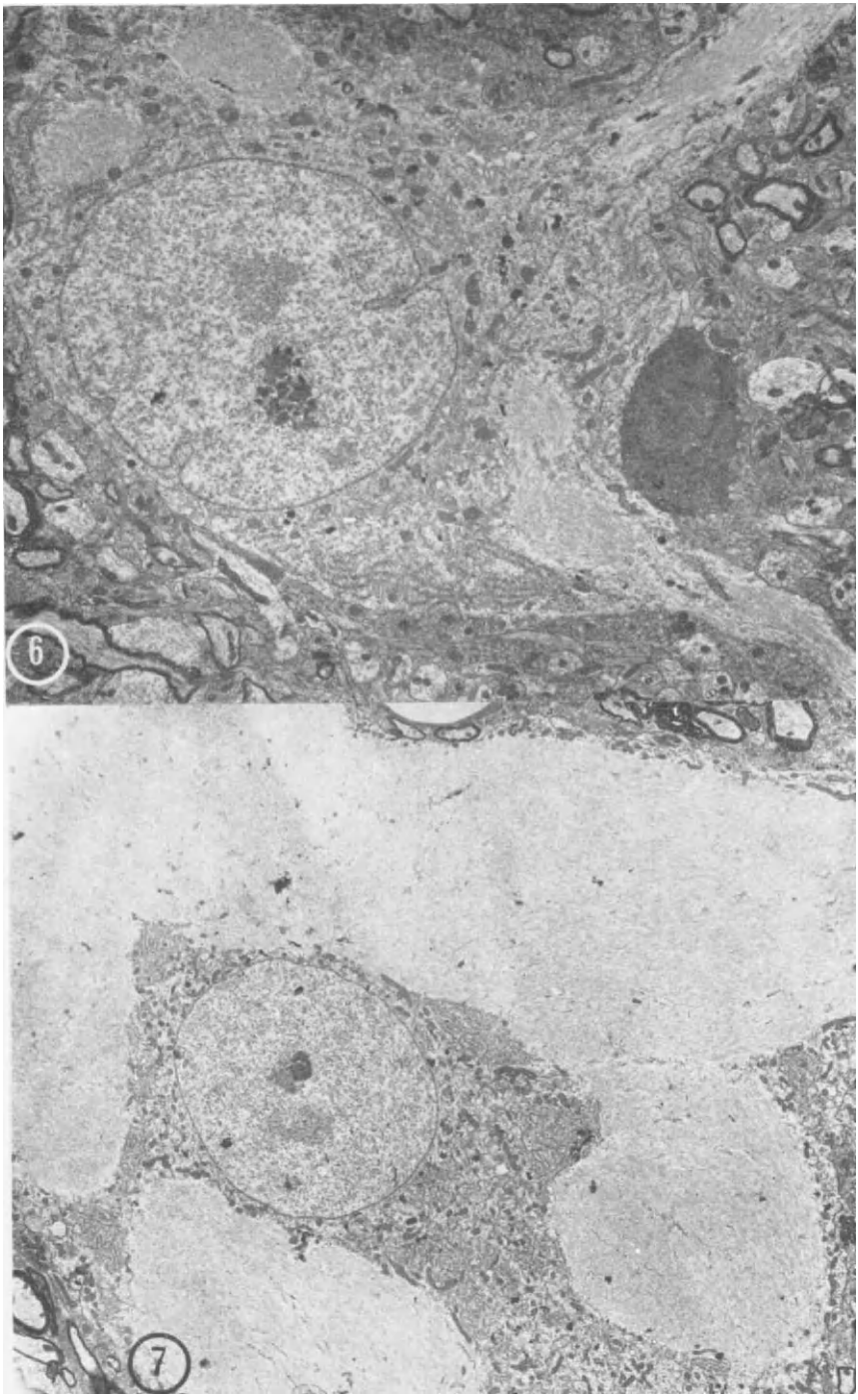

FIG. 6. Early changes in an aluminium-treated rabbit neuron consist of small islands of filaments in the perinuclear and hillock regions. The other organelles are normal. × 13 600.

FIG. 7. Later changes in an aluminium-treated rabbit neuron consist of large masses of filaments distending the cell. Note that the nucleus and residual cytoplasm are still well preserved. × 2750.

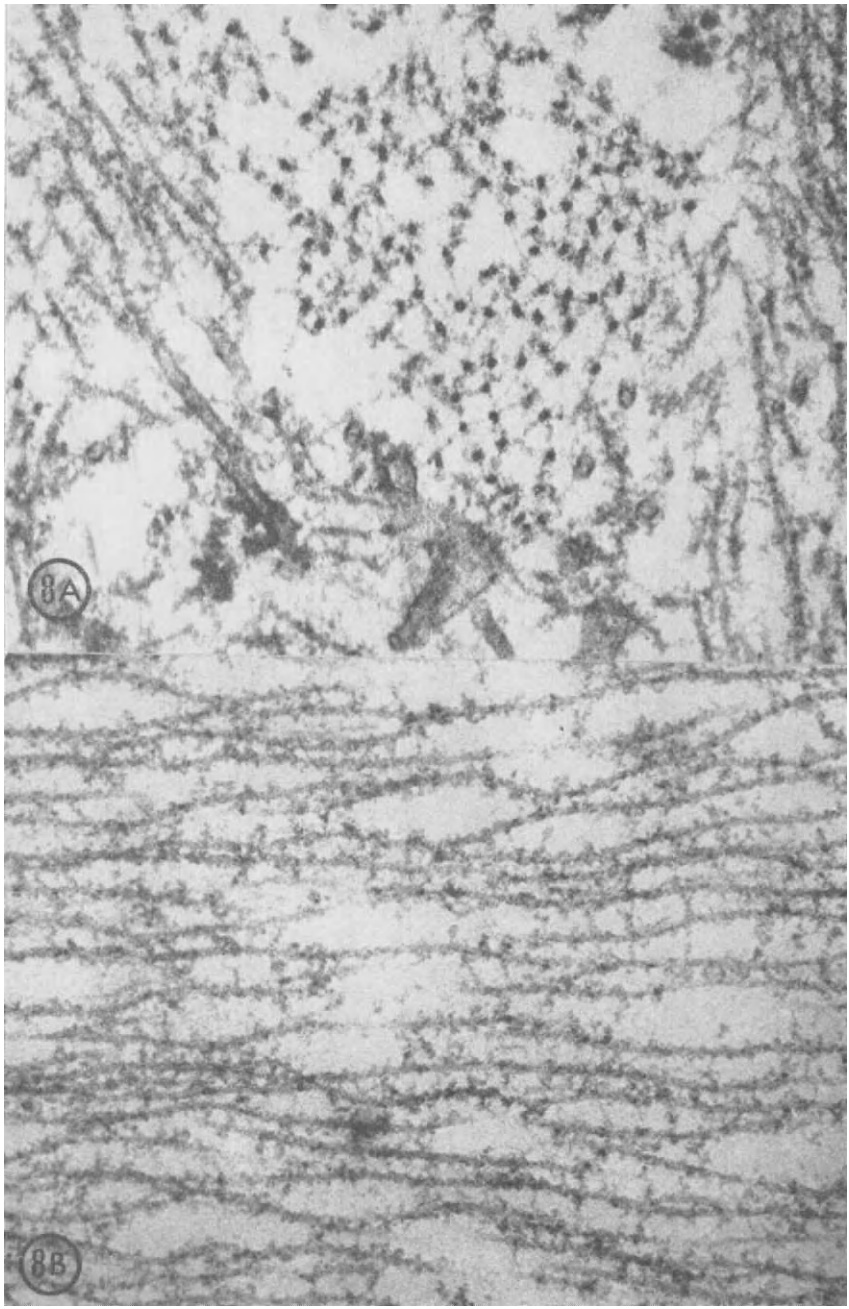

FIG. 8A. Aluminium-induced intraneuronal filaments have circular cross-sections. Note the delicate and indefinite cross branches. × 102 000.

FIG. 8B. Aluminium-induced intraneuronal filaments in longitudinal section also reveal short branches at right angles along the sides. × 114 000.

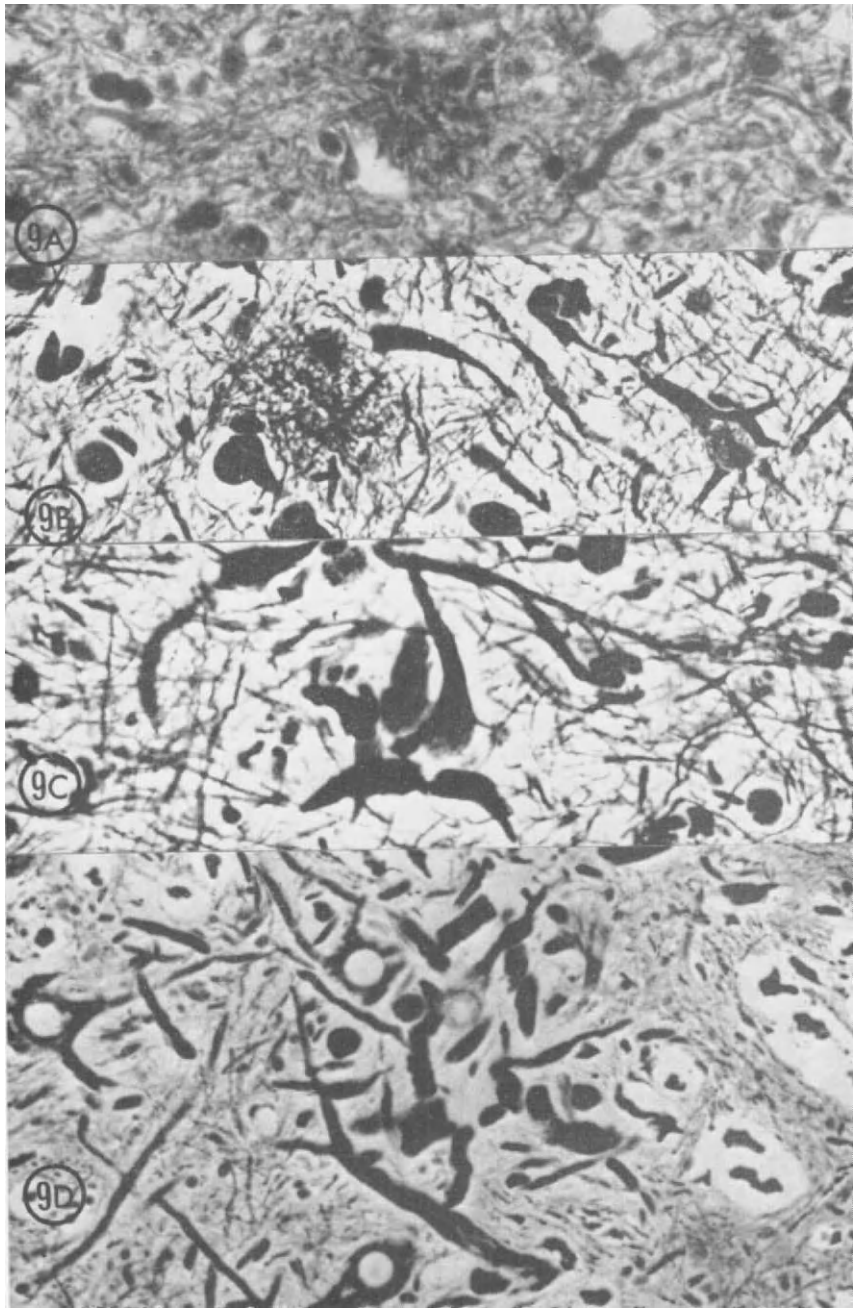

FIG. 9A, B. Plaque-like lesions in the cortex of rabbits chronically treated with aluminium. × 560.

FIG. 9C, D. Plaque-like lesions in the cortex and inferior olive, respectively, of aluminium-treated rabbit. The coarsely argyrophilic rods probably represent altered proximal neurites. × 560.

found to be affected. Occasionally a presynaptic terminal contained aggregates of mitochondria, dense bodies and filaments.

The configuration of the individual filaments did not change throughout the entire time course. They were about 10 nm wide, with a small poorly defined lumen, and of great length, often exceeding several microns (Fig. 8A, B). Small side branches, about 4 nm thick and arranged at right angles to the filaments at irregular intervals, were seen to interconnect those filaments which were closer than about 100 nm. It is possible that these branches simply represented coagulation of the cytoplasmic matrix rather than preformed elements.

The neurons in animals with chronic lesions displayed, in general, changes identical to those in the acute stages. There was, however, some evidence of recovery, especially with the more soluble compounds such as aluminium chloride. The highest concentration of chronically affected neurons in the cat was achieved with subcortical injection of the paste. Dead neurons were few with any of these compounds, and in the electron microscope a residue of filaments was never seen without a surrounding plasma membrane.

The neuropil in the most affected areas of these animals with chronic lesions displayed occasional aggregates of argyrophilic rods and granules (Fig. 9A, B, C, D) which bore a striking resemblance to the human senile plaques of the primitive type, as von Braunmühl (1957) described them. These plaque-like lesions were usually positive in a periodic acid-Schiff reaction, as are the human lesions (Margolis, 1959). Congophilia, however, was absent. Another sporadic chronic change was the argyrophilic, axonal or dendritic spheroid.

Electron microscopy of the neuropil from these chronically treated animals revealed numerous widely scattered single neurites containing masses of filaments or aggregates of degenerating mitochondria and dense bodies (Fig. 10A, B). These abnormal neurites were rarely found in groups, but when they were so located they corresponded to the plaque-like lesions seen with the light microscope. In no case has amyloid been present in these areas, and the number of affected neurites was always small. The spheroids were filled with masses of filaments and sometimes mitochondria and dense bodies.

To increase the number of degenerating terminals, undercutting the cortex has been combined with aluminium treatment. This double procedure induced larger plaque-like lesions composed of up to 15 neurites (Fig. 11). Still, amyloid was not present in the few animals so far studied up to ten weeks after operation.

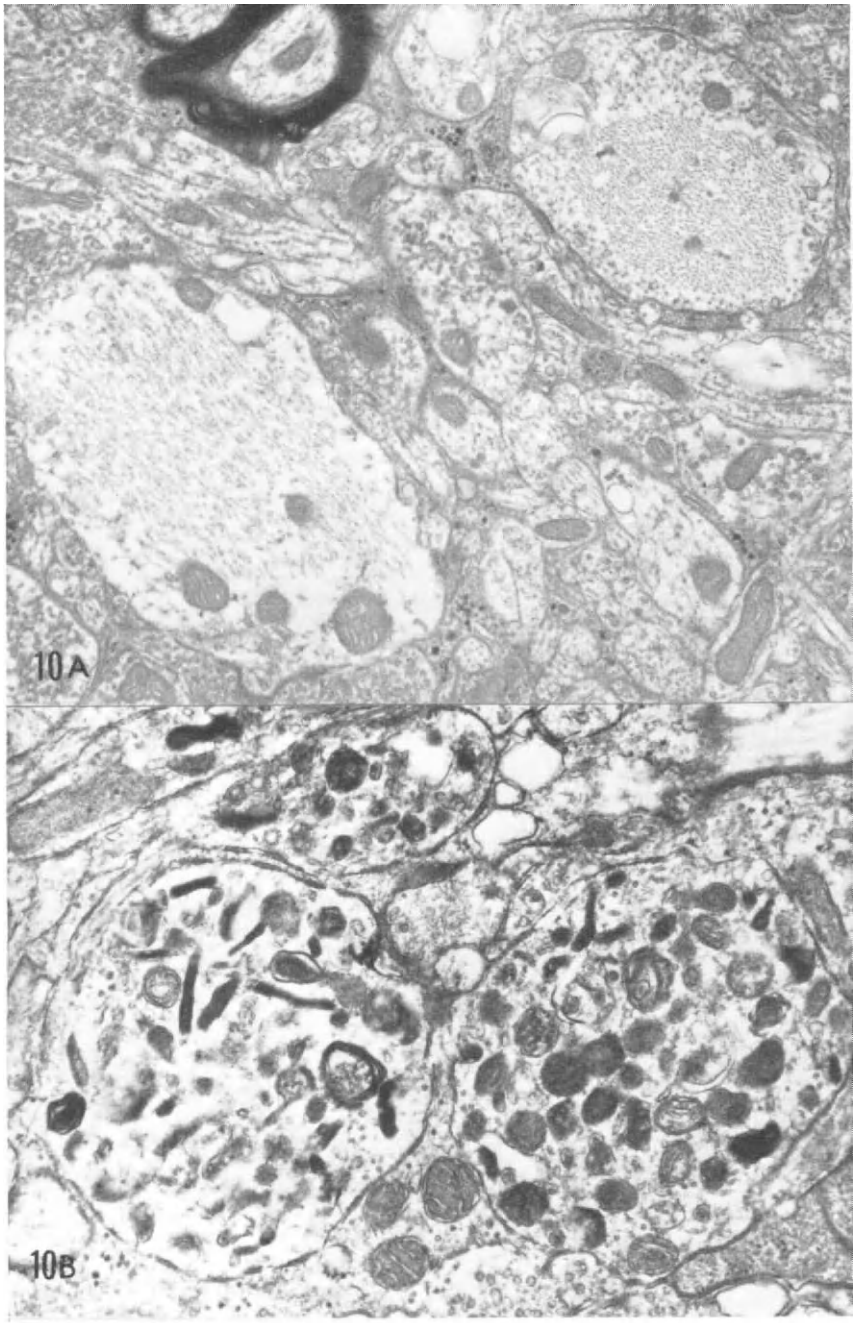

FIG. 10A, B. Aluminium-induced plaque-like lesions are made up of altered neurites containing filaments (A), and dense bodies (B). Note preservation of peripheral tubules in A. A: × 13 500. B: × 25 800.

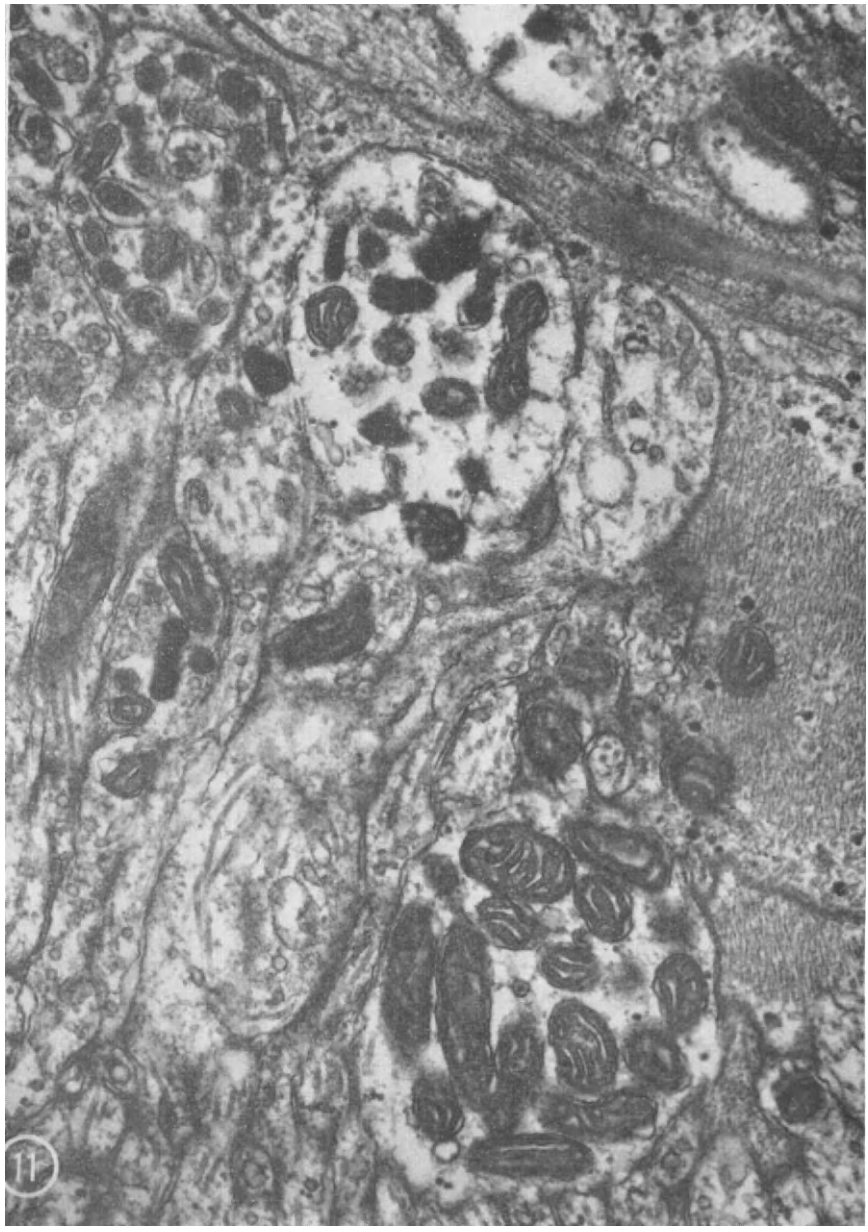

FIG. 11. This experimental plaque was induced by undercutting the cortex of an aluminium-treated cat. The abnormal neurites are filled with aggregates of filaments, degenerating mitochondria and dense bodies. Compare with human lesion. × 28 000.

(*b*) *Spindle inhibitors.* Animals treated with these compounds responded very quickly relative to those given aluminium, and the cellular changes were more limited to the area of injection. All areas seemed susceptible except the cortex, where light microscopy did not show obvious lesions other than total destruction leading to a scar or cyst. The neuronal changes elsewhere, as seen with the usual colorations, were similar to those with aluminium, but the cleared zones in the cytoplasm were more apt to be multifocal in a given cell. Argyrophilia was slightly less intense than with aluminium.

Electron microscopy in the first five days showed large bundles of filaments apparently identical to those in aluminium-treated animals. Spheroids were also similar. Other important somatic cytoplasmic changes not seen with aluminium, however, were caused by the spindle inhibitors. Microtubules disappeared, mitochondria clumped, and there was a marked reduction of rough endoplasmic reticulum (ER) (Fig. 12). Some remaining rough endoplasmic reticulum was directly involved in the formation of autophagic bodies (Wiśniewski *et al.*, 1970) (Fig. 13), while elsewhere it was broken into short segments. Certain neuronal nuclei displayed proliferation of nuclear pores.

The recovery phase has been studied mainly with colchicine. This reversal of the morphological changes was uneven, but was often seen from the fifth day after doses of 30 to 50 μg of this drug. It should be noted that clinical recovery was minimal. Within the neurons the numbers of filaments were notably reduced, while there was a marked proliferation of normal-appearing microtubules of the usual 24 nm width (Fig. 14). There was also a great increase in the amount of smooth endoplasmic reticulum. While most neurons recovered in this fashion, others showed signs of continued degeneration with augmented autophagia. Higher doses permitted only a few neurons to recover.

Spinal nerve roots were normal during the first five days, but then the ventral roots underwent obvious degeneration of the Wallerian type (Fig. 15). Many axis cylinders died in this way, although relatively few of the corresponding neurons failed to recover. The dorsal roots maintained their integrity even in the presence of high doses, but the loss of the anterior roots accounted for the failure of clinical recovery.

DISCUSSION

As a result of treatment with either aluminium salts or spindle inhibitors, mammalian neurons produce masses of 10 nm filaments in their cytoplasm.

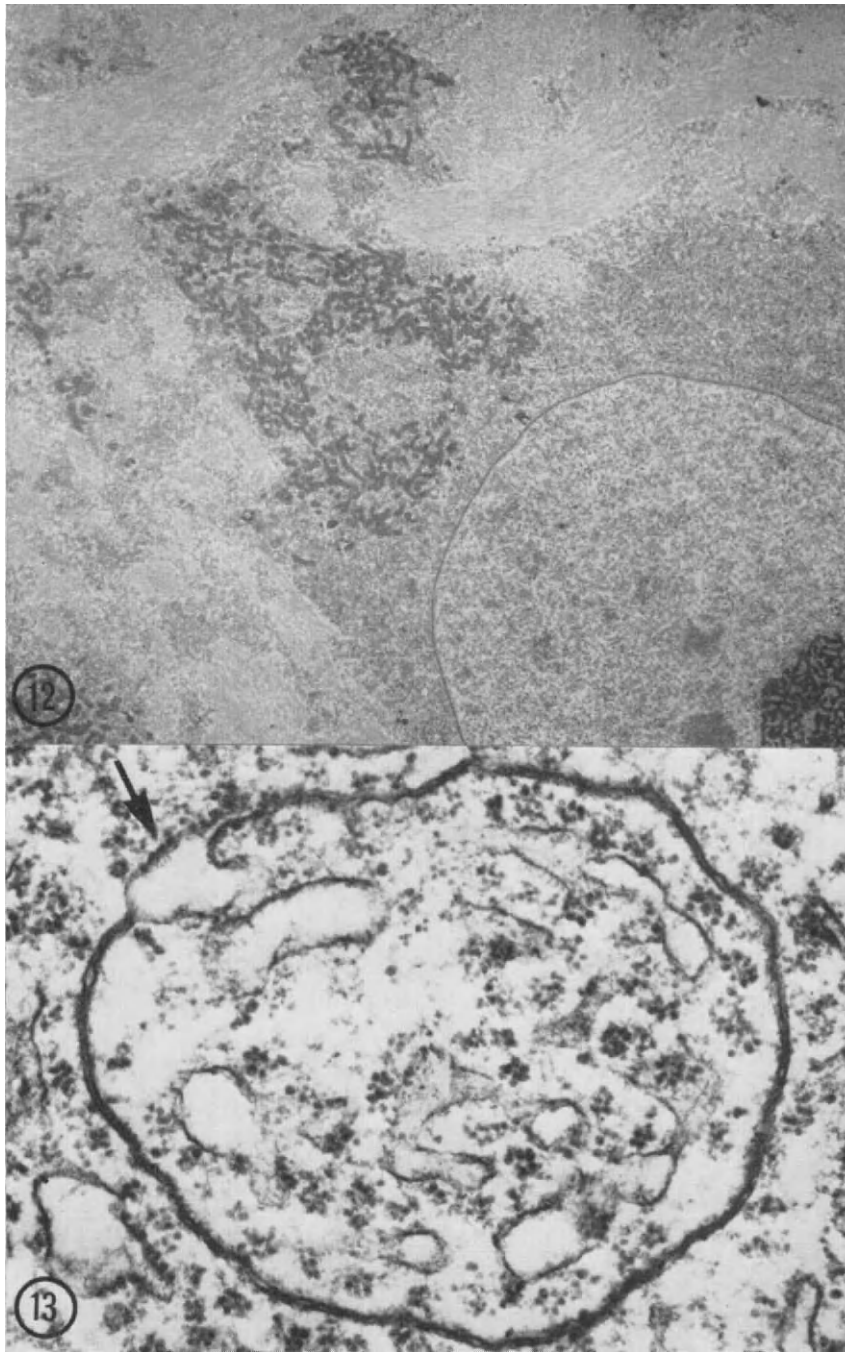

FIG. 12. Spinal motor neuron of colchicine-treated rabbit has large clusters of filaments. The mitochondria are clumped, and the endoplasmic reticulum is broken into short segments. Nuclear pores are increased in number. × 4100.

FIG. 13. An autophagic body in a colchicine-treated spinal motor neuron. The membrane is formed from rough endoplasmic reticulum, and ribosomes are still attached at the arrow where compaction is not complete. The contents appear unchanged at this stage. × 54 600.

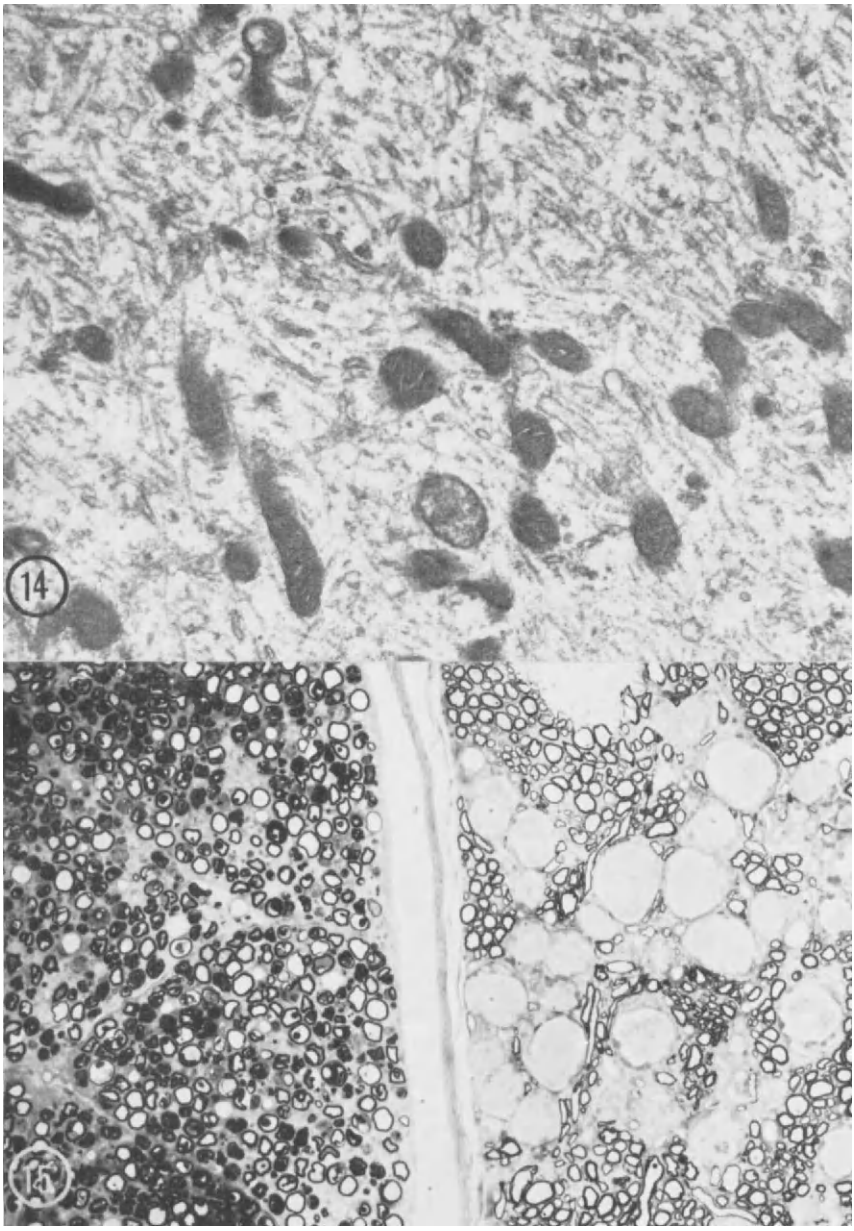

FIG. 14. Recovery stage of spinal motor neuron, two weeks after treatment with colchicine. Great numbers of neurotubules are visible. × 18 500.

FIG. 15. Spinal roots from the rabbit illustrated in Fig. 14. The anterior root on the left displays numerous figures of Wallerian degeneration. The posterior root and ganglion on the right are normal. × 350.

These argyrophilic elements are substantially different at the morphological level from the twisted tubules which make up the argyrophilic tangle of Alzheimer's neurofibrillary cell change. The abnormal human tubules measure up to 22 nm in width and constrict to about 10 nm every 80 nm along their length. The abnormal filaments are morphologically identical to normal axonal filaments. Normal human neurotubules, on the other hand, are 24 nm in diameter. As a consequence one must consider three structural elements in this intraneuronal situation: twisted neurotubules, normal neurotubules and 10 nm filaments. Still-incomplete chemical analyses of these neuronal fibres, each isolated from the tissue, will ultimately reveal the similarities and differences among them at the molecular level.

The experimentally induced plaque-like lesions are composed exclusively of abnormal neurites. The size of these argyrophilic aggregates is directly proportional to the extent of neuronal damage. Their number and size can be greatly enhanced by undercutting the cortex, the essential aspect of which is interruption of the neurites. These altered terminals represent the common ground between the human and the experimental lesions. This observation leads to the conclusion that the human plaque might well be the result of neuronal incompetence and Wallerian degeneration of the terminals, or that it is a cortical form of a dying-back phenomenon.

The differences between the human plaque and the experimental lesion seem to be substantial, but are perhaps secondary. First, the animal lesion is smaller, but this is simply a matter of the number of neurites one can alter experimentally. As indicated by our study of the spinal roots, aluminium-treated neurons, in spite of extensive neurofibrillary accumulation, maintain their processes well so that few neurites are altered. This situation may be analogous to that in Guam-Parkinsonism dementia, where there are many neurons with tangles made of twisted tubules, but no argyrophilic plaques. The spindle inhibitors do cause degeneration of the neuronal processes, but their somatic changes are reversible. The cortex, where dendrites are prominent and thus susceptible to plaque formation, is so sensitive to these drugs as to become necrotic rather than develop selective neurofibrillary degeneration.

A second major difference between human and experimental plaques is the presence of amyloid in the former. This is undoubtedly important, and is still not understood. We might theorize that the amyloid in the plaques is the result of the generalized vascular amyloidosis which exists very commonly in humans beyond a certain age. All the animals so far used were quite young, but older ones might well be tested. However,

von Braunmühl (1956) and Osetowska (1966) have both noted primitive-type plaques without amyloid in old dogs. The former did find evidence of amyloid in the cerebral vessels of his aged animals. Electron microscope studies of canine lesions are currently under way in our laboratory, since these primitive plaques might be found to have amyloid, as do the human ones when sought at high magnification.

The mechanism by which aluminium acts is wholly unknown. It is interesting that it is not effective as an inhibitor of mitosis (Shelanski and Robbins, 1968, personal communication), nor does it cause dissolution of microtubules. The metal is highly specific in that organelles other than the filament are unaffected. In this latter respect, the Alzheimer neurons are similar to the aluminium-treated cells.

The literature on the biological effects of spindle inhibitors is extensive. It is well known that the drugs bind to the 6S protein subunit which makes up microtubules and causes these structures to depolymerize (Inoué, 1964). Neurotubules in the cell bodies are far more sensitive to this effect than those in the neurites. It is not certain whether the tubules depolymerize directly into filaments or into subunits which then form filaments (Schmitt, 1968).

The functions of the microtubule, especially as regards axoplasmic flow, are discussed briefly in our previous contribution to this volume (Terry and Wiśniewski, 1970). The point here is that in neurons treated with low doses of spindle inhibitors this mechanism becomes deranged, so that the neurites suffer from lack of their usual somatic support. This is probably the cause of their degeneration in the anterior roots. This is not a local effect on the roots since the posterior fibres remain intact, as do the dorsal root ganglia. Similar disruption with aluminium of axoplasmic and dendritic flow may fairly be presumed to be the cause of degeneration and aggregation of neurites to form the plaque-like lesions. It is partly from this analogy that we hypothesize a relationship between tangle and plaque in the human disease based on disruption of axoplasmic flow possibly due to the formation of abnormal microtubules.

That the perikaryonal changes in animals are, at least in part, reversible offers a ray of hope for the potential treatment of human disease.

SUMMARY

As a result of treatment with either aluminium salts or spindle inhibitors, mammalian neurons produce masses of 10 nm filaments in their cytoplasm. These argyrophilic elements are substantially different at the ultrastructural

level from the twisted tubules which make up the argyrophilic tangle of Alzheimer's neurofibrillary cell change.

The experimentally induced plaque-like lesions in animals with long survival after aluminium treatment are composed exclusively of abnormal neurites. The size of these argyrophilic aggregates is directly proportional to the extent of neuronal damage. Their number and size can be greatly enhanced by undercutting the cortex. These altered terminals represent the common ground between the human and the experimental lesions. This observation leads to the conclusion that the human plaque might well be the result either of neuronal incompetence and Wallerian degeneration of the terminals, or that it is a cortical form of a dying-back phenomenon.

Acknowledgements

The authors gratefully acknowledge the technical assistance of Lawrence Gonzales and Karen Berkman.

ADDENDUM

Our very recent studies of aged dogs revealed that the canine primitive plaque lacks twisted tubules, but does contain amyloid deposits which are independent of vascular and perivascular aggregates of amyloid. The latter do not appear to cause a reaction in the surrounding neurites. These observations further strengthen our hypothesis that the plaque is a result of degeneration of neuronal processes rather than a primary amyloidosis.

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DISCUSSION

Friede: What exactly do you mean by "neurite"?

Terry: Stedman's dictionary (16th edition) says that a neurite is either an axon or a dendrite and I am using the word deliberately in that sense. Those are the cell properties that I can't identify.

Barondes: Is the marked proliferation of tubules or filaments in the aluminium- and colchicine-treated animals seen primarily in the cell bodies and dendrites, or is it also in the axons?

Terry: It was predominantly in the cell bodies, but it is also seen to some extent in neurites.

Barondes: One way to distinguish neurites is that dendrites may have ribosomes whereas axons do not. Are the axons affected or just the dendrites?

Terry: Myelinated axons clearly are not, but other unidentifiable processes are.

Shelanski: The spindle inhibitor effects have been reported in glial cells as well as in neurons and in HeLa cells, but this effect is more general than the specific aluminium effect Dr Terry described. In HeLa cells treated with extremely high doses of aluminium we have never seen an abnormal filament.

Gonatas: How long does it take for the change in the filaments to occur after the introduction of colchicine or aluminium? Is this really a metabolically mediated process?

Terry: The spindle inhibitors are effective within 24 hours, while aluminium acts more slowly. A few neurons with tangles are seen in five to seven days, but at 10–12 days there are great numbers of affected neurons in the anterior horn, spinal cord, basis pontis and so on, but not so much in the cortex. Embree (Embree, Hamberger and Sjöstrand, 1967) has shown active incorporation of leucine into these filaments, so I think it is an anabolic process, stimulated by aluminium. He also found, by X-ray methods, that the cell mass doubled.

Dayan: You are suggesting the possibility of a "dying-back" process, and you have seen degenerating anterior roots. Have you evidence of a disto-proximal gradient of degeneration in peripheral nerves?

Terry: No.

Shelanski: Before there was any visible change in the distal axon in the peripheral nerve, Dr Wiśniewski and I saw a block in electrical transmission in vincristine-treated animals, apparently in the terminal axon,

which then became more generalized (Shelanski and Wiśniewski, 1969). Unfortunately we never made any more measurements.

Sourander: Are there any electron microscopic studies on neuronal changes induced by hibernation or dehydration for example? Light microscopically such changes resemble the tangles seen in Alzheimer's disease (Jackson, 1925; Alexander, 1934; Stern and Elliott, 1949).

Kidd: Boycott, Gray and Guillery (1961) kept lizards at 20 and 30°C for several weeks, and where there was increased neurofibrillary staining under the light microscope, they found large numbers of perfectly normal-looking neurofilaments in the electron microscope. Where there were large ring-shaped synapses in light microscope pictures they saw large ring-shaped bundles of neurofilaments and so on in the electron microscope. Where the synapses disappeared no neurofilaments were seen.

Sourander: It has been claimed that Alzheimer-like changes could develop rapidly in so-called toxic psychosis (Rothschild and Kasanin, 1936).

Shelanski: Dr Prineas in Dr Terry's laboratory studied intoxication with both tri-*o*-cresyl phosphate (TOCP) and acrylamides. With acrylamides there was an increase of neurofilaments in the axon (Prineas, 1969*b*). In TOCP a slight increase in neurofilaments was seen in the neuronal soma (Prineas, 1969*a*).

Hirano: We observed essentially the same phenomena as Dr Terry after we had implanted vinblastine directly into the brain. The crystalloid structure was seen in both neurons and oligodendroglia within 30 minutes, which was the earliest specimen we examined, and this was associated with the disappearance of microtubules. Bensch and Malawista (1969) found similar structures in cultures of fibroblasts and leucocytes within 30 minutes after exposure to *Vinca* alkaloids.

Terry: Do you think that the microtubular proteins were depolymerized and then crystallized in response to the implantation of a massive local dose?

Hirano: This is certainly a reasonable proposal.

Shelanski: These crystals are composed solely of microtubular protein and there need be no other constituent in them, as I shall show later.

Gonatas: In other words, in this case one can suggest that it is not a metabolically mediated process.

Shelanski: For the crystallization you need no metabolic mediation.

Dayan: What is the time course of the metabolic turnover of microtubular protein *in vivo* for comparison with the experimental demonstration that it takes several days for the aluminium-induced effect to appear?

Barondes: In the mouse the protein turns over fairly rapidly; it has a half-life of about four days. It represents a substantial part of the total soluble protein of mouse brain.

Terry: The mouse is not susceptible to aluminium.

Friede: This microtubular protein forms much larger crystals than are equivalent to the number of microtubules present in an axis cylinder. When you cut a microtubule, does it retract or coil as if it were elastic? There may be redistribution along the fibre axis. Are there any data to support this?

Shelanski: There are two problems here, coming from the use of the term "microtubular crystals". First of all there is no clear evidence that these things are true crystals. Second, these are not made of aggregates of microtubules, but of microtubular protein. There would be no way of packing normal microtubules together into the crystalline patterns. Therefore, the crystal must be formed either from a pre-existing pool of microtubule subunits or from the recruitment of subunits by depolymerization of existing microtubules, or both.

Friede: I understand that. I am still impressed by the difference in mass between the crystal and the precursor material.

Shelanski: These tubules are of great lengths and the crystal when it is formed is really not of great length. I don't think they snap, but rather that the tubule is broken down and the subunits are mobilized to this point. We find that the synthetic rates of soluble proteins in vinblastine-treated cells in supernatants do not differ from those in untreated cells. Specifically, there is no "turning on" of microtubule protein synthesis in *Vinca*-alkaloid-treated neurons or HeLa cells.

Roth: What is the significance of these changes in hibernating animals? If they reflect structural damage, these animals will suffer cerebral damage that progresses with each season's hibernation, unless there is some means of reversing the changes.

Terry: There is good evidence that the filamentous type of tangle is reversible. There is no evidence bearing on the twisted tubule, the Alzheimer-type tangle, because there is no model.

Shelanski: The other thing is that the microtubule is a temperature-sensitive organelle. It depolymerizes at 4°C.

Friede: Increased density of filaments in hibernation does not necessarily mean damage. If lowering the temperature lowers the rate of protein synthesis, there may be less protein dispersed between the filaments, and their spacing may decrease. When the rate of protein synthesis is increased again, the filaments would assume normal spacing.

Roth: Is it established that this change is of the filamentous type? Is anything known beyond the fact that it is of neurofibrillary character?

Terry: The material from the animals of Boycott, Gray and Guillery (1961), cited by Dr Kidd, was filamentous in the electron microscope.

Hughes: What determines the choice of these substances that you use, Dr Terry? They are very far removed from any conceivable aetiology of Alzheimer's disease.

Terry: The effect of Holt's adjuvant was first noted by Dr I. Klatzo (1963, personal communication) who was trying to study the effect of killed bacteria on the brain. He noted vacuoles in the neurons, and reported this as a method of inducing oedema in neurons. At the time I had just been looking at Creutzfeldt-Jakob disease and was interested in vacuolated neurons, so I applied his technique to the rabbit, as he suggested. The electron microscope pictures were full of filaments. At the time my rabbit and human preparations were not very good, and I really couldn't tell the difference between the aluminium-induced tangle and the human one.

Hughes: Does the injected material come into direct contact with the neuron?

Terry: Histochemical methods and electron probe studies show that there is aluminium in the tangle. I tested human neurons with Alzheimer changes in the electron probe at the same time and by the same technique and did not find any aluminium (Terry and Peña, 1965). Aluminium might of course have disappeared from the human tangle because of the time that had passed. Robbins and Gonatas (1964) reported several years ago that colchicine produced filaments in the intermitotic cell, the resting HeLa cell. Then Peterson and Murray reported (1966) that organized myelinated tissue cultures developed filaments within the neurons in response to colchicine. So I took it from there in 1966. It would be far too risky to treat an Alzheimer patient with colchicine intrathecally, but it would be very interesting if it could be done. Could one depolymerize the twisted microtubules?

Hughes: Many people used to be treated for gout with colchicine.

Terry: Even with enormous doses of colchicine parenterally, not enough material passes the blood-brain barrier to affect the nervous system. It has to be put into the cerebrospinal fluid.

Barondes: Could you study the possible effect of colchicine on the abnormal tubules by incubating colchicine *in vitro* with bits of brain obtained by biopsy of patients with Alzheimer's disease?

Kidd: I immersed pieces of biopsy material from three of Dr Hughes' patients in Ringer solution as a control, and also in colchicine. The effect was completely nil, because the Ringer-treated material was worse than the colchicine, but this is certainly an idea which should be tried again.

Roth: Had you some therapeutic purpose in mind when you did this, Dr Terry?

Terry: Yes. We know that the colchicine-induced filamentous tangles are reversible into large numbers of normal microtubules and then the cell settles down to its normal microtubule content. If one could depolymerize twisted tubules into either neurofilaments or something else, the cell might then recover and it would be a worthwhile therapeutic method. But it would be too risky.

Shelanski: Dr Wiśniewski and I saw more extensive changes in the cells of spinal ganglia than in those of the anterior horn. We saw no cortical lesions, but we saw one lesion of the facial nucleus in a patient with facial palsy.

Roth: Patients with Alzheimer's disease sometimes become demented in a matter of months. Rapid progression appears more common in cases of early onset. If animal experiments showed that depolymerization and recovery of cells could occur, cautious therapeutic experiments would in my opinion be justified in this hopeless kind of case.

Gonatas: Dr Barondes, the microtubular protein has a very fast rate of turnover. Is there any microtubular protein or precursor in the cerebrospinal fluid?

Barondes: We haven't done that. We have no idea where the protein goes. We have worked only with mice and it is hard to get any cerebrospinal fluid from them. Dr Friede told me that secretion of neuronal or glial protein into the cerebrospinal fluid was studied in fish by Rahmann (1968).

Friede: Rahmann's findings suggest the discharge of labelled material into the cerebrospinal fluid; this may give the answer to the question about the fate of axonal flow once the synapse is reached.

Shelanski: Dr C. A. Miller in our department is working on the skate, which has a caudal neurosecretory system in the spinal cord that secretes its products into the cerebrospinal fluid.

Dayan: This is exactly the situation in the adrenal medulla where chromogranin proteins are released when the cells are stimulated.

Barondes: Chromogranin secretion should certainly be studied in the central nervous system and I imagine this will be done soon. In the radioautography studies of McEwen and Grafstein (1968) on transport of labelled

proteins to nerve terminals in the optic tectum, grains representing labelled proteins were not found beyond the nerve endings. They could not find evidence for secretion into the postsynaptic neuron. However secretion into the intercellular space is certainly possible.

Friede: So much material moves down the fibre that its ultimate fate presents a problem. The process of discharging synaptic vesicles, for example, might involve some leakage of protein.

Barondes: There may in fact be a mechanism for this. Eylar (1965) suggested that addition of sugars to proteins may be a prelude to their secretion. We have shown that there is incorporation of sugars into proteins at nerve terminals (Barondes, 1968). Addition of sugars to proteins at nerve endings may lead to their secretion.

Terry: The suggestion that failure of antioxidation protection has something to do with the formation of twisted tubules is apparently not altogether impossible and it would seem to be worth while treating patients with large doses of vitamin E and vitamin C. I believe this has been done anyway, based on the idea that lipofuscin has something to do with senile dementia. Lipofuscin is a polymer, essentially, and is thought to be the result of this same oxidation. Are there any results from such therapy?

Roth: All sorts of vitamins have been given to aged and senile subjects without benefit as far as I know. Years ago there was a vogue for using vitamin E in a variety of disorders.

Terry: I doubt that its dose was adequate to do anything.

Roth: Possibly not. Recently some investigators have tried to apply the techniques employed in the preservation of rubber to the prolongation of life. Harman (1968*a*) added 0·5 per cent butylated hydroxytoluene (BHT), a non-toxic antioxidant, to the diet of mice and claimed a 30–40 per cent extension in the mean span of life.

Gonatas: I couldn't follow the reasoning for the antioxidant theory in tangles. Lipofuscin may increase because there is a defective auto-oxidation protection, but how is this linked with the tangles?

Terry: If thiol groups arranged at periodic intervals along a tubule were oxidized to form disulphides, the walls of the tubule might be constricted at those locations. This is a hypothesis which might be tested experimentally.

Shelanski: If the outer pairs of tubules of the sperm tail are put in an oxygen-rich atmosphere, the tubules, already hard to solubilize, become even more so, and one has to use very strong denaturing agents to dissolve them. R. E. Stephens has found that the less soluble tubule of the outer pair of tubules has one more disulphide group than the more soluble. So

in the microtubules these factors are related to solubility, and this might apply also to Alzheimer tubule.

Roth: Both ethoxyquin and BHT are of very low toxicity. Ethoxyquin seems to have the additional property of scavenging free radicals from the liver and Harman (1968*b*) claimed even better results with the use of this substance.

Sourander: A very interesting juvenile case of Alzheimer's disease (Løken and Cyvin, 1954), one of the few which has been published, was clinically a case of juvenile amaurotic idiocy, with retinitis pigmentosa-like changes, but histopathologically a typical case of Alzheimer's disease. There was some accumulation of sudanophilic material, probably lipofuscin, in cortical nerve cells. My impression is that this case was a combination of juvenile amaurotic idiocy and Alzheimer-like changes.

Gonatas: Have you any information about the effect of centrophenoxine (meclofenoxate) on lipofuscin and its ability to reduce the number of the granules?

Terry: No.

Roth: Any of the antioxidant agents ought theoretically to be capable of interfering with the formation of lipofuscin.

Terry: These experiments were with filaments; we have not yet been able to produce twisted tubules experimentally. We hope to do that, and one of the better bets is in the olive in transneuronal degeneration. But that is a very difficult experiment. The hypertrophic olive has tangles. The question is what kind of tangles are they?

Friede: We made an attempt to increase neuronal lipofuscin in rats with diets rich in polyunsaturated fatty acids with and without vitamin E. The results were entirely negative.

Terry: We currently have a colony of rabbits on a vitamin E-deficient diet which we hope to combine with these other experimental methods.

Kidd: Your experiments with spindle inhibitors show there is a good chance of recovery of the cell. This is a very hopeful point.

Roth: As far as inflammatory changes are concerned, good results were recently claimed in three cases of Alzheimer's disease (Chynoweth and Foley, 1969) treated with large doses of corticoids.

Hughes: I tried corticoids in two cases, followed by biopsy. These cases seemed to improve after the biopsies, but after a while without corticoids they relapsed to their previous states. From the histological appearances afterwards such treatment seems hopeless unless the disease can be diagnosed two to three years earlier, before these changes have occurred.

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ALZHEIMER'S DISEASE AND RELATED CONDITIONS
G. E. W. WOLSTENHOLME & MAEVE O'CONNOR

BIOCHEMISTRY OF NEUROFILAMENTS AND NEUROTUBULES

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NEUROFIBRILS—fibrillar argyrophilic structures observed at the light microscopic level—are seen in small numbers in normal neuronal cell bodies and processes. In a number of conditions, including Alzheimer's disease, Guam–Parkinsonism dementia complex, sporadic motor neuron disease and vincristine neuropathy there is a striking increase in the amount of fibrillar argyrophilic material present in the neuron. Electron microscopic studies have revealed that while all these diseases show a proliferation of fibrillar organelles in the affected areas, there exist marked morphological differences between the fibrils in different diseases.

In the normal neuron two major types of fibrillar structures are present. The neurofilament is a structure 8 to 10 nm in diameter with a length greater than can be determined with the electron microscope. Its fine structure is not firmly established. The neurotubule (microtubule) is 24 nm in diameter and has a wall approximately 5 nm in width composed of 12 to 13 protofilaments, 5 nm in diameter, arranged to form tubules. Each protofilament is composed of linearly arranged 4 to 5 nm globular subunits (Grimstone and Klug, 1966). The microtubules in the brain appear to differ little if at all from microtubules in other locations.

A number of diseases and experimental intoxications are characterized morphologically by the proliferation of 9 to 10 nm filaments which appear identical in their morphology to normal axonal neurofilaments. In intoxications with the mitotic spindle inhibitors vincristine, vinblastine, colchicine and podophyllotoxin, as well as with aluminium (Terry and Peña, 1965), the proliferation is most marked in the neuronal perikaryon (Wiśniewski, Shelanski and Terry, 1968). In intoxications with acrylamide the major proliferation of neurofilaments is in the axon (Prineas, 1969*a*), while in tri-*o*-cresyl phosphate-treated animals the major changes are in the terminals (Prineas, 1969*b*). Similar neurofilamentous proliferation occurs in sporadic motor neuron disease (Schochet *et al.*, 1969).

A clear increase in the number of normal-looking neurotubules is seen only in the recovery stages of colchicine- and vinblastine-induced neurofibrillary proliferation (Wiśniewski, Shelanski and Terry, 1968).

Alzheimer's disease and its related conditions show an abundance of 22 nm tubular structures with constrictions approximately every 80 nm (Terry, 1963). These structures have been observed only in diseased human neurons, never in normal neurons and never in other tissues. Their morphological fine structure is also uncertain.

In this paper we shall review the isolation and characteristics of the microtubule subunit protein and examine the evidence for homology between the neurotubule and the microtubule. We shall discuss the mechanism of action of the anti-mitotic drugs, vinblastine and colchicine, and how these actions relate to the proliferation of neurofilaments and the formation of crystalline cytoplasmic arrays on intoxication with drugs of this group. This will be related to the effect of these agents on axoplasmic transport, and the relationship between neurofibrillary degeneration in general and axoplasmic flow will be discussed. A new method of isolating mammalian neurofilaments and preliminary biochemical data on these filaments will be presented, and the hypothesis that neurotubules and neurofilaments are polymorphic assembly forms of the same protein subunit will be examined. Finally, an attempt will be made to relate these findings to Alzheimer's disease.

THE NEUROTUBULE (MICROTUBULE)

The microtubule is ubiquitous in its distribution in the Protista, plants and animals. They are morphologically indistinguishable whether they occur in neurons, as the spindle fibres in the mitotic apparatus, as the central pair or outer nine fibres in cilia and flagella or in any other situations. They appear to be involved in the maintenance of form in certain cells and cellular organelles (Tilney and Porter, 1967). They are necessary for the orientation of nuclei in virus-induced syncytia, and for the orderly array of reovirus in infected cells (Dales, 1963). They are the major structural elements of motile structures such as cilia and flagella and are felt by many to be involved in intracytoplasmic motile phenomena such as mitosis and axoplasmic transport (McEwan and Grafstein, 1968).

Many microtubules are depolymerized by exposure to high hydrostatic pressures, low temperatures, or the drugs known as mitotic spindle inhibitors, which include colchicine, vinblastine, vincristine, griseofulvin and podophyllotoxin.

The observation that colchicine caused a reversible loss of birefringence in the mitotic spindle (Inoué, 1964) led to a study of the kinetics of uptake and mechanisms of colchicine action (Taylor, 1965) which revealed that it was likely that colchicine bound to a single intracellular macromolecule. The affinity constant was sufficiently great for colchicine to be used as a tag to isolate a macromolecule which would be of importance in the mitotic apparatus. A large number of tissues were assayed for colchicine binding activity to test its correlation with mitotic activity. The correlation was reasonably good except in brain and axoplasm, which had low mitotic activity and the highest binding activity (Borisy and Taylor, 1967*a*). However, if the correlation was made with the apparent number of microtubules in the tissue then the agreement was much better in all tissues.

The macromolecule was identified as a protein with a sedimentation velocity of 6S and a molecular weight slightly in excess of 100 000 (Borisy and Taylor, 1967*b*). It was possible to show the origin of this protein from the microtubule by demonstrating that the central pair of microtubules in the sperm tail of the sea urchin could be selectively dissolved and that this was accompanied by the appearance in solution of a single protein with a sedimentation velocity of 6S and high levels of colchicine-binding activity (Shelanski and Taylor, 1967). The protein was shown to have a molecular weight of 120 000 and to be composed of two similar monomers of 60 000 daltons each (Shelanski and Taylor, 1968). The amino acid composition vaguely resembled that of actin and other proteins of the class which have since been called tektins (Mazia and Ruby, 1968). However, the protein did not undergo a globular-fibrillary transformation like actin. Similar results were obtained by another group of workers on the outer doublet microtubules of cilia (Renaud, Rowe and Gibbons, 1968) and sperm tails (Stephens, 1968*a*). A major difference was the lack of colchicine-binding activity in the outer doublet preparations. This difference could be accounted for in part by the difference in isolation methods necessitated by the relative insolubility of the outer doublet microtubules. Both outer doublet and central pair microtubules have GTP bound to their subunits. A similar protein could also be identified with the microtubules of the sea urchin mitotic spindle (Borisy and Taylor, 1967*b*).

The first clear evidence for the homology of the spindle microtubule and the neurotubule came from the electron-microscopic studies of Gonatas and Robbins (1964/1965) showing the origin of neurotubules in a centriole during neurogenesis. The ready solubility of microtubules after cell disruption made it necessary to seek a means other than subcellular fractionation to identify the subunits of the neurotubule. This was done by

developing a method for purifying colchicine-binding activity from brain homogenates. Ammonium sulphate fractionation and DEAE-Sephadex chromatography led to the purification of a single protein with a sedimentation velocity of 6S and molecular weight, monomer, GTP and colchicine-binding characteristics identical to those of the sperm tail central pair microtubule (Weisenberg, Borisy and Taylor, 1968). There were small differences in the amino acid compositions which were within the limits of variation expected in such widely separated species. In addition to establishing the chemical similarity of neurotubules and microtubules this method enabled purification of gramme quantities of protein which greatly facilitated biochemical studies. Microtubule protein accounts for 2 per cent of the total protein and 10 per cent of the soluble protein of adult pig brain. As much as 20 to 50 per cent of the soluble protein of neonatal mouse brain (Dutton and Barondes, 1969), isolated neuroblasts (Shelanski, unpublished observation) and cloned neuroblastoma cells (Olmsted *et al.*, 1970) is microtubule protein. The rates of metabolism of this protein and its variation with age will be discussed elsewhere in this symposium (Barondes and Feit, 1970).

MITOTIC SPINDLE INHIBITORS

Colchicine, podophyllotoxin, griseofulvin and the *Vinca* alkaloids are best known for their arrest of mitosis in metaphase. In each case this effect appears to be a direct result of the depolymerization of microtubules by these agents. However, the effects of colchicine and the *Vinca* alkaloids on interphase cells and post-mitotic differentiated cells are also of interest. In these cells microtubules are seen to break down, and in their place many more 8 to 10 nm filamentous structures appear (Robbins and Gonatas, 1964; Wiśniewski, Shelanski and Terry, 1968). With the *Vinca* alkaloids one also sees the formation of crystalline arrays of hexagonally packed circular profiles which have been called "microtubular crystals" (Bensch and Malawista, 1968, 1969). With colchicine treatment the filamentous proliferation appears reversible and the recovery stage shows a greatly increased number of normal-looking microtubules as well as a marked increase in the amount of smooth endoplasmic reticulum. These observations led to the hypothesis that the microtubule and the microfilament were polymorphic assemblies of the same protein subunit (Wiśniewski, Shelanski and Terry, 1968). Several biochemical investigations were undertaken to achieve a better understanding of these phenomena.

It is clear that colchicine binds to the subunit protein of the microtubule

(Borisy and Taylor, 1967*a*, *b*; Shelanski and Taylor, 1967, 1968) but the binding of vinblastine has not been so well established. Our first attempts to demonstrate binding of vinblastine to pure microtubule protein were hampered by the fact that it completely precipitated protein from solution in the presence of divalent cations and all colchicine-binding activity was precipitated. When we added vinblastine to the supernatants of the brain and cell homogenates, all colchicine-binding activity was again precipitated and the protein was considerably purified (Marantz, Ventilla and Shelanski, 1969). Olmsted and co-workers (1970) demonstrated independently that redissolving the protein and a second precipitation with vinblastine resulted in even greater purity. The concentration dependence and ion dependence of this reaction have been studied in detail by Weisenberg and Timasheff (1969).

In the polarizing microscope the vinblastine-induced precipitates of microtubule protein were birefringent. In sections examined in the electron microscope the precipitates were well ordered structures with a ladder-like array of 9 nm diameter "rungs" approximately 20 nm apart and surrounding areas of circular cross-section with individual diameters of 36 nm (Bensch *et al.*, 1969). Negative contrast techniques applied to similar precipitates showed the crystals to be of identical appearance to the microtubular crystals observed *in vitro* after treatment with vinblastine (Marantz and Shelanski, 1970) (Fig. 1). The unusual feature revealed was that the microtubular crystals were not arrays of tubules but rather a coiled-spring type of arrangement in which individual spirals, viewed from above, appeared as circular elements. These elements measured 44 nm in diameter, considerably more than in sectioned material *in vivo*. The difference is probably due to differences in dehydration between sectioned and negative contrast preparations. The individual subunits composing these helical elements in the crystal are globules with a centre to centre spacing of 5 nm—identical to that of the normal microtubule subunits.

The binding of vinblastine to these arrays is assayed by first subjecting the crystals to rapid column chromatography to remove unbound vinblastine and then precipitating the protein with perchloric acid. Bound vinblastine is released by this procedure and is measured by colorimetric assay. All attempts to identify bound GTP in the vinblastine-induced precipitates have been unsuccessful (Marantz and Shelanski, 1970; Olmsted *et al.*, 1970). If the amount of GTP bound to the microtubule protein is assayed before and after precipitation by vinblastine, partial release of the nucleotide is observed (Ventilla and Shelanski, 1970).

The vinblastine-induced precipitates can be redissolved by dialysis against vinblastine-free buffer for several hours. However, the sedimentation velocity of this redissolved material is of the order of 20 to 25S (Weisenberg and Timasheff, 1969; Marantz, Ventilla and Shelanski, 1969) and the molecular weight is in excess of 10^6 daltons compared to a 6S, 120 000

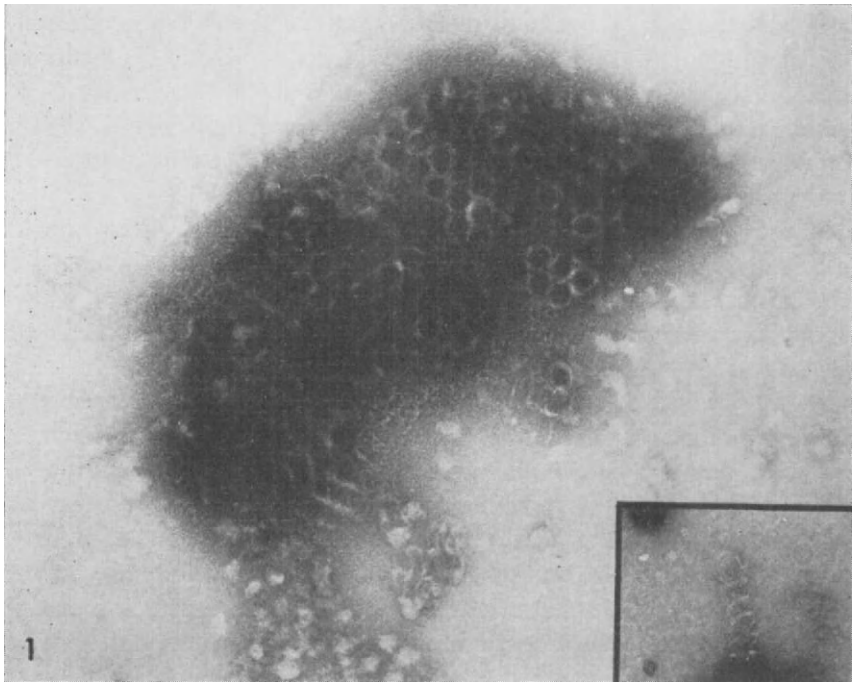

FIG. 1. Microtubule crystal induced *in vitro* by dialysis of 10^{-5}M microtubule protein against 10^{-4}M-vinblastine in 0·01M-phosphate buffer containing 10^{-3}M-$MgCl_2$. The hexagonal packing of circular profiles 44 nm in diameter is evident. At the periphery occasional helical structures are seen. *Inset:* isolated helical filament from microtubular crystal. Diameter is 7 nm. Negative contrast. ×87 000.

dalton starting material. These hydrodynamic data are compatible with a linear strand and studies of the morphology of this entity are now under way.

Precipitation effects such as are seen with vinblastine do not occur with colchicine or podophyllotoxin. It is interesting that colchicine and vinblastine do not compete for the same binding site (Wilson and Friedkin, 1967), and therefore differences in their action are not unexpected.

NEUROFILAMENTS

The results of treating cells with colchicine and the *Vinca* alkaloids suggest an interconvertibility of neurofilaments and neurotubules. To test this hypothesis we attempted to isolate spindle-inhibitor-induced filaments from brains and from HeLa cell cultures. In all cases the filaments went into solution rapidly, making isolation difficult. Separations done in the presence of hexylene glycol—an agent known to decrease the solubility of the neurotubule (Kirkpatrick, 1969)—were more successful, but no highly purified preparations have yet been obtained.

Normal neurofilaments presented a simpler problem. J. Turnbull and W. T. Norton have developed a method for isolating axons from bovine white matter, the details of which were kindly made available to us before publication. This axonal material proved to be a rich source of neurofilaments, relatively free from contamination by other tissue elements. The method is based on the fact that myelinated axons are obtained intact if white matter is loosely homogenized in salt/sucrose medium. Because of the high lipid content of these myelinated fibres they can be floated free from other tissue particles by repeated centrifugation in high density media.

The axons obtained by a modification of this procedure were demyelinated by homogenizing them in distilled water, and the cytoplasmic contents were centrifuged away from the myelin in a sucrose solution. The pellet was composed of mitochondria, membranous vesicles and filaments (Fig. 2). Repeated centrifugation of this material produced filaments estimated to be approximately 95 per cent pure (Fig. 3).

Biochemical studies on mammalian neurofilaments are still in a preliminary stage. The smallest subunit obtainable has a molecular weight of 85 000 daltons, considerably higher than the microtubule. The filaments are insoluble at low ionic strength and neutral pH and only slightly soluble in 0·6M-KCl. They dissolve readily in detergents such as sarkosyl and in 2M solutions of guanidine-HCl or urea. The filaments can be reconstituted after treatment in 2M-urea simply by removing the urea. The sedimentation velocity of the protein in detergent is approximately 3S. No GTP was found bound to the filament, and the filaments lacked both GTP and colchicine-binding activity (Shelanski, Turnbull and Norton, 1970).

Neurofilaments have been isolated from the axoplasm of the squid giant axon (Davison and Taylor, 1960; Huneeus and Davison, 1969). Much information was obtained on these structures (Huneeus and Davison, 1969) and their biochemical characteristics are quite similar to those of the mammalian neurofilament.

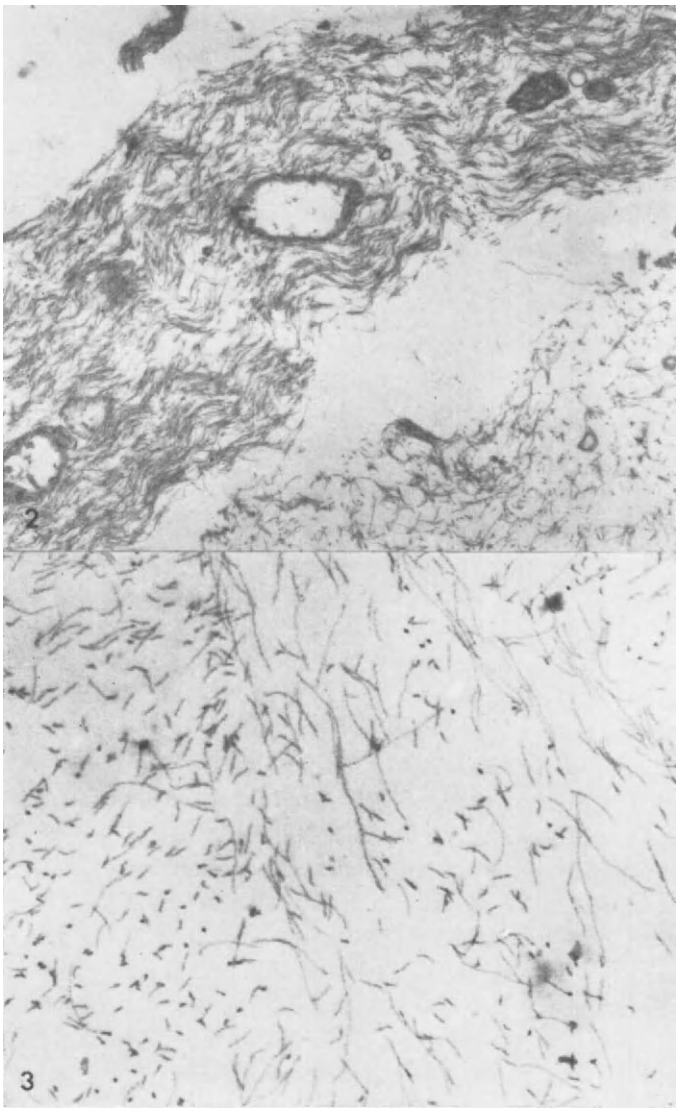

FIG. 2. Preliminary stage in the purification of neurofilaments. The filaments are closely packed, with degenerated cellular material in the bundle. × 16 800.

FIG. 3. Sonicated neurofilament preparation after centrifugal purification. The preparation is relatively free of cytoplasmic contaminants × 28 000.

It is not at all clear that the filaments isolated from mammalian axons and from squid axoplasm are chemically related to the neurofilaments induced by aluminium, tri-*o*-cresyl phosphate, acrylamide or the spindle inhibitors, in spite of the fact that they are morphologically indistinguishable. They certainly appear to differ from the filaments induced by colchicine and vinblastine, at least in their solubility characteristics.

AXOPLASMIC TRANSPORT

The unique geometry of the neuron, with most of its synthetic machinery concentrated at one end, necessitates a mechanism for moving newly formed products from the site of synthesis to distant parts of the neuron. That this mechanism was a proximal-distal transport process was first established by Weiss and Hiscoe in 1948. More recent studies have demonstrated that axoplasmic flow can be resolved into two broad components: a faster particulate component with a velocity of the order of 100 nm per day, and a slower component composed of both soluble and insoluble material and accounting for approximately 90 per cent of the total radioactivity transported (McEwen and Grafstein, 1968). It has been postulated that microtubules might serve a role in the axoplasmic flow process and that one of the effects of the neurofibrillary proliferation in Alzheimer's disease is a disruption of axoplasmic flow (Suzuki and Terry, 1967).

This hypothesis has not been experimentally verifiable in Alzheimer's disease but it has been tested in other experimental neurofibrillary proliferations. Direct application of colchicine or vinblastine to the nerve appears to block axoplasmic transport completely (Kreutzberg, 1969; Schlaepfer, 1969). If colchicine and radioactive leucine are injected into the eyes of experimental animals the rapid component of axoplasmic flow decreases (Karlsson and Sjöstrand, 1969). In our laboratory neurofibrillary proliferation was induced in anterior horn cells by subarachnoid injection of either colchicine or vinblastine and the axoplasmic transport was measured by the method of Ochs (Ochs, Johnson and Ng, 1967). The slow component of axoplasmic transport was unaffected in these experiments while the fast component was virtually abolished. Preliminary electrophysiological studies in these animals show that neurofibrillary proliferation precedes the earliest electrical deficits by 24 to 48 hours (Shelanski and Wiśniewski, 1969). If the distance between the cell bodies and the terminals were of the order of 200 mm and the flow normally took place at a rate of 100 mm per day this would be a reasonable delay before the block in flow was functionally apparent. In the isolated crayfish nerve cord, Davison and his

co-workers have demonstrated a blockade of both fast and slow components with colchicine (Davison, personal communication).

In acrylamide intoxication a block in the slow component of axoplasmic transport has been demonstrated (Pleasure, Mishler and Engle, 1969).

DISCUSSION

From the evidence presented the neurotubule is clearly the only well-characterized fibrillar protein in the neuron and is not a structure specific for the neuron. We have been able to isolate a structure from axons which appears to be the normally occurring neurofilament. This latter structure differs from the microtubule in subunit molecular weight, nucleotide binding and solubility, suggesting that it is composed of an entirely different protein. This means that either we must abandon our earlier suggestion that neurotubules and neurofilaments are, at least when mitotic spindle inhibitors are used to induce neurofibrillary proliferation, polymorphic forms of the same protein, or we must conclude that more than one type of neurofilament is possible. This latter view is supported by the fact that the solubility characteristics of the neurofilaments in colchicine- and vinblastine-treated cells are closer to those of the microtubule than to those of the axonal neurofilament, and by the fact that the *Vinca*-alkaloid-induced microtubular crystals often seen within neurofibrillary bundles, apparently arising from the neurofilaments, consist exclusively of microtubule protein. The continuity of filaments and crystals in *Vinca*-alkaloid-treated cells has been demonstrated, together with considerable evidence for their interconversion (Krishan and Hsu, 1969).

There may therefore be two or more chemically distinct structures which are called neurofilaments on morphological grounds. It remains to be seen whether this is true in normal as well as drug-treated neurons.

Neurofibrillary proliferation seems to be accompanied by alterations in axoplasmic transport in the cases studied. With the metaphase-blocking anti-mitotic agents this proliferation might occur through the direct breakdown of the microtubules postulated to have a key role in axoplasmic flow. Such proliferation is not seen with the other agents used and other hypotheses would have to be advanced for their effect on flow, including such possibilities as physical blockage, interference with energy sources for flow and energy coupling, or any of a wide range of phenomena. At the moment no good evidence exists for any of these mechanisms, leaving us with only the observation that derangements in transport occur when these drugs are given.

In two non-neuronal systems the basis for cytoplasmic movement appears to reside in an actin-myosin contractile system. The more completely established of these is the streaming system in the slime mould *Physarum* which depends on actin and myosin, very closely related in all properties to the muscle proteins (Adelman *et al.*, 1968; Adelman and Taylor, 1969). Evidence for the second system, the chondrocyte, is more indirect and comes from the work of Ishakawa (1968) who has demonstrated binding of heavy meromyosin to 5 nm filaments in these cells. There is no binding to 10 nm filaments in the same cells, nor is the heavy meromyosin bound to microtubules. The existence of an actomyosin-like contractile system in brain has been reported (Puszkin *et al.*, 1968) but conclusive protein chemical studies are still lacking.

The work discussed above is only indirectly applicable to Alzheimer's disease. It is now clear that Alzheimer's disease is a primary neuronal disease (Terry and Wiśniewski, 1970) and it is therefore likely that the twisted tubular structures are directly related to the pathological process rather than being of a reactive nature. Experiments aimed at isolating these structures are underway in our laboratory, but no results are yet available. Three major possibilities exist for the nature of this tubule:

(1) It represents a mutation in a microtubule gene with the production of a faulty tubule.
(2) The structure is a viral product or the product of a gene unrelated to the microtubule.
(3) The structure represents a secondary modification, perhaps by oxidation of thiols or some similar mechanism in a normal cellular constituent like the microtubule.

The first hypothesis is attractive because it would represent an alteration in a known structural protein, as has been suggested elsewhere in this symposium (Pratt, 1970). However, since some normal-looking microtubules are still present in affected neurons, and since normal microtubules exist in other tissues of patients with Alzheimer's disease, it would be necessary to assume that more than one gene exists for the production of microtubule protein. This possibility is supported by evidence showing that the A and B subfibres of the sperm tail microtubules have small differences in their peptide maps (Stephens, 1970). Abnormal proteins are also found in flagellar mutants of *Chlamydomonas* (Warr *et al.*, 1966; J. Rosenbaum, personal communication). The exact control mechanism for turning on the production of abnormal tubule protein late in life is not clear, but

age-dependent control mechanisms are known, for example in the transition from foetal to adult haemoglobin.

The second hypothesis enjoys the advantage that slow virus infections are currently receiving a great deal of attention in neurology. Certainly viruses such as the measles virus (Raine *et al.*, 1969) are known to produce tubular cytoplasmic products in infected neurons. The possibility also exists of a mutation in a gene other than the microtubule gene, or the expression of a normal but previously repressed gene. Numerous proteins including haemoglobin S and cytochrome P-420 are known to be capable of self-assembly into tubular forms (Murayama, 1966; Shoeman, White and Mannering, 1969). These tubules are not identical in substructure to the microtubules.

The third possibility is that the normal microtubular subunit protein—or some other normal product—is being secondarily altered as the result of the pathological process. For example, oxidation of SH groups might lead to changes in the solubility or assembly characteristics of the protein. An alteration in the amount of GTP available might result in abnormal assembly since GTP appears to be required for microtubule assembly *in vitro* (Stephens, 1968*b*). The aggregation of microtubules and microtubular crystals is related to concentrations of both monovalent and divalent cations (Marantz, Ventilla and Shelanski, 1969; Stephens, 1968*b*; Weisenberg and Timasheff, 1969). Finally, plant alkaloids such as vinblastine induce an unusual assembly of microtubule subunits, and substances capable of inducing other polymorphic forms might exist in certain disease states.

These suggestions are all speculative but they demonstrate the wide variety of alterations which might induce secondary changes in tubular assembly.

SUMMARY

The evidence is reviewed that the organelles seen at the light microscopic level as argyrophilic neurofibrillary proliferations are of different types at the ultramicroscopic level. These structures fall into three classes: 9 nm diameter neurotubules and the 22 nm twisted tubule of Alzheimer's disease. The microtubule is a readily soluble structure whose subunits have a molecular weight of 60 000, are dimeric, and have a sedimentation velocity of 6S in the dimeric form.

The isolation of a 9 to 10 nm neurofilament from mammalian white matter is described. Its smallest subunit has a molecular weight of 85 000, and the protein is highly insoluble at low ionic strength and neutral pH and differs in other ways from the microtubule protein. The possibility is

raised that other neurofilaments, especially when neurofibrillary proliferation is induced with spindle inhibitors, are composed of different protein subunits and that this subunit might be microtubule protein.

Axoplasmic flow, postulated to be disturbed in Alzheimer's disease, is altered in the presence of neurofibrillary proliferation.

The problem of Alzheimer's disease has been discussed briefly and to the extent that extrapolations can be made from studies on microtubules and filaments from laboratory animals.

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DISCUSSION

Pratt: How are the slow and fast components of flow determined experimentally?

Shelanski: McEwen and Grafstein (1968) allowed anatomy to do the experiment for them. The eye of the goldfish has an uncrossed optic tract going back to the tectum, which is stratified into synaptic and fibre layers. They injected tritiated leucine into the eye and measured its incorporation into protein both radioautographically and by scintillation counting. They counted the intact nerve, the fibre layers and the synaptic layers. After 12 hours there was labelled material in the synaptic layer of the tectum, but almost none in the fibre layers or optic nerve. The material which very quickly reached the synaptic level is the fast component; this is a 90 per cent insoluble component in the sense that when the tissue is homogenized in a detergent-containing buffer, 90 per cent of the radioactivity remains in the pellet after centrifugation at 100 000 ***g***. This component is thought to move in the goldfish at a rate of 40 mm/day. In a mammal it probably moves at 100 mm/day. A second invasion of

labelled material into the nerve occurs later and goes on for many days. This component moves at a rate of 0·4 mm/day in the goldfish, and it is about 50/50 soluble/insoluble. There is some question of whether this slow component ever gets into the synaptic layer, though it enters the fibre layer in the tectum. The microtubule protein is present in both these components but to what extent we do not yet know. We think probably 20 per cent of the slow component is microtubule protein. And we think that much of the soluble labelled material in the fast component is microtubule protein. These experiments are awaiting clarification.

Gonatas: You mentioned that when one infects HeLa cells with reovirus one sees reorientation of reovirus particles along the spindle tubules. If one destroys the spindle tubules with colchicine one still sees reovirus particles, but in a more random arrangement. In some of Dr Margolis's material of reovirus-infected central nervous system of mice, we found, as expected, that the orientation of the reovirus particle along the neurotubules was identical to that along the spindle tubules. This is perhaps a reinforcement of the original thesis that neurotubules and microtubules are homologous structures (Gonatas and Robbins, 1964). But in the reovirus infection, I am not convinced that the function of the microtubules is just for orientation. The neurotubules appear to be gradually coated with an osmiophilic material which is really very similar or almost identical structurally to the capsid of the reovirus. Gradually the neurotubule disappears and then one sees the formation of the complete viral particles. In addition to orientation, the microtubule might lend some protein for the formation of the viral capsid (personal observations).

Shelanski: The reovirus has a cell-derived outer protein coat which could possibly come from the microtubule. You were the first to establish the homology between neurotubules and microtubules, Dr Gonatas, and chemically this has been well borne out.

Strich: Does the twisted tubule bind colchicine?

Shelanski: There is no increased colchicine-binding activity in the Alzheimer brain pieces we have had. In autopsy material control is difficult. Since we cannot isolate either the intact microtubule or the intact Alzheimer tubule we have no way of knowing whether it binds. Colchicine is very soluble radioautographically, so this is not a good approach.

Kidd: Rose (1965) separated neurons from the neuropil by the crude method of pushing the material through nylon cloth and then spinning it down on a gradient. When I undertook some electron microscopy for him I wasn't too impressed with the healthiness of the neuron, but I felt that however sick these cells were, they could easily retain their twisted

filaments. There would probably still be the same proportion of normal and abnormal perikarya in the pellet, but at least the bulk of the brain, which is neuropil, would have been lost. One might be inclined to feel that the affected cells are of a slightly different density from the unaffected.

Shelanski: This is a very useful idea. Dr I. Tellez-Nagel in our laboratory is beginning to apply a technique of cell separation devised by Norton (1969) to the Alzheimer brain. It gives a higher yield and is clearer than any other method we have seen.

Terry: The reoviruses are strongly congophilic in this preparation, and this is further evidence of the non-specificity of amyloid stains.

Kidd: Congo red was used in the 19th century as a stain for axons.

Taylor: Which way is the reovirus transported along the tubule in the experimental infection?

Gonatas: We are still working on this material. We did not see reovirus in presynaptic terminals or in myelinated axons, which have few tubules and lots of filaments. We saw virus particles either in perikarya or in dendrites along microtubules.

Terry: Could denaturation of non-specific protein material in cytoplasm lead to formation of filaments which might be confused with all these other elements that we think exist *in vivo*?

Taylor: That is always a possibility, I am afraid. There are several cases of fibres produced from globular proteins. Formation of fibres from insulin at acid pH is one example.

There is also increasing evidence of polymorphism in protein structures. Originally it was thought that the geometry of interacting regions on a protein subunit determines the structure uniquely and thus, for example, a filament or tubule could assemble spontaneously in only one way. But it is now becoming clear that different kinds of structures can be formed from the same protein under different environmental conditions. One might well generate a filament from what would normally have been a soluble protein in some disease state. In the microtubule we seem to be dealing with what may be extreme polymorphism. In the presence of colchicine a filamentous form is produced while with vinblastine we obtain an open helical structure. In the Alzheimer condition one sees what is perhaps a twisted tubule or a double helix structure. Thus a variety of structures may be formed through the binding of a small molecule to the protein subunits.

Our studies were originally concerned with the question of how colchicine works, but I don't think we have ever answered this question.

It is rather surprising that microtubule protein has a single specific binding site for colchicine, and probably for vinblastine. Since the cell has not previously seen these molecules, and their structures do not appear to resemble the amino acid side chains which interact in order to polymerize the subunits, one suspects that there are specific regulator sites on the protein. It is possible that there are small molecules present in the cell which control tubule polymerization and depolymerization by binding to these sites and the action of the drugs is due to their resemblance to the normal control factors. In the disease the twisted tubule may be produced by the binding of some metabolite in the same way that vinblastine produces an altered structure.

Strich: Are the amyloid filaments in any way related to any of the long thin structures which you have been talking about?

Shelanski: Biochemically, no. Morphologically one always has this problem with filaments in the 10 nm range.

Terry: Even in electron micrographs the amyloid fibre has quite a distinctive appearance. The diameter is the same as in the neurofilament but the wall thickness of the amyloid is much thinner so the lumen is a lot larger than in the neurofilaments. There is no problem about differentiating between those two.

Strich: But there is the problem of relating them. In Alzheimer's disease there must be some relation between the amyloid deposition, the degeneration in the plaque and the tangle. Or do you think they are completely unrelated phenomena?

Shelanski: The evidence of Dr Hirano is very much against the idea that twisted tubules give rise to amyloid. In the Guam-Parkinsonism dementia complex the twisted tubule exists without amyloidosis.

Friede: Structures with the same morphology may not have the same function, for example the tubules in axons and those in cilia.

Taylor: I don't for a moment think that the functional significance in the axon is the same as in the cilium. Again one can take this in a number of ways. In the cilium, of course, one knows that there is a distinct enzyme which can be seen on the outer doublet. The enzyme is attached to the outer doublet, and these are modified tubules, so one can take the view that there is a generalized tubule system which can be operated on in various ways and modified for different functions. If one takes such a broad view, it is rather hard for people to argue with you, but one is more or less forced to do that because the microtubule occurs in so many places that it is unlikely to have a single function. In the cilium it is modified in a particular way and binds an enzyme, which hydrolyses ATP. On the question of

colchicine having no effect on cilia we have always held the view that colchicine is effective in depolymerizing a tubule only if the tubule itself is in a state of dynamic equilibrium in which the subunits are coming on and going off. The cilia tubules are stable and colchicine has no effect, but if cilia are amputated they will not grow again in the presence of colchicine.

Nevin: Are you implying that the twisted tubules, or tangles, in the Alzheimer lesion are not formed by the same protein as normal tubules?

Shelanski: This is our major problem. We have no idea of what twisted tubules are composed.

Taylor: Proteins can form different structures in which the symmetry relating the units is different, through the influence of the environment but without any large-scale change in the tertiary structure. Haemoglobin will crystallize to give crystal structures of different symmetries. Tropomyosin tends to form an open net structure, like a piece of window-screening, when precipitated near the isoelectric point, but it yields microcrystals with molecules oriented parallel to each other when precipitated with magnesium chloride (Caspar, Cohen and Longley, 1969). Therefore, even though structures form spontaneously and are states of minimum free energy for the system, more than one structure is possible through interaction with other molecules making up the environment. One could presumably affect the structure in various ways through the binding of small molecules. As Dr Shelanski pointed out, the protein-protein interaction sites on a subunit can give three or four different types of contacts of the subunit with its neighbours, so by blocking one or more of these contacts, one could produce quite different structures.

Shelanski: With the microtubule protein we measured the circular dichroism spectra in ultraviolet light, which gives, at least in an ideal system, a measure of the amount of α helix and β pleated sheet structure. We see no statistically significant changes in α helix content on any of these interactions.

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ALZHEIMER'S DISEASE AND RELATED CONDITIONS
G. E. W. WOLSTENHOLME & MAEVE O'CONNOR

METABOLISM OF MICROTUBULAR PROTEIN IN MOUSE BRAIN*

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MICROTUBULES are abundant in axons, cilia, and sperm tails (Shelanski and Taylor, 1970), and are believed to play some role both in maintaining the structure of these elongated cellular processes (Tilney and Porter, 1967) and in certain motile functions (Schmitt, 1968). Brain and axoplasm are exceptionally rich in a protein which is presumed to be the subunit of microtubules (Borisy and Taylor, 1967). This protein binds colchicine (Borisy and Taylor, 1967), is precipitated by vinblastine (Marantz, Ventilla and Shelanski, 1969), and, when disaggregated, has a molecular weight of approximately 60 000 (Weisenberg, Borisy and Taylor, 1968; Shelanski and Taylor, 1968). Because of its particular abundance in neurons, metabolic studies of this protein in brain were begun. The purpose of this brief preliminary report is to demonstrate that the synthesis and metabolism of this protein is active both in developing and adult brain, that the protein is transported to nerve terminals at a uniquely rapid rate and that the protein may exist in both soluble and particulate forms in brain homogenates. Metabolic techniques of this type will help to elucidate the mechanism of neuronal pathology believed to be due to primary or secondary microtubular abnormalities.

SYNTHESIS AND METABOLISM

For the purpose of this study we consider that soluble (100 000 ***g*** supernatant) protein which binds colchicine, is precipitable by vinblastine and consists of monomers of 60 000 daltons is microtubular protein. Were microtubular protein less abundant in the soluble fraction of brain, these

* Supported by Grants MH-12773 and K3-MH-18232 from the United States Public Health Service.

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characteristics might not be sufficient to allow simple purification for metabolic studies. Because of its abundance and the development of polyacrylamide gel electrophoresis techniques (Maizel, 1966) which allow easy separation of proteins on the basis of molecular weight (Shapiro, Vinuela and

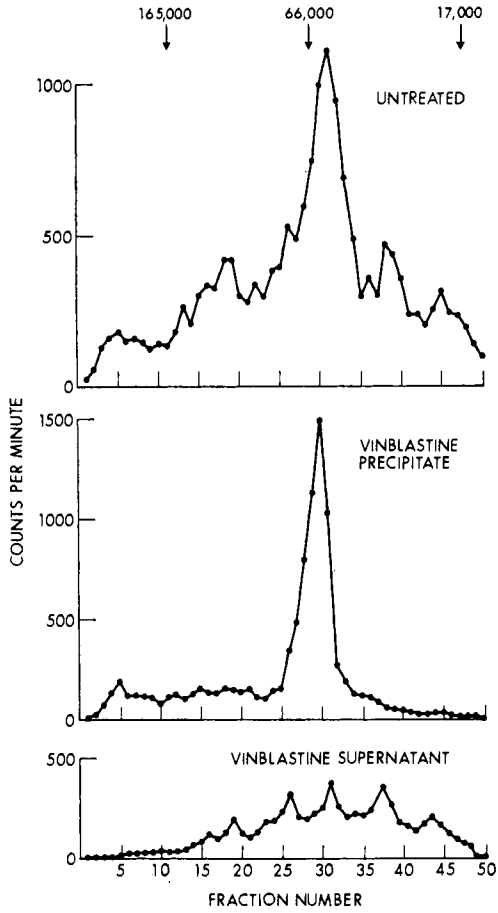

FIG. 1. Gel electrophoresis of 100 000 *g* supernatant protein from 6-day-old mouse brain obtained 24 hours after intracerebral injection of [^{14}C]leucine. Electrophoresis was for 9 hours at 80 V on polyacrylamide gels by the method of Maizel (1966). The untreated supernatant (top), vinblastine-precipitated protein (middle) and supernatant protein remaining after vinblastine precipitation (bottom) were run simultaneously. Molecular weight markers were a mixture of non-reduced human gamma globulin (165 000), bovine serum albumin (66 000) and haemoglobin monomers (17 000). They were run simultaneously in a separate gel and localized by staining with Coomassie blue. For details see Dutton and Barondes (1969).

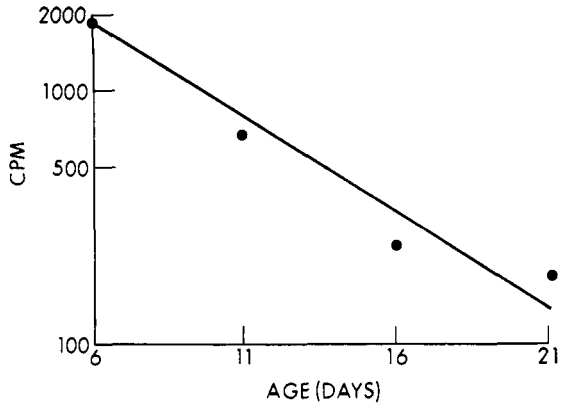

FIG. 2. Turnover of microtubule protein. Mice, 5 days of age, were each injected intracerebrally with 2 μCi[^{14}C]leucine and three were killed at each age indicated. The 100 000 *g* supernatant was isolated, 0·2 mg was electrophoresed and the fractions were counted. Counts/min (CPM) of 60 000 mol. wt. protein in 0·2 mg of soluble brain protein were determined. Since the protein content of brain increased markedly during the period studied, corrections were made for dilution of the labelled protein and these corrected results are shown on the ordinate. For details see Dutton and Barondes (1969).

Maizel, 1967), the study of microtubular protein metabolism is considerably simplified. This is particularly true of developing brain, where microtubular protein metabolism is especially prominent; but metabolic studies in adults may also be done conveniently.

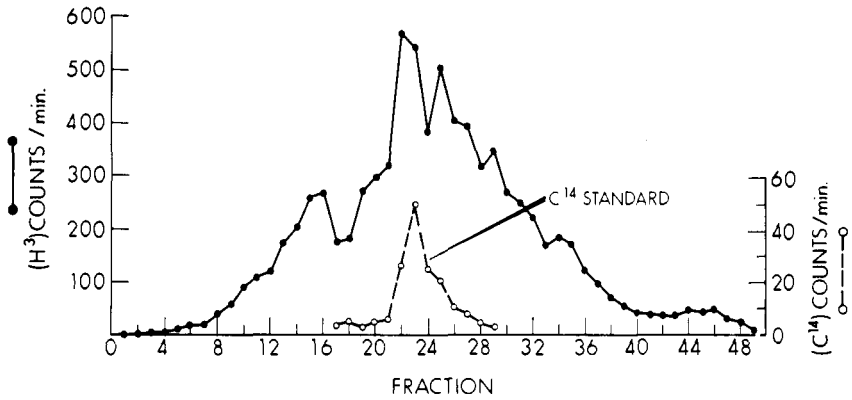

FIG. 3. Gel electrophoresis of 100 000 *g* supernatant protein from 70-day-old mouse brain obtained 90 minutes after intracerebral injection of [^{3}H]leucine. Purified ^{14}C-labelled microtubular protein obtained by vinblastine precipitation of labelled soluble protein from 5-day-old mouse brain was run as an internal standard.

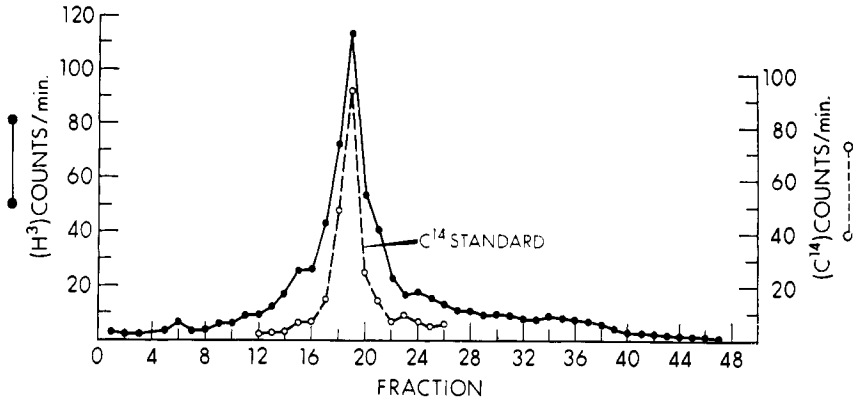

FIG. 4. Gel electrophoresis of purified ^{3}H-labelled microtubular protein from 70-day-old mouse brain. The 100 000 *g* supernatant protein shown in Fig. 3 was precipitated with vinblastine (Marantz, Ventilla and Shelanski, 1969), redissolved by dialysis against 0·01M-$MgCl_2$, 0·01M-sodium phosphate, pH 7, then against buffer alone. The protein solubilized by dialysis was electrophoresed. Purified ^{14}C-labelled microtubular protein from 5-day-old mouse brain was run as an internal standard. For details see Feit and Barondes (1970).

Polyacrylamide gel electrophoresis of soluble protein obtained from six-day-old mice injected one day previously with radioactive leucine demonstrates a single major peak, with a molecular weight of about 60 000 which contains about 40 per cent of the total labelled soluble protein (Fig. 1). The protein in this peak was precipitated by vinblastine whereas many other labelled proteins are not (Fig. 1). More than 95 per cent of the colchicine-binding activity was also precipitated by vinblastine (Dutton and Barondes, 1969). When gels were stained with Coomassie blue (Shapiro, Vinuela and Maizel, 1967), some weakly labelled contaminants of the microtubular protein were found in the vinblastine precipitate. The microtubular protein may be further purified (Olmsted *et al.*, 1970) by removing the vinblastine by dialysis and reprecipitating the microtubular protein with vinblastine.

The metabolism of the labelled microtubular protein was studied by determining the decrease in label in this peak at a number of times after injection of [^{14}C]leucine. In mice injected with labelled amino acid five days after birth, the labelled microtubular protein has a half-life of three or four days (Fig. 2). In brains of young adult (70-day-old) mice, only about 15 to 20 per cent of the soluble protein labelled with radioactive leucine migrates in the molecular weight range of approximately 60 000 (Fig. 3). For metabolic studies of this protein, vinblastine precipitation was followed

by dialysis. Even without a second vinblastine precipitation, the protein dissolved by dialysis is quite pure, as determined both from radioactivity in gel fractions (Fig. 4) and by staining of the gels. When the disappearance of labelled microtubular protein in young adult animals was studied with this technique, a half-life of approximately four days was found.

We conclude from these studies that microtubular protein is actively synthesized both in developing and in young adult mouse brain. Preliminary studies with 18-month-old mouse brain show similar results. In all cases the labelled microtubular protein turns over quite rapidly. Although rapid turnover may not be characteristic of all brain microtubular protein these studies suggest that microtubules may be in a constant state of re-formation rather than inert structural elements.

TRANSPORT OF MICROTUBULAR PROTEIN TO NERVE ENDINGS

Although microtubules are not demonstrable in nerve endings, the possibility that they play a role in the transport of proteins through the axon and to the nerve terminal has been considered (Schmitt, 1968; Karlsson and Sjöstrand, 1969; Kreutzberg, 1969). Studies of the transport of labelled protein including microtubular protein were made by determining the rate of appearance of labelled protein in various fractions of the brain, including the soluble and particulate components of isolated nerve-ending fractions, at various times after intracerebral injection of radioactive leucine. The labelled amino acid was rapidly incorporated into protein and within one hour of its injection its incorporation was completed (Barondes, 1968). All subcellular fractions of brain with the exception of the nerve-ending fractions were maximally labelled at about one hour after injection of the labelled precursor. In the ensuing hours and days the specific activity of most brain fractions fell, whereas the specific activity of both the soluble and the particulate components of the nerve-ending fraction rose, presumably due to transport of labelled protein from the nerve cell bodies to the nerve terminals (Barondes, 1964, 1968). Even three hours after injection there was relatively little labelled protein in the soluble component of the nerve-ending fraction, whereas in the interval between three hours and one day there was a marked increase in the specific activity of the soluble protein of nerve endings and a marked decrease in the specific activity of the soluble protein from whole brain (Fig. 5). The small amount of labelled protein present in the soluble component of the nerve-ending fraction as early as 90 minutes after injection of the precursor in young adult mice was found to be composed largely of protein with a molecular

weight of approximately 60 000 (Fig. 6). The profile of the labelled soluble proteins of the nerve-ending fraction (Fig. 6) contrasts strikingly with that of the soluble protein from whole brain also obtained after 90 minutes of labelling in the same experiment (see Fig. 3), where a wide spectrum of labelled proteins was found. This difference in profile suggests that the

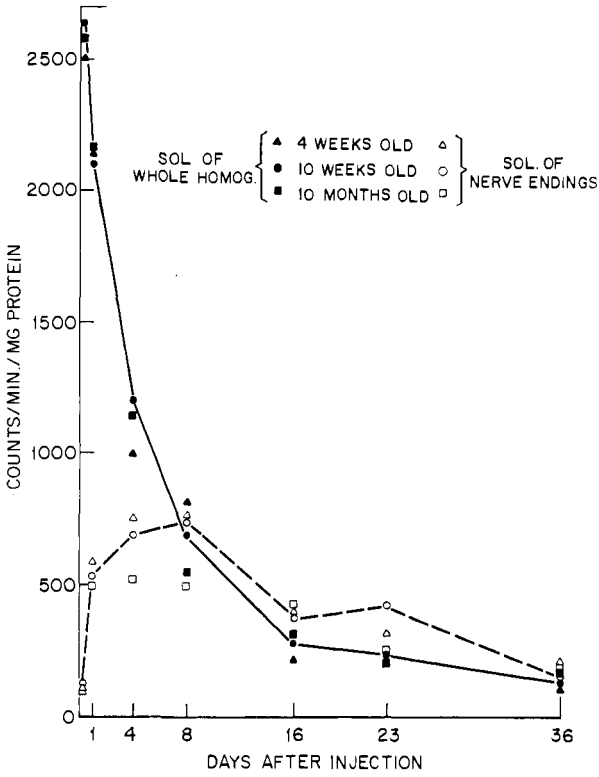

FIG. 5. Transport of soluble protein to nerve endings. Mice (ages indicated) were injected intracerebrally with [^{14}C]leucine, their cerebral hemispheres were removed at the indicated times after injection, homogenized and fractionated. The soluble protein of the nerve-ending fraction (Whittaker, Michaelson and Kirkland, 1964) is obtained by lysis of these particles with water. The specific activity of soluble protein from whole brain and from nerve endings was determined as described by Barondes (1964, 1968).

labelled soluble protein associated with the nerve-ending fraction is not due to contamination with soluble protein from whole brain. The labelled protein of 60 000 molecular weight found in the soluble fraction of the nerve endings was precipitable by vinblastine and is presumed to be microtubular protein.

The appearance of labelled microtubular protein in the nerve-ending fraction within 90 minutes of injection of precursor may be due either to rapid transport of the protein from a perikaryal site of synthesis or to local synthesis of microtubular protein at nerve endings. Since incorporation of radioactive leucine is completed within one hour of injection and since subsequent experiments showed further increases in labelled microtubular

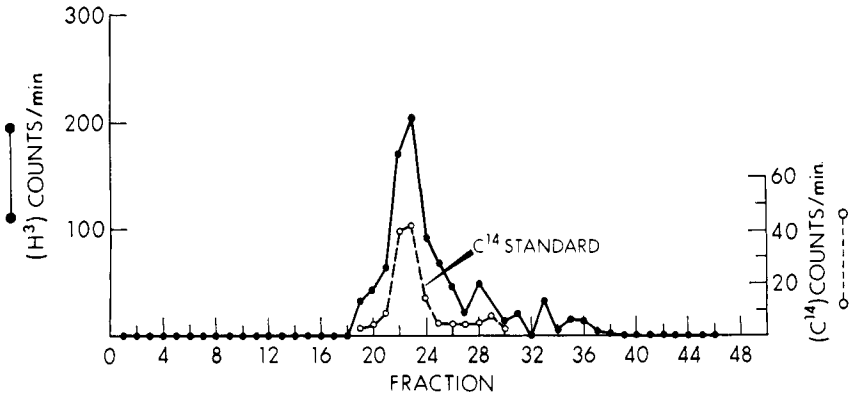

FIG. 6. Gel electrophoresis of ^{3}H-labelled soluble protein from the nerve-ending fraction obtained from 70-day-old mice injected with [^{3}H]leucine 90 minutes before they were killed. The same animals were used for the material in Fig. 3. No purification with vinblastine was done in this experiment. Purified ^{14}C-labelled microtubular protein from 5-day-old mouse brain was run as an internal standard. For details see Feit and Barondes (1970).

protein in the soluble component of the nerve-ending fraction for many hours thereafter, it seems quite likely that the microtubular protein is being transported to nerve endings from the cell body. Other labelled soluble proteins are also transported to the nerve ending for hours and days after administration of the radioactive amino acid, as shown by the progressive appearance of labelled proteins of diverse molecular weights on polyacrylamide gels after electrophoresis of the soluble protein of the nerve-ending fraction. The reason for the particularly rapid transport of microtubular protein to nerve endings is not known.

SOLUBLE AND PARTICULATE COLCHICINE BINDING PROTEINS

In studies of the subcellular distribution of colchicine-binding protein in tissue culture cells, it was found that almost all of this protein was soluble, and that it was present in the 100 000 *g* supernatant after homogenization and centrifugation at 4°C (Borisy and Taylor, 1967). In studies of the

colchicine-binding activity of mouse brain we found that, although the amount of [^{3}H]colchicine bound per milligramme of protein was quite high in the soluble protein prepared from brain as compared with other organs, there was also considerable particulate colchicine-binding activity (Table I). Since the particulate fraction contains considerably more protein

TABLE I

SOLUBLE AND PARTICULATE COLCHICINE-BINDING ACTIVITY IN VARIOUS ORGANS

	Counts/min per mg protein		
Organ	*Soluble*	*Particulate*	*Soluble: % of total*
Brain	98 600	48 400	43
Heart	6000	2800	67
Liver	4900	2100	76
Lung	10 400	2500	88
Kidney	7800	3200	61
Testis	62 500	2400	96

Soluble and particulate components of whole brain homogenates were separated by centrifugation at 100 000 ***g*** for one hour. Colchicine binding was studied by incubation of aliquots of the soluble or washed particulate fractions in 0·01M-$MgCl_2$, 0·01M-sodium phosphate buffer, pH 7·0, containing 1 μCi[^{3}H]colchicine. After incubation, [^{3}H]colchicine bound to soluble protein was determined by filtration through DEAE-impregnated filter paper (Weisenberg, Borisy and Taylor, 1968). [^{3}H]Colchicine bound to the particulate fraction was determined by carefully layering 1·0 ml aliquots of reaction mixture over 7·0 ml of 10% sucrose in 0·01M-$MgCl_2$, 0·01M-sodium phosphate buffer, pH 7·0, followed by centrifugation for one hour at 100 000 ***g*** using the Spinco 50 rotor. After centrifugation, the supernatant was aspirated and the pellet was counted. This procedure gave results identical with those of a more laborious washing procedure involving five successive re-homogenization and centrifugation steps. Soluble and particulate colchicine-binding activity was linear over a wide concentration range. For details see Feit and Barondes (1970).

than the soluble fraction, we found that about half of the total colchicine-binding activity of brain was particulate (Table I). This was true even after vigorous homogenization and repeated washing of the particulate fraction in both isotonic and hypotonic media. Colchicine binding by the particulate component of brain homogenates resembled that of the soluble component in that it did not occur when incubations were conducted at 4°C, was inactivated by urea, thiol reagents and detergents, and was stimulated considerably by addition of vinblastine (Feit and Barondes, 1970).

Both the microsomal and nerve-ending subfractions of the particulate component of brain are relatively rich in colchicine-binding activity (Table II). Subfractionation of the microsomal fraction by sedimentation through a step-wise sucrose gradient indicates that only the subcomponent of the microsomes which sediments to the 1·0–1·2M-sucrose interface is rich

in colchicine-binding activity (Feit and Barondes, 1970). Attempts to solubilize this particulate colchicine-binding material have been frustrated by the fact that the various solubilization procedures we have so far used have destroyed the colchicine-binding activity upon which we rely heavily to identify the protein. Studies making use of vinblastine precipitability and

TABLE II

COLCHICINE-BINDING ACTIVITY IN SUBCELLULAR FRACTIONS OF BRAIN

Fraction	*Counts/min per mg protein*
Soluble	45 132
Crude nuclear	4 624
Myelin	10 423
Nerve endings	34 928
Mitochondria	13 383
Microsomes	47 912

Subcellular fractionation was done without magnesium or phosphate buffer, which were found to influence this procedure. Colchicine-binding activity is lost rapidly under these conditions. For details see Feit and Barondes (1970).

the molecular weight parameter are in progress, but it is not yet clear whether or not the particulate and soluble colchicine-binding proteins are identical. The only evidence for such identity is the presence of a prominently labelled protein with a molecular weight of approximately 60 000 in the particulate component of the nerve-ending fraction (obtained by centrifugation after lysis of the nerve-ending fraction with water: see Barondes, 1968) 90 minutes after intracerebral injection of [^{3}H]leucine. The intriguing possibility that microtubules may be converted into membranous structures, as proposed from morphological studies (Pellegrino de Iraldi and De Robertis, 1968) receives tentative support from these studies.

SUMMARY

Because of the abundance, active metabolism, vinblastine precipitability and colchicine-binding activity of microtubular protein, it has been possible to study the metabolism and distribution of this protein in brain using relatively simple procedures. After intracerebral injection of radioactive leucine, approximately 40 per cent of the labelled soluble brain protein of five-day-old mice and 15 to 20 per cent of such protein from 70-day-old mice is microtubular protein. At both ages this labelled protein has a half-life of about four days. Microtubular protein is virtually the only labelled protein in the soluble component of the nerve-ending fraction 90 minutes after injection of radioactive amino acid. It is apparently transported to nerve endings at a particularly rapid rate whereas other labelled soluble proteins are transported more slowly. Colchicine-binding

protein, which may be identical with microtubular protein, is present not only in the soluble fraction of brain homogenates but also in the particulate fraction, particularly in the microsome and nerve-ending subfractions. The techniques for studying the subcellular distribution and metabolism of microtubular protein should have important applications in experimental neuropathology.

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DISCUSSION

Gonatas: Which is the particulate synaptosome fraction? Is it related to synaptic vesicles, mitochondria or synaptic membranes?

Barondes: The synaptosome fraction contains all these components but we do not know which are responsible for binding colchicine. There is some speculation that microtubules turn into synaptic vesicles, and we have attempted to determine whether synaptic vesicles were particularly rich in colchicine-binding activity. However, the synaptic vesicle preparations which we have made are not sufficiently pure to answer this.

Dayan: Have you considered using a labelled antibody against microtubular protein?

Barondes: This would certainly help to decide whether the soluble and particulate materials which bind colchicine are identical, but there has been difficulty in obtaining good antibodies to microtubular protein.

Shelanski: Antisera to microtubule protein have been made and we are currently planning some experiments utilizing this material.

Dayan: Where does all this protein go? There isn't much protease activity, but what about retrograde flow?

Barondes: I don't see how retrograde flow resolves the problem. My guess is that the protein is degraded.

Friede: You showed the relative concentration of protein in various organs. Is there a correlation between the concentration of the protein and the density of microtubules in these organs?

Barondes: I can't answer that except in the crudest way. Neurons and sperm cells have a high concentration of microtubules and of microtubular protein. Other organs have less of both. One problem in this estimation is that much microtubular protein may be in the cell in a non-aggregated form, that is as soluble precursor rather than identifiable microtubules. In addition, we do not know how many microtubules are actually *fixed* as such for morphological studies. Some might disaggregate during fixation.

Friede: How much tubular material is there in hepatic cells?

Barondes: There is not much colchicine-binding protein in the liver.

Shelanski: E. Robbins and I found (unpublished) that approximately 15 per cent of the colchicine-binding activity in the HeLa cell is in a vesicular fraction. However, unlike what you saw, Dr Barondes, these vesicles can be lysed with distilled water shock and all the colchicine-binding activity is released.

Friede: How sure can you be of distinguishing between microtubules and tubules of smooth endoplasmic reticulum in fractionated tissue? Is it possible that the membranes seen in the tissue fractions are membranes of smooth endoplasmic reticulum?

Shelanski: We feel confident we can tell the unit membrane from the microtubule wall.

Barondes: It is certainly clear that an organ like the liver, which is rich in smooth endoplasmic reticulum, does not have a lot of particulate colchicine-binding activity, whereas the brain, which is also rich in smooth endoplasmic reticulum, has a lot of colchicine-binding activity in the microsomal fraction.

Shelanski: Other proteins of 60 000 molecular weight are known. The definitive analysis of this protein is going to depend on its isolation, and on showing that either its total amino acid composition or at least portions of the peptide map are similar to those of the microtubular protein.

Barondes: As you know, we use two criteria—it is a protein of 60 000 molecular weight, precipitable by vinblastine. Neither of these is completely specific for microtubular protein but the two together decrease the chance for error. Nevertheless further tests like those you suggest must be made.

Terry: Do you really not see any recognizable microtubules in those pellet fractions?

Barondes: No. This is not to say there is not some sort of sequestered precursor, but we can't get it out even with very vigorous homogenization.

Shelanski: Have you any evidence for mitochondrial synthesis of this protein?

Barondes: We have considered this possibility because of the rapid appearance at nerve endings of what seems to be microtubular protein. We plan to check this by determining whether its synthesis is blocked by cycloheximide, which should not inhibit mitochondrial protein synthesis.

Terry: If all the counts were in mitochondria would that rule out transport? Mitochondria might move too.

Barondes: There is good evidence for transport of mitochondria in axons.

Friede: In tissue culture one can see mitochondria moving both ways. I can also accept movement of smooth endoplasmic reticulum and protein. However, considering the length of a microtubule it seems hard to imagine that it moves down the axon.

Shelanski: There is no direct answer to that, but in cilia microtubules add subunits distally while other systems appear to do so proximally. Whether the whole tubule moves or whether subunits are added at opposite ends is not known.

Friede: From changes in cat fibres I got the impression that filaments and tubules do not move. They may grow by apposition at the end but I doubt that the whole core of filaments and tubules moves. It might not be surprising if the axis cylinder were of uniform calibre; the process is more difficult when the calibre of the axis cylinder changes, yet filaments and tubules retain the same spacing.

Barondes: You should recognize that these filaments and tubules are composed of individual protein molecules aggregated in a particular array. It is not clear to me whether the aggregates, once formed, behave as a unit, or whether there is constant exchange of the protein molecules in the aggregate.

Could energy be released by aggregation of the subunits? Could that be harnessed for movement?

Taylor: I suppose so, but I don't know how.

GENERAL DISCUSSION

Tomlinson: Professor Roth and Dr G. Blessed have carefully assessed a group of old patients for evidence of dementia. I then examined the brains of the cases coming to autopsy and attempted to quantitate the findings before I knew the category in which these patients had been placed by the clinicians. First, the senile plaques were counted in 12 standard areas of the cortex and a mean plaque count was obtained. Secondly, I attempted to estimate the quantity of neurofibrillary change within the hippocampus and also within the neocortex, on a five-point scale. Finally I attempted, after the above survey had been completed, to estimate the number of hippocampal pyramidal cells in Sommer's sector which showed granulovacuolar degeneration.

Originally, 28 patients who were considered shortly before death to show no evidence of dementia were studied in this way. Senile plaques were found in 22 (78 per cent) of them; the mean plaque count in this group was only three per field (range 0 to 13 per field). Only two of these "control" cases showed more than ten plaques per field. Neurofibrillary change was found in the hippocampus in 55 per cent, but in only two of the 28 were the changes severe. In the neocortex neurofibrillary change was only found in three of the 28 cases and then in small numbers of neurons only. Granulovacuolar degeneration was present in 55 per cent of cases, but only once was it present in more than 10 per cent of the neurons in Sommer's sector.

Fifty demented patients were assessed in an identical fashion, but this was a miscellaneous group in which cerebral softening clearly played a significant part in a good many. I shall therefore refer here only to those who showed (*a*) no specific cause for dementia other than Alzheimer-type change, and (*b*) no gross arteriosclerotic softening. In this group there were 25 cases in which the only significant change in the brain was plaque formation and neurofibrillary change. In these 25, that is 50 per cent of the whole group, tangles were found in every case in the neocortex. They were heavy in the hippocampus in 20 of the 25 and in the neocortex were present in greater numbers in all these cases than had been seen in the controls. Plaque counts were much higher than in the controls; the mean was 22 compared with three for the controls, and in 16 of the 25 the

mean plaque count was more than 18 per field, i.e. 50 per cent greater than in any control. In the remaining nine cases the plaque count was around the upper limit of what had been found in the controls. After seeing the publications of Woodard (1962, 1966) on granulovacuolar degeneration we went back and found that 23 of the 25 cases showed it in more than 10 per cent of the cells in Sommer's sector, as compared with one out of 28 controls. The correlation between the mean plaque counts and the degree of dementia assessed clinically in the controls and in dementia was highly significant statistically. When the dementia score was plotted against the mean percentage of cells in Sommer's sector showing granulovacuolar degeneration a very similar and significant correlation was seen.

In the last three years I also looked at 270 brains from patients dying in the general wards of an acute hospital. These had not been assessed for intellectual deterioration in life and the investigation is not yet complete. The patients ranged from the third to the tenth decade. Senile plaques occur in significant numbers in the fourth and fifth decades and are present in over 70 per cent of patients above 70 years of age. However, some 20 per cent of people above the age of 80 show no senile plaques and a similar proportion show no granulovacuolar degeneration. All the people examined in the tenth decade showed tangles in the hippocampus. This material was reviewed to see how many cases fell within the same quantitative range of abnormality as the assessed dementia cases with Alzheimer-type change. One case occurred in the sixth decade, two in the seventh and 15 in the eighth and ninth decades. These, by all the standards that we had found in the assessed cases, appeared to be cases of Alzheimer's disease, at least pathologically. Hospital notes were available for only 13 of these 18 because many of these patients came in from road accidents and other forms of sudden death. Nine of the 13 had records which either stated that the patients were thought to be demented, or a series of statements were made which suggested that they probably were demented.

This investigation seems to show a direct relationship between the quantity of change found, of the types we have been discussing here, and the occurrence of dementia. The following hypothesis might be made from these findings: in old age, light microscopic changes of the same kind as those seen in demented people of Alzheimer type are common, but in most old people the changes do not reach the degree seen in the demented cases. In a relatively small proportion, even in very advanced age, either from genetic or perhaps many other unknown causes these changes reach a magnitude at which functional disability of the cortex is reflected in

recognizable dementia. It seems clear that there are some old people in the community who are not recognized as cases of Alzheimer's disease, and in these cases any small contributory damaging factor to cerebral function could well result in obvious dementia. Perhaps some of the dramatic cases Dr Strich described were of this sort. If any additional damage to the brain may, in a proportion of cases, result in dementia then it is perhaps not surprising that correlations of the kind that have been described are not complete. In fact it is perhaps surprising that they are as high as they appear to be from this investigation.

Terry: There are then a few patients with high concentrations of plaques and tangles who are not demented. Have you attempted to assess the neuropil intervening between the lesions?

Tomlinson: The intervening neuropil in the apparently non-demented cases with high plaque counts didn't seem any different from that in the cases of dementia. Of course it is tempting to suppose that these are cases of subclinical Alzheimer's, but on that we have no real information.

Dayan: We examined brains from 58 people over the age of 60, who were mentally normal as far as we could tell, and found increasing numbers of plaques and tangles in the older patients when these lesions were counted in the frontal cortex and hippocampus by a standardized technique. (The lack of lesions in younger normal subjects may just reflect the size of the sample examined.) These results were compared with the findings in cases of senile dementia in people over the age of 60. The results were very similar to yours, Dr Tomlinson, as the cases of dementia showed more plaques and tangles than the controls at both sites examined. There is an interesting overlap between the controls and the cases of dementia in the numbers of plaques per unit volume of cortex. However, the numbers of tangles in the frontal cortex seem to correlate more closely with the dementia than the numbers of plaques. The findings in the hippocampus were similar, but there was a statistically significant excess of tangles there in such cases as compared with the frontal cortex.

Tomlinson: The close correlation with tangles in the neocortex in dementia is clear in our series. There is an overlap in plaque formation but very little overlap in tangles in the neocortex.

Roth: We have not yet conducted a reliability study of our technique for assessing the severity of neurofibrillary change. The inter-observer reliability of our plaque-counting technique has proved to be extremely high.

Terry: Many lesions beneath the resolution limit of the light microscope might well be significant. We took serial 1 μm sections before and after

electron microscope sections. At neither end were plaques visible to the light microscope, but in between there were many lesions in the electron microscope.

Roth: But the light microscope was adequate to reveal a significant correlation between the quantitative amount of cerebral damage as shown by our indices and the severity of mental impairment. Both the plaque counts and the measures of total volume of softening showed clear threshold effects. A high proportion of the normal aged population showed some change of both kinds, but dementia was manifest only in those in whom the plaque counts reached an average of 14–15 per field or, in the case of softening, a total of approximately 50 ml.

There is not necessarily any conflict of evidence here. Light microscopy may have the advantage of providing an overall picture of damage and making it possible to examine statistical differences between groups, but electron microscopy will perhaps reveal finer and more meaningful differences within the demented and within the normal group of subjects.

Dayan: Many years ago Simchowicz (1911) pointed out that there appears to be a correlation between the type of dementia and the distribution of lesions in particular areas of the brain. So when cases of dementia are considered, we may be dealing with damage concentrated particularly in certain parts of the cortex.

Tomlinson: In cases with very heavy plaque formations in the neocortex the neuropil in between the defined plaques often does not look normal, whereas in the non-demented patients one often saw scattered plaque formation with apparently normal surrounding cortex.

Friede: You showed tangle counts for hippocampus and for cortex but only one curve for plaque counts. Are the plaque counts for cortex only?

Tomlinson: The plaques within the hippocampus were not counted in the mean plaque count estimations.

Friede: If you tabulated gross brain weight in your diagram would you get a proportionate curve or a disproportionate one?

Tomlinson: One would get a disproportionate curve. At least in the group we have looked at, there was no significant difference in the mean between the control series and the aged dements.

Roth: Brain weight appears to be highly correlated with softening.

Jacob: Did you see senile dementia cases without senile plaques?

Tomlinson: In the total of 50 cases there were five in which no morphological basis for the dementia could be defined with certainty. One of these had neither cerebral softening of any degree, nor any well-defined specific process, nor plaques nor tangles, nor even marked atrophy.

Friede: Does the considerable normal variation in brain weight conceal atrophy or is there no atrophy?

Tomlinson: I was only talking about brains from old people and this did not include any presenile examples. My impression would be that in presenile Alzheimer's disease there is considerable and often gross atrophy, but that in the senile group atrophy is not marked when compared with mentally normal old people, in whom a considerable amount of atrophy is often present. Brain weight after the age of 65 is an insensitive indicator. In the small group that we have examined it is probably too insensitive to demonstrate any significant difference.

Barondes: Are you implying that if one lives long enough one will start showing these kinds of changes?

Tomlinson: From the sample of the ordinary population one wouldn't draw that conclusion. Even above 90 years some people do not show plaques or tangles in the neocortex, and some of them only have very minimal changes in the hippocampus.

Barondes: But they all have changes?

Tomlinson: All the ones that we saw had changes in the tenth decade within the hippocampus.

Barondes: That seems to have important implications for the aetiology.

Roth: There appear to be two main types of degenerative change in the central nervous system in old age: cerebrovascular disease and the processes underlying the Alzheimer—senile dementia groups of disorders; there is, of course, some measure of overlap between them. The differences between normal elderly subjects and those manifesting an unequivocal dementia appear, in our findings (Roth, Tomlinson and Blessed, 1967; Blessed, Tomlinson and Roth, 1968; Tomlinson, Blessed and Roth, 1968), to be quantitative in respect of each type of change. Perhaps the different clinical end-results arise from differences in the rate of development of the same processes in normal and demented subjects. However, such formulations presuppose that methods of observation employed in such studies were sufficiently sensitive to record all that was relevant. It is possible, of course, that those in the demented group exhibit some qualitative morphological change that has eluded us.

Barondes: It depends on whether you are a lumper or a splitter! In the absence of any reason to think otherwise one can say that a parsimonious way of looking at it is that this is a continuum.

Roth: If there is a continuum, determined perhaps by a difference in the rate of development of the relevant degenerative process, the prospects

of further enquiry would be hopeful. The question arises of what regulates the rate of development of the process.

McMenemey: What about the very old people who were reported to have no plaques or tangles?

Roth: With a graded variable, which the process represented by plaques and tangles appears to be, there is bound to be little or no change at the extreme end of the distribution curve.

Barondes: The invariable occurrence of tangles in the hippocampus in the tenth decade which Dr Tomlinson reports is particularly striking when one recognizes that the technique of the light microscope is not very sensitive. One wonders what the results would be if you did the same study with the electron microscope.

Shelanski: This leads one to think there is an ageing factor in everybody and just some controlling factor which differs. An analogous case is carcinoma of the prostate. Few people would say that ageing causes carcinoma of the prostrate, yet if a male population ages for long enough the incidence is about 97 per cent.

Barondes: Why is that not due to ageing?

Shelanski: I don't know what the aetiology is. In the sort of thinking we are doing about cancer today, most of us would not be willing to accept just ageing as an aetiological entity.

Barondes: The same is true with Alzheimer's disease. It need not be "ageing"; it could just be an invariable consequence of having lived long enough to have been exposed to enough of something—like radiation.

Hughes: Dr Tomlinson, where there are no plaques, especially if there is atrophy, how do you know the plaques haven't dropped out? Do we know anything about the survival of amyloid in brain tissue?

Tomlinson: I don't know. It is a mere factual observation that a small number of patients who dement in old age apparently have no identifiable specific process.

Hughes: I get the impression that these cases are very common.

Dayan: Amyloid is remarkably inert. Once it is deposited in the body it is difficult to get rid of it.

Hughes: Even in ten or fifteen years?

Terry: All the other elements of the plaque may be labile and disappear, but the amyloid will probably last for ever.

Shelanski: The amyloid is resistant to degeneration by mild chemical means (Miller *et al.*, 1968). Procedures such as treatment with cyanogen bromide or urea are necessary.

Roth: I should like to return briefly to some other evidence relating to

the cerebral lesions associated with dementia in old age. In a proportion of cases with predominantly cerebrovascular lesions, plaques and tangles are present. In the Newcastle material the effects of softening on dementia scores were potentiated by plaque formation; the process represented by plaque formation had made an independent contribution statistically to the variation in respect of dementia in this group of patients. This potentiation of one process by the other provides some supportive evidence for the causal association between each process and dementia in old age.

Corsellis: We have been comparing the intensity of plaque formation in the cerebral neocortex with the severity of cerebral atherosclerosis in 667 patients dying in a mental hospital. Fig. 1 shows that some degree of atherosclerosis was found increasingly often as age advanced, until over 90 per cent of the nonagenarians were affected, at least to a slight degree (degree 1). Fig. 2 shows that senile plaque formation taken as a whole followed the same trend. The difference came with the more severe degrees of change (2 and 3). Whereas moderate or severe cerebral atherosclerosis went on increasing to the end, moderate or severe degrees of senile plaque formation showed a definite tendency to fall away after the age of 85. There was also the suggestion of a peak during the 60s which appears to be made up of cases of presenile dementia, that is of Alzheimer's disease.

The sample is of course relatively small and it is inevitably selected, but the findings seem to suggest that the senile degenerative process does not advance with age so relentlessly as cerebrovascular disease appears to do. Can anyone explain this apparent difference?

Roth: Supposing the intensity of plaque formation is more or less normally distributed and that we follow a cohort of subjects from the age of 70 years onwards. Then, if those with higher concentrations of plaques have a higher mortality rate than those with fewer plaques (and there is ample evidence to validate this assumption), those reaching the age of 90 or more years will be a population of survivors among whom those with few plaques would be over-represented.

Tomlinson: My figures for mean plaque counts in the different decades are almost identical to what Dr Corsellis finds, with this tailing off above the mid-80s.

Roth: Different mortality rates among those with differing degrees of plaque formation could be expected to produce this phenomenon.

Corsellis: Wouldn't this explanation apply equally to the incidence of cerebrovascular degeneration?

Roth: What one would like to know is whether the total volume of

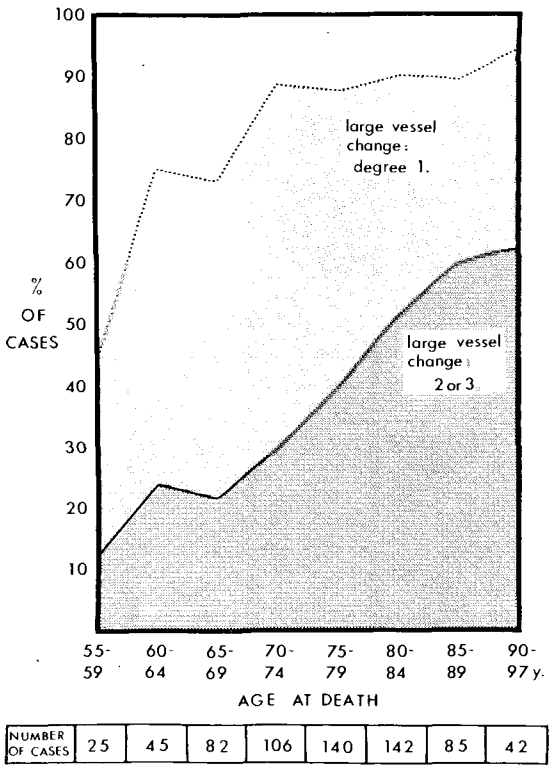

FIG. 1 (Corsellis). Incidence of atherosclerosis of large cerebral vessels in a series of 667 patients dying in a mental hospital. (Light shading shows a slight degree of change; dark shading shows changes estimated as moderate to severe).

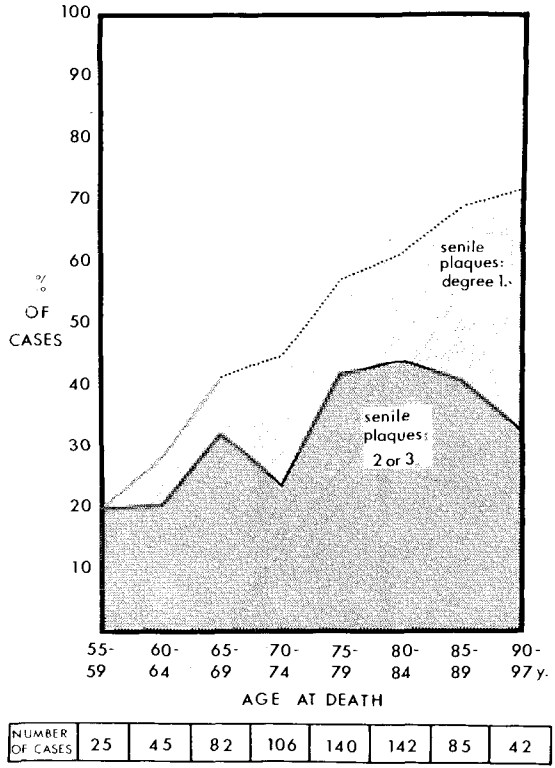

FIG. 2 (Corsellis). Incidence of senile plaque formation in the same series as in the previous figure. (Light shading shows cases with only a few plaques; dark shading shows those with moderate to severe degrees of plaque formation.)

softening, which has been shown to be related to dementia, changes with age like your measures of change in large vessels, or more like plaque counts.

Tomlinson: I showed the age distribution with plaques. In the general population no doubt it starts in the 30s, but plaques are few in number. One interpretation which is perhaps oversimplified is that a small proportion of the population is highly resistant to plaque formation. Perhaps if one hasn't started to form plaques by the age of 80 one never will.

McMenemey: Is there a sex difference in your findings, Dr Corsellis?

Corsellis: They are more or less the same. Of course in the 90s not many men are left (six out of 42)!

Friede: Does the reduced life expectancy refer to the entire material including cerebrovascular disease and Alzheimer's disease?

Roth: The life expectancy for all types of dementia taken together is markedly reduced (Kay, 1962). It is also greatly reduced for senile dementia alone (Larsson, Sjögren and Jacobson, 1963). The mortality rate for subjects with dementia due to cerebrovascular disease is little better than that of the senile dementia group (Roth, 1955) and it must therefore also be markedly above that of a normal population of comparable age composition.

Polak: At this symposium various hypotheses on pathogenesis have been based on the electron microscopic features of the changes in nervous tissue in pathological senility. However, it would be premature to accept these as definitive, and I believe that the resources of optical microscopy have not yet been exhausted. With adequate histochemical techniques, in particular the silver techniques of del Rio Hortega, most interesting results are obtained on which theories can be based that may then be confirmed in the electron microscope. In fact, Terry and Wiśniewski pointed out in the abstract of their paper for this meeting that "The classical light microscopists have described several sorts of plaques, depending upon the distribution of amyloid, and correlates have been found by electron microscopy."

However, the different published descriptions of senile plaques depend on the fact that each researcher generally uses only one silver technique, which is seldom specific for more than one or two of the components of senile plaques. Consequently, theories about the evolution of plaques vary according to the techniques employed.

We consider that the senile plaque, not the tangle, is the fundamental lesion of normal and pathological senescence. The tangle has been noted by several authors in many other pathological conditions involving a chronic degeneration of the neuron.

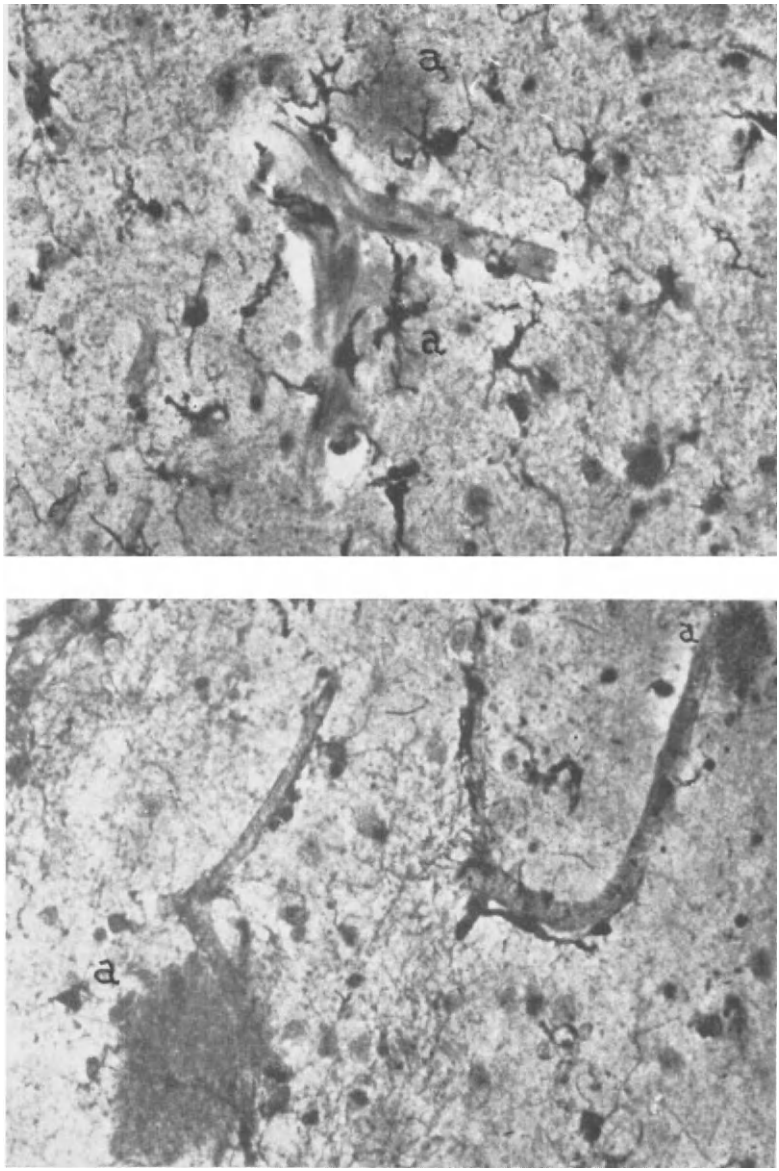

FIG. 1 (Polak). A: Hypertrophic microglia around a blood vessel. Two argyrophilic zones (a) corresponding to the early senile plaque are related to the vessel. Del Rio Hortega-Polak technique.

B: Blood vessel with hypertrophic adventitial microglia. Argyrophilic zones (a) related to the vessel correspond to the early senile plaque. Del Rio Hortega-Polak technique.

In order to obtain adequate preparations for study, a series of silver techniques must be used to show the various components of the plaque (namely nerve cell bodies, dendrites, axons, astrocytes and their processes, oligodendrocytes, microglia cells, amyloid, degenerative granules, etc.).

Contrary to the opinion of Friede and Magee (1962), who reported that 92 per cent of the plaques they studied had no relation with blood vessels, we believe that most if not all of the plaques are intimately related with capillaries (Fig. 1A, B). The younger the plaque, the more obvious is this relationship, because later on degenerative phenomena profoundly alter the various constituent structures within it.

According to the picture of the senile plaque we have built up in our studies of "physiological" and pathological senility, we believe that scrutiny of serial sections in the electron microscope will show abnormalities of the endothelium in blood vessels crossing a plaque, and even perhaps in those not yet surrounded by an optically visible plaque.

The recognized presence of senile plaques in the white matter and in the pineal body seems to mitigate against the view held in the past that plaques always originate in nerve cells. The occasional presence of nerve cell remains in the centre of plaques in the cortex only means that they have been caught up in the developing plaque.

In different brains and sometimes in different parts of the same brain we have observed senile plaques that react positively for amyloid (Fig. 2A, B), and others that are positive for mucopolysaccharides. We have also observed plaques that have positive reactions for both amyloid and mucopolysaccharides. However, these histochemical differences were not accompanied by morphological differences when silver stains were used.

An interesting and early feature in the senile brain is the diffuse though sometimes focally accentuated hyperplasia of the microglia. One sees large cells, sometimes amoeboid, the normally fine processes being diminished in number, and shorter and thicker (Fig. 1A).

With slight technical modifications one can also obtain silver staining of the capillaries, when hypertrophied adventitial microglia are clearly seen detaching themselves from the vascular wall to merge with the interstitial microglia (Fig. 1B).

Foci of hypertrophic microglia, five to ten in a group, are often the pointer to an incipient senile plaque. In the already constituted plaque the microglia stand out because of their irregular shape, hypertrophy and dense staining with silver. Sometimes they are amoeboid (Fig. 2A) and may at first glance be mistaken for oligodendrocytes.

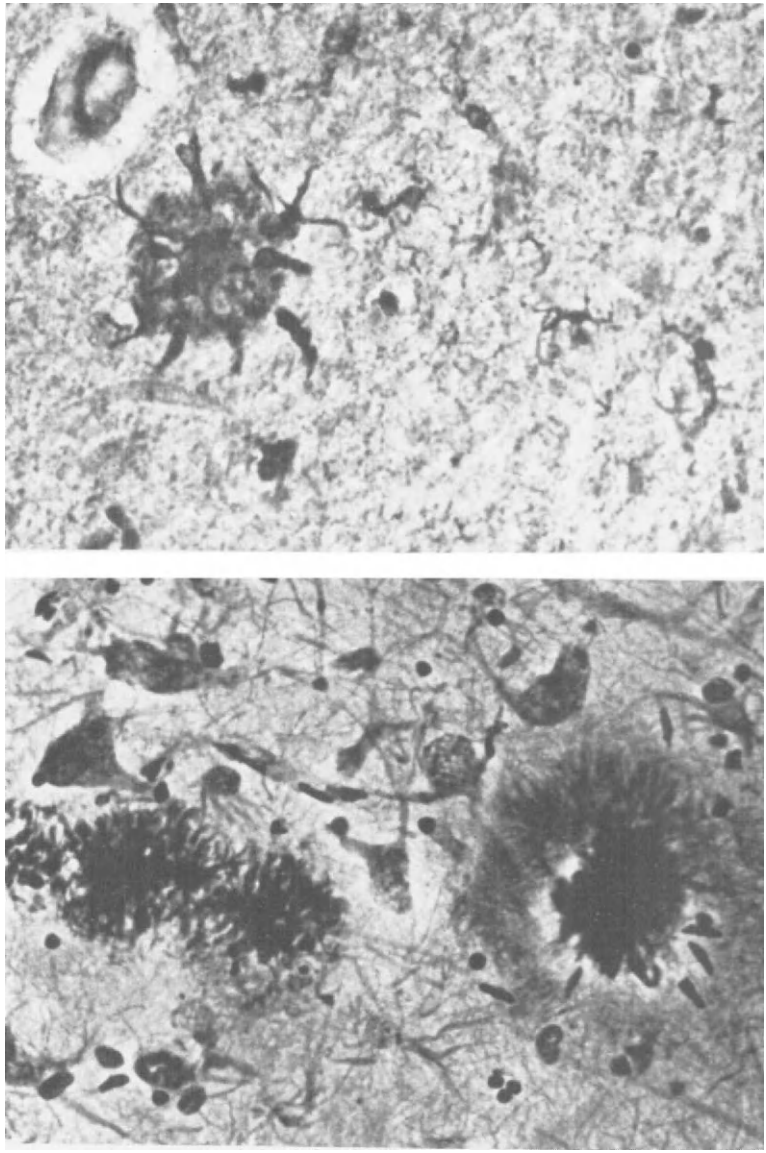

FIG. 2 (Polak). A: Senile plaque with amyloid in the centre, surrounded by microglia. Del Rio Hortega-Polak technique.
B: Fibrillary amyloid in the centre of the plaque. Del Rio Hortega double impregnation.

Del Rio Hortega (1929) observed fibrillary degeneration in the glioepithelial cells but not in oligodendrocytes in senility. This is of importance in our consideration of the possible role of blood vessels in the pathogenesis of the lesions seen in senility. The relation between the astrocytes and glioepithelial cells, and the blood vessels, would explain the penetration in the cytoplasm, through the "sucking feet", of substances capable of provoking gliofibrillary alterations. By this same mechanism, recalling the concept of "angiogliona" elaborated by del Rio Hortega (1939, 1942), one could explain Alzheimer's neurofibrillary degeneration.

Bülow (1956) has shown that the staining of plaques by aniline techniques appeared to be dependent on the pH, and Hiroisi and Lee (1936) found mucin in some cases. Morel and Wildi (1952) showed that the activity of acid phosphatase decreases in the plaques when their argyrophilia increases, and Friede and Magee (1962) demonstrated intense enzymic respiratory activity in the new plaques and marked decline in the old ones, as well as the relative hyperglobulinaemia met with in the aged. From these observations and our own, I venture to suggest the following hypothesis as an explanation for the sequences of changes in the brain in senility.

(1) In both "physiological" and pathological senescence there are, to a greater or lesser degree, alterations in the endothelial lining of the capillaries, with an increase in their permeability. This morphological fact permits the passage of substances, sometimes normal and sometimes not, out of the blood vessel towards the nervous parenchyma (in both the glial compartment and the intercellular one). These substances stand out histologically because of their discrete argyrophilia (Fig. 1A, B).

(2) Such substances provoke an immediate mobilization of the interstitial and advential microglia, followed by alterations of the astrocytes and of the oligodendrocytic membranes, whose fragility is well known. These in turn give rise to alterations of the axons, dendrites and neuronal soma (intense staining with silver, fragmentation, fibrillary degeneration, argyrophilic balls).

(3) Simultaneously microglial mobilization increases and there is a generalized swelling of these cells, both interstitial and perivascular, which migrate towards the proteic deposits which constitute the initial stage of the senile plaque (Fig. 2A).

(4) Finally we get irregularly distributed astrocytic hyperplasia and glioepithelial and astrocytic structural changes which can be regarded as in the nature of reaction to the above.

McMenemey: Have you any explanation for the degeneration of the astrocytes you saw?

Polak: The fibrillary degeneration of the astrocytes and of the ependymal cells is probably related to the modification of the blood vessels. We don't find this type of degeneration in the oligodendroglia because these are not connected with vessels and have no glial fibres. It is a rare finding in astrocytes, but very common in ependymal cells.

Terry: Are non-neoplastic ependymal cells invariably associated with blood vessels?

Polak: Practically all the normal cells are connected with vessels. With ependymal cells, Golgi staining and del Rio Hontega's triple impregnation must be used for the prolongations to be seen.

Hirano: In 1967 we reported that ependymal cells could occasionally be found directly abutting on the blood vessels (Hirano and Zimmerman, 1967). This is, however, a rather infrequent finding.

Terry: I don't doubt that it is. I just wondered if all ependymal cells were connected with blood vessels. In actual fact there are relatively few differences between Dr Polak's observations and those of the electron microscopists. The differences almost exclusively concern the hypotheses based on the observations. Dr Polak doesn't demonstrate endothelium and yet he postulates that the endothelium is at fault. He showed that blood vessels pass by some of these plaques, but he doesn't show that all plaques are associated with the vessels. We have done serial 1 μm sections and repeatedly demonstrated no vessels. So I just can't see a primary place for the blood vessels in this affair. We know that amyloid in general is secreted by cells and, except in certain circumstances, it does not come from the plasma. Something causes cells to secrete components that precipitate in the fibrillar form. The microglia, or whatever pass for microglia in the nervous system, are analogous to the reticuloendothelial cells which make amyloid in other organs. I would therefore suggest that the so-called microglial cell is what makes the amyloid in the plaque and that the amyloid does not come from the blood vessel.

Polak: With a light microscope and a frozen microtome, one may in fact be in a better position to study the relation of vessels to plaques than the electron microscopist. Since the sections are thicker than serial sections one uses more material to find the relation with the vessels. I don't say that the vessels are diseased, but I would ask the electron microscopists to study the endothelium of these vessels, because I believe there is something in the capillaries in this situation.

I don't agree with the term "microglia-like cells", derived from Feigin's

paper (1969) about the mesenchymal tissues of the nervous system in which he said that microglia hardly exist in the normal human brain, and that del Rio Hortega was only able to see these cells in rabbit brain because rabbits have infectious diseases.

First of all I don't believe that all the rabbits (newborn and adults) in the world are ill. Secondly, working with del Rio Hortega's technique in frozen sections, we can stain the microglia in human brains and in different animals in both normal and pathological conditions.

Jacob: The capillary network in the cortex is so dense that one can always construct relations or contacts between senile plaques, amyloid substances and vessels, but one cannot prove anything.

Gonatas: It is not necessary to postulate that amyloid always has to come from some blood-borne factor. Amyloid has been experimentally produced in splenic explants, where no blood vessels are connected with the bloodstream. Direct observations, including labelling of newly formed amyloid by radioautography, show that amyloid can be formed *in vitro* without necessarily any contribution of the blood vessel (Bari, Pettengill and Sorenson, 1969).

Polak: But how many types of amyloid do you have? There are many amyloids.

Gonatas: Morphologically (ultrastructurally) there is only one type of amyloid.

Barondes: Under what conditions was amyloid formation induced?

Gonatas: Splenic amyloidosis was induced in mice by casein. Splenic explants were cultured and new amyloid was detected by electron microscopy and radioautography.

Shelanski: I know of no evidence that there are many chemically different amyloids. The bulk of the evidence today is that there is one amyloid. Miller and colleagues (1968) conclude that amyloid is a filament formed by the polymerization of a single subunit. Ben-Ishay and Zlotnick (1968) point out strongly, at least in the case they studied, a cellular origin rather than a circulatory origin for the amyloid.

Friede: My own interpretation of the order of events in the development of a senile plaque, based on histochemical observations, is quite similar to that given by Dr Terry (Friede and Magee, 1962; Friede, 1965). Plaque formation may begin with the accumulation of high molecular weight polysaccharides in the neuropil, accompanied by an increase in oxidative enzyme activity, which evidently corresponds to the accumulation of mitochondria in the neurites. At this phase, the plaque has a uniform structure throughout. Subsequently, there are

retrogressive changes in its centre, with a decrease in enzyme activity and accumulation of coarse deposits which are positive for periodic acid-Schiff and sometimes also for lipid stains. It is this later phase of plaque formation that is associated with microglial invasion. Dr Polak proposed a different concept which I welcome as it emphasizes the difficulties involved in the reconstruction of an order of events from the observation of various phases. Enzyme histochemical preparations as used in our laboratories are usually 30 μm thick, which facilitates studying the relations between the tissue's components. As Professor Jacob said, capillaries are so numerous in grey matter that they may be included by chance. Actually, there is no cell type in the brain, except the ependyma, that will not on occasion be included in a plaque. I have seen pyramidal cells right in the centre of plaques and capillaries running through plaques. But is that significant? Here again we agree about observations, but we disagree about the interpretation. In my opinion the occasional association of plaques with capillaries is not significant.

Sourander: Dr Shelanski, if only one type of amyloid is present, and it is very inert, how can one explain J. Waldenström's observation (quoted from Bergstrand, 1956) based on repeated liver biopsies, that amyloid may disappear when children with tuberculosis and amyloidosis of the liver recover?

Shelanski: Although it is difficult to dissolve in the test tube, amyloid may be susceptible to digestion by cellular enzymes.

Terry: There are other possible explanations. Liver regenerates activity and it can perfectly well regenerate a nodule free of amyloid.

McMenemey: This possible dissociation of the twisted tubules from the amyloid is important. It would be helpful if we could have a biopsy from what Stern and Reed (1945) called the abiotrophic form of Alzheimer's disease. Theirs was a presenile case with a particularly small brain but no plaques and no tangles. Then in the older age group there are the cases Dr Jacob has already referred to: very demented people with atrophied brains, rather fatty cells and so on, but no plaques and no tangles. I never regarded this case of Stern and Reed's as one of Alzheimer's disease but I suppose one could call it a presenile equivalent of the so-called dementia senilis simplex. Then there was the case of Grünthal and Wenger (1940) where one of two siblings with clinical Alzheimer's disease had no plaques. It would be interesting to see if there was a change in the neurites or tubules in those cases and yet no amyloid.

Mutrux (1953) made the point that plaques are to be expected in the inner layer of the cortex in Alzheimer's disease, but in the outer layer in

senile dementia. I believe that this is in general true and I wonder what is a likely explanation.

Many years ago Neubürger and Rösch (1935) described what have since been called Neubürger bodies in the brains of middle-aged people who died of carcinoma. I don't know of any recent work on this topic but when I last talked to Dr Neubürger he said that he thought they were probably ordinary senile plaques. I think a further study would be helpful.

Another useful line would be studies on boxers' brains. Ferguson and Mawdsley (1965) reported one recently in which tangles were found, but no plaques at all. The changes were rather extensive, and the midbrain was heavily affected. There was a little about this in Roberts's recent monograph on boxers' encephalopathy (1969). I believe this is not the only case in which there were tangles without plaques.

Strich: That is right. Some had plaques and tangles (Brandenburg and Hallervorden, 1954; Corsellis and Brierley, 1959), some had only tangles (Grahmann and Ule, 1957), and one had congophilic angiopathy as well (Brandenburg and Hallervorden, 1954). But there are very few post-mortem reports.

Corsellis: I believe the five Cardiff cases (Payne, 1968) showed remarkably little in the way of plaques or tangles. I have twice encountered a history of a boxing career in demented patients, and in both the pathological diagnosis was Alzheimer's disease. The ages at death were 56 and 63, but it is impossible to say how important the boxing was.

McMenemey: When it comes to quantitative studies on the distribution of plaques and tangles, it would be useful to have a standardized counting procedure laid down so that we could all be on exactly the same wave length. Probably we all began our studies on the lines of Grünthal (1926) who scored with pluses and compared several parts of the brain.

Roth: A generally accepted counting technique would be of great value. It would be equally desirable to develop standardized techniques for clinical examination and measurement of other pathological changes such as tangles, softening and atheroma in vessels.

Dayan: Von Braunmühl (1957) was unable to confirm the findings of Neubürger and Rösch (1935), and in a series of 20 brains of patients dying with carcinomas I too was unable to find such lesions.

Friede: I agree with you, Dr Dayan. We screen every case of malignancy because we are interested in carcinomatous neuropathy and cerebellar degeneration.

Corsellis: Indirectly I agree with this too. One rarely sees plaques

under the age of 60, in the cerebral neocortex of schizophrenic patients, about one-third of whom die from carcinoma.

Terry: In the case of Portuguese amyloidosis found on Crete the families were traced back three or four centuries. Amyloid deposits were seen in the molecular layer as well as in the leptomeninges and there was no indication of any plaque formation; that is, there were deposits of amyloid surrounded by normal neuropil, so amyloid by itself does not induce a plaque (Pavlou and Terry, 1969).

Barondes: Is this primarily a large neuron problem or a small neuron problem? In the hippocampus, for example, are the big cells mostly affected, or the small cells?

Terry: It is not the big motor cells, but neurons of moderate size. The pyramidal layer of the hippocampus is made up of medium-size neurons, not giant cells.

Barondes: So there is no clear correlation, and all kinds of cells are affected.

Tomlinson: In severely affected cases of Alzheimer's disease, certainly both plaque formation and tangles are spread throughout all cortical layers.

Sourander: Are there any studies of the chromosomes in Alzheimer's disease? Recently I have seen an example of Down's syndrome who reached the age of 51 and showed cerebral changes indistinguishable from a typical case of Alzheimer's disease. Similar cases have been reported in the literature (cf. Crome and Stern, 1967).

Roth: "Unto him that hath shall be given and from him that hath not shall be taken away even that which he hath." This appears to apply to many phenomena: the acquisition and the loss of wealth, the development and decline of university departments, the growth and deterioration of intelligence and the fate of one's endowment of neurons. The development of dementia in mental defectives is very interesting. If mongols not only dement early but accumulate senile plaques and tangles to a significantly greater extent than normal subjects of comparable age, this would accord well with the view already expressed here that dementia appears when the reserve capacity of the brain (which one would expect to be smaller in mongols) has been encroached upon beyond a certain threshold point. The Danish observations (Nielsen, 1968) on aneuploidy in senile dementia have not yet been independently confirmed. Nielsen's hypothesis that increased loss of chromosomes with age, mainly of X chromosomes in women, was part of the aetiology of senile dementia is interesting and open to simple experimental tests.

Jacob: Another fact of interest is that we never find senile plaques or congophilic angiopathy in the grey matter of the spinal cord.

Polak: There are plaques in the pineal gland.

Roth: There must be different kinds of neurons because the spinal reflexes are immutable whereas the neuronal processes at the cortical level are mutable.

Terry: Some neurons are definitely more susceptible to this sort of lesion. Even though the experimental tangle is different from the human tangle, it is clear that tangles are not induced in any cells just because the drug is injected into that area. When aluminium is injected into the cortex of the convexity tangles are very rare there; rather they are very common in the anterior horn cells of the cord or in the basis pontis in rabbits. Those are the susceptible neurons and I suggest that the same thing is true of the human tangle—some neurons are susceptible and their dendrites will undergo the degeneration which leads to plaque formation.

Roth: If this were the case one would expect a considerable measure of consistency in the site of accumulation of plaques and tangles.

Terry: Not necessarily. Some neurons are susceptible to tangles and if their processes are involved they will induce plaque formation. On the other hand some neurites might perhaps be involved primarily, without the participation of the cell body, and those neurites would themselves cause plaque formation. The cell body may even be able to recover from a tangle. One can manipulate the hypothesis to satisfy all these disparate data.

Tomlinson: In human cases there is a good deal of evidence that there are highly susceptible areas. In almost every case the amygdaloid nucleus is perhaps more heavily involved than elsewhere, and the hippocampal gyrus and the neighbouring temporal gyri are heavily implicated in many cases. But in senile dementia the caudate cortex is almost devoid of plaques and tangles, although in the tissues of the third ventricle and the hypothalamus they tend to occur again. They never occur in the neurons in the spinal cord and very rarely in the cerebellum.

Dayan: Tangles have been described in many other diseases in which plaques have not been found. It would be of interest to know whether these tangles are identical with those found in Alzheimer's disease as the discordance between the two groups of conditions is striking.

Roth: Are twisted tubules found in all these other conditions?

Terry: They are found in most conditions that are listed, including post-encephalitic Parkinsonism and Guam-Parkinson dementia. The Pick body has short segments of twisted tubule. In several other diseases where

tangles have been reported, such as sporadic motor neuron disease or infantile neuronal dystrophy, they are filamentous aggregates.

Dayan: What about the tangles in subacute sclerosing panencephalitis, where we have some clues about the aetiology?

Terry: I haven't looked at them.

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ALZHEIMER'S DISEASE AND RELATED CONDITIONS
G. E. W. WOLSTENHOLME & MAEVE O'CONNOR

CHAIRMAN'S CLOSING REMARKS

PROFESSOR M. ROTH

I have the sad and unenviable task of winding up a closely packed three-day programme of scientific communication, discussion and dialectic which has rarely failed to provide something illuminating or unexpected for some of us. For the organization of the symposium and the gracious social events and warm hospitality that have helped to make the exertions so pleasant and memorable we owe a debt of gratitude to the Ciba Foundation.

I should also like to express our warm appreciation for the dedicated work of preparation undertaken over a long period by Dr McMenemey, whose brain child this symposium was.

As Dr Pratt made clear in his paper, the genetic situation in Alzheimer's disease appears complex. It is clearly unprofitable to think overall in terms of classical Mendelian phenomena, that is, in terms of major autosomal or sex-linked genes. In so far as we deal with heredity, polygenetic hypotheses alone seem viable and these have the disadvantage of being difficult to demolish by means of a critical attack, being reconcilable with so many types of data. But it appears possible that the disorder is heterogeneous. These are isolated cases in which the genetic factor appears particularly clear-cut. Dr Jacob's paper and the discussion that followed it and Dr McMenemey's earlier work suggest that some heredofamilial cases may have distinctive clinical features and they appear also to be of relatively early onset. Major genes may operate in these sub-groups and biochemical investigations focused on them might, therefore, prove rewarding.

There has been a general consensus here that plaques and tangles are pathological phenomena. How then is their occurrence in normal elderly subjects to be explained? One possible answer which arises from Dr Tomlinson's report of the Newcastle studies is that Alzheimer's disease and its senile variants arise from quantitative intensification of changes that in early stages and limited extent do not cause manifest clinical deficits. These seemingly develop only when the changes seen in normal elderly, and less often in middle-aged people, extend and aggregate beyond a

certain threshold point. However, such views rest on light microscopic and statistical evidence. It has become clear in our discussions that the quantitative hypothesis must be submitted to the critical test of ultramicroscopic investigations. Do the plaques and tangles observed in normals exhibit ultramicroscopic changes really indistinguishable from those demonstrated in indubitable cases of Alzheimer's disease? Neurosurgery in well-preserved elderly subjects should provide acceptable opportunities for enquiries aimed at answering such questions.

The wide-ranging paper by Dr Sourander and Dr Corsellis's careful neuropathological investigations have brought out the importance of a relatively new subject: the contribution of lesions in the limbic system to certain aspects of symptomatology in Alzheimer's disease. In relation to the amnestic symptoms which predominate in the first stage of the disorder, we have been presented with two separate lines of evidence which do not at present converge. The presence of limbic lesions in amnestic syndromes has been known for more than half a century and the presence of lesions in the hippocampus and entorhinal cortex in Alzheimer's disease provides a plausible explanation for the memory defects.

The other line of evidence that comes from the work of Dr Gonatas and his associates suggests that there is a consistent enlargement of the presynaptic terminals in Alzheimer's disease and that this may play a role in the morphogenesis of the senile plaque. This is certainly the kind of finding one would hope for if one were seeking some generalized unitary change as the most likely explanation for the failure of subjects with this disorder to learn and form enduring memories. For the present, there appears to be no link between these two kinds of tentative morphological explanation. The plausibility of an explanation in terms of a localized lesion is certainly increased by the presence in many cases of Alzheimer's disease in its later stages, as Dr Sourander and Dr Corsellis have indicated, of the full Klüver-Bucy syndrome.

Both these lines of evidence provide a stimulus for further enquiries, as does the material reported by Dr Tariska which suggests that investigation of the role of extracerebral physical disease and metabolic disturbances in Alzheimer's disease has perhaps been neglected in recent years.

The papers by Dr Strich and Dr van Bogaert and the discussions that followed have clarified a number of issues, including the relationship between congophilia and amyloidosis. There appears to be a wide consensus that congophilic change is not a reliable indicator for the presence of amyloid. Dr Strich's paper also makes clear that the possible role of trauma and other precipitating factors in Alzheimer's disease deserves

further exploration, despite the fact that the subject bristles with difficulties. If the interesting speculations about the possible reversibility of the polymerization which may underlie some types of neurofibrillary change are to be brought down to earth and to find application in treatment, these cases would appear to merit special attention. If experimental evidence can be strengthened and a sufficiently good case made to render therapeutic experiment ethically justifiable, it is these cases with a step-like onset and rapid progression that suggest themselves for enquiry.

The observations that have come from the ultrastructural studies of Drs Terry, Kidd, Gonatas, Shelanski and Barondes are impressive for their depth and variety, and for the comprehensiveness and cohesion of the explanations they have provided for many of the phenomena of Alzheimer's disease as observed with traditional light microscopic techniques. Dr Terry's group has advanced evidence suggesting that the basic morphological abnormality underlying the tangle consists of bundles of twisted microtubules measuring about 22 nm in diameter and narrowing at intervals of 80 nm to about 10 nm. The succession of changes culminating in the formation of the senile plaque is possibly secondary to proximal degeneration related to the tangle; enlargement of the neurites is followed by gradual accumulation of amyloid rendering the plaque congophilic. Dense bodies, possibly lysosomal in origin, appear and are initially seen with distended axons and dendrites and degenerating mitochondria within the plaques. Ultimately plaques develop which are just masses of amyloid without neuronal elements. The argyrophilia in the plaques is to be related to the twisted tubules and lipofuscin, and to the amyloid and dense bodies. Even certain biochemical activities have been tentatively established in association with certain structural changes. Thus the dense bodies are held to account for the acid phosphatase and the mitochondria for the oxidative activities in the plaques. Such differentiation of the pathological changes could not have been conceived even seven or eight years ago and it raises the scientific study of Alzheimer's disease to a new level.

We have also learnt that neurofibrillary change is not a uniform morphological change. The work of Dr Wiśniewski, Dr Terry and Dr Shelanski has made it clear that the filamentous type of neurofibrillary change is non-amyloid and non-specific and that it can be produced by a variety of agents. At the same time, this form of change provides experimental opportunities that can be created at will. Microtubular protein, which has drawn so much attention here, is present in abundance and readily precipitated by vinblastine, as Dr Barondes has shown.

Dr Friede's observations reveal an orderly and strict relationship between the size of the axon, on one hand, and the size of the nerve cell body, its protein metabolism, the thickness of the sheath and the number of neurofilaments and microtubules, among other associations. Such basic observations make clear some of the mechanisms controlling the calibre of axons. They may, in addition to their intrinsic significance, help with the interpretation of the many complex and baffling changes encountered in pathological states. It is rare in biological observations to see the points describing the correlations between variables nestling so closely to the regression line.

The aetiological basis of the Parkinsonism-dementia syndrome which is endemic in Guam remains, for the present, obscure. Dr Hirano's investigations have made it clear that, although senile plaques are uncommon in this condition, tangles are widely distributed and their morphology is closely similar to those observed in cortical biopsy specimens from patients with Alzheimer's disease. The concentration of tangles both in the cerebral cortex and in the brain stem, particularly the substantia nigra, accords well with the two most conspicuous clinical features of the disorder and confirms that where tangles are found in abundance they are likely to be closely related to the pathogenesis of the disorders concerned rather than some remotely related epiphenomenon. The relative absence of plaques in this phenomenon clearly calls for close clinical and pathological comparisons between it and Alzheimer's disease.

This symposium has brought together clinical investigators, geneticists and scientists investigating the morphology of Alzheimer's disease with classical light microscopic techniques and also with ultrastructural and experimental techniques—an exchange of observations and viewpoints that has, I believe, been fruitful in many ways. Although the ultrastructural observations have called for revision of some of the views formulated on the basis of traditional clinico-pathological observations, it is the agreements rather than the discrepancies that are so striking. It is clear that hypotheses about the aetiological basis of Alzheimer's disease and related disorders formulated in terms of detailed neuronal structure will in future come from ultrastructural investigation. However, many of the hypotheses that emerge in this manner, as well as others that come from clinical and epidemiological observation, will need to be taken up with the aid of light microscopic studies to provide relevant overall pictures of the location, distribution and diversification of the changes within the brain. Quantitative investigations undertaken with the aid of light microscopy should complement the more differentiated and qualitative ones

provided by the electron microscope. However, to exploit to the full the traditional methods of observation, whether clinical or pathological, the methodology employed in the past will no longer always suffice; techniques of proven reliability and careful statistical analysis will be essential.

As Dr McMenemey made clear in his interesting introduction, Alzheimer was a clinical psychiatrist as well as a neuropathologist and his first paper in 1906 took the form of descriptions drawn from both types of observation. Here something that Freud said about psychopathology in general appears apposite: "I have no inclination to keep the domain of the psychologist floating as it were in the air without any organic foundation. Let the biologists go as far as they can and let us go as far as we can; some day the two will meet." Our discussions have shown in an unmistakable manner that in this important group of organic disorders there are many meeting points and some of them are beginning to show promise as growing points.

I should like to thank you all for your stimulating contributions and the effort and cooperation you have given to make our symposium a success.

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*Author and Subject Indexes compiled by William Hill

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Printed by Spootiswoode, Ballantyne & Co. Ltd., London and Colchester