

Do environmental neurotoxins or novel infectious agents play a role in the genesis of Alzheimer's disease?

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A brief historically oriented review is given of selected lines of evidence pointing to a primary genetic role of both environmental neurotoxins and novel infectious agents in the pathogenesis of different forms of dementia. Although there is at present no proof that such agents cause Alzheimer's disease, such hypotheses remain viable as a basis for additional research. Moreover, further consideration should be given to a potentially important pathogenetic role for age-related pathophysiological changes of the cerebral vasculature.

Introduction

As the title indicates, we shall draw upon our limited experience of changes in monkey brains induced by the glutamate-like excitatory toxicity of the seed of *Cycas circinalis*, which contains the neurotoxin β -methyl-amino-alanine (β MAA), as well as the glycoside cycasin, a hepatotoxin and oncogenic substance¹. As the title further indicates, we shall also draw upon our limited experience of changes in the human brain due to the globally distributed infectious agent which produces spongiform encephalopathy or Creutzfeldt-Jakob disease (CJD). Of special interest are the unusual cases with long duration and formation of Kuru-like plaques, in addition to the more constant presence of membrane profiles often called 'prions'². A slow virus infection, subacute sclerosing panencephalitis (SSPE), will also be considered briefly. The brains of such subjects may rarely show filamentous aggregates or even discrete helical-type filaments in degenerating nerve cell processes, in addition to the classical intranuclear myxovirus inclusions (Dastur and Manghani, unpublished data). Paired-helical filaments have been seen in both CJD³ and in Guam parkinsonism-dementia complex (PDC)⁴.

We shall try to demonstrate the histological or fine structural similarities in the brains of these two diverse groups of disorders, and the neuropathological changes reported in the literature in Alzheimer's disease (AD) and senile dementia. Two papers in the present issue^{5,6} have described the histopathologic and fine structural changes in AD and in older subjects without dementia.

They consist of depletion of neurons and nerve fibres, the development of neurofibrillary tangles (NFT) in some of the remaining pyramidal neurons, the formation of neuritic plaques (NP) in the cortex, and some degree of astrocytic reaction⁷.

Finally, we shall consider the morphological findings in the context of the considerable body of data on cerebral circulation and metabolism in normal and pathological ageing in man⁸.

Observations

The findings, mainly morphological, based on light and electron microscopy, will be considered under the headings of the several different topics mentioned in the Introduction. Much of the text will be in the form of detailed legends to figures.

The brain in aged men

A few sections of brains from patients over 60 years in age, autopsied at the J. J. Hospitals, Bombay, made available by S. A. Barodawala were examined at our Department.

Figure 1a (O-718), Silver-impregnated section [by Gros-Bielschowsky (G-B) method, $\times 630$]; 70-year-old patient with mild dementia; frontal cortex showing depleted neurons, the remaining neurons appearing argyrophilic and 'flame'-shaped. All of them probably are late-stage 'neurofibrillary tangles' (NFT). In the neuron at the top left, argyrophilic threads representing clustered neurofilaments can be seen. This brain had also shown some degree of amyloid angiopathy.

Figure 1b (Q-402), (G-B stained section, $\times 800$); 60-year-old 'control' subject who had pulmonary tuberculosis, showing two very large pyramidal neurons, probably in the early stage of NFT. Because the section has gone through the centre of this nerve cell, its apical dendrite appears thicker than in the neuron on the right, and it also shows a normal-looking nucleolated nucleus.

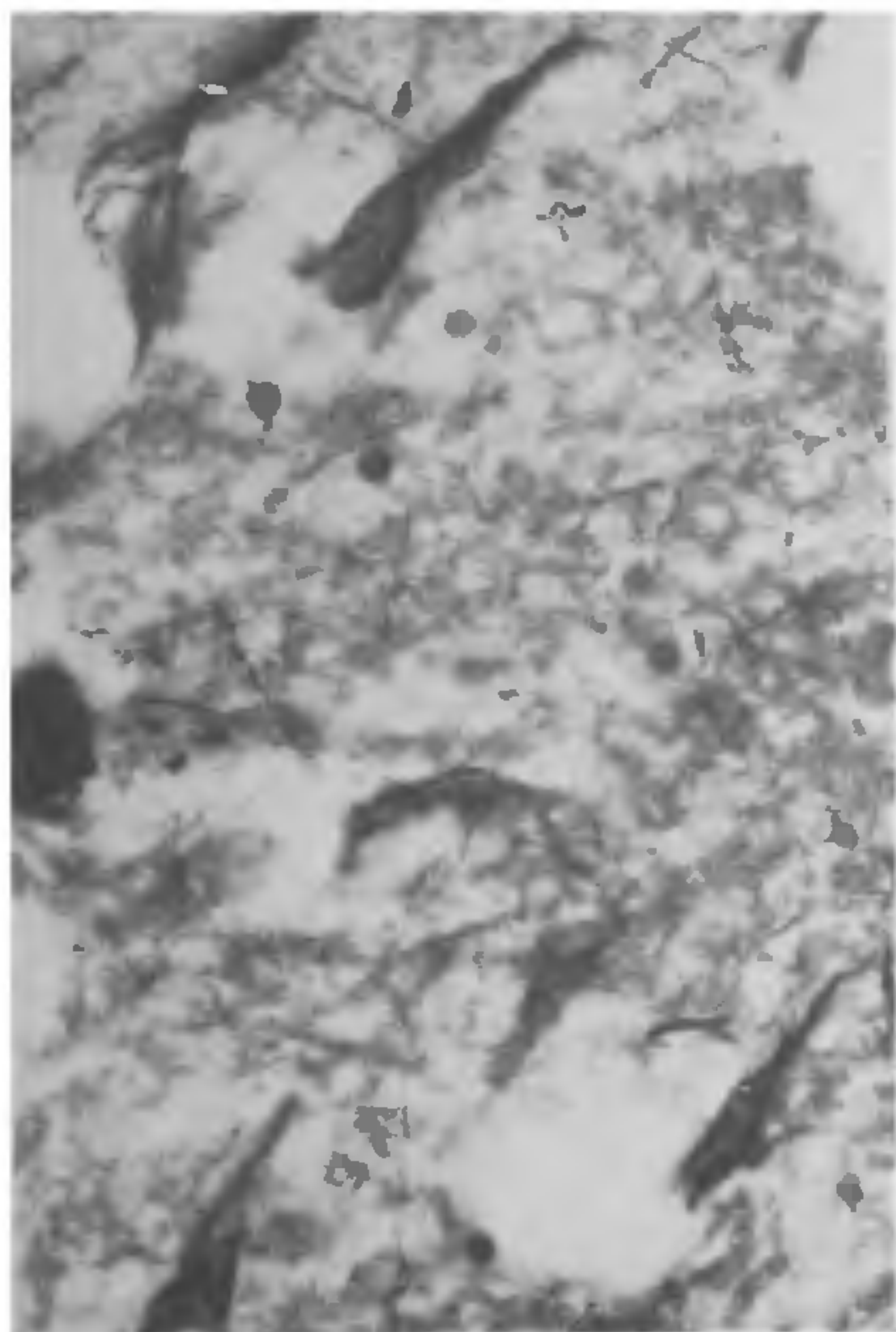


Figure 1a.

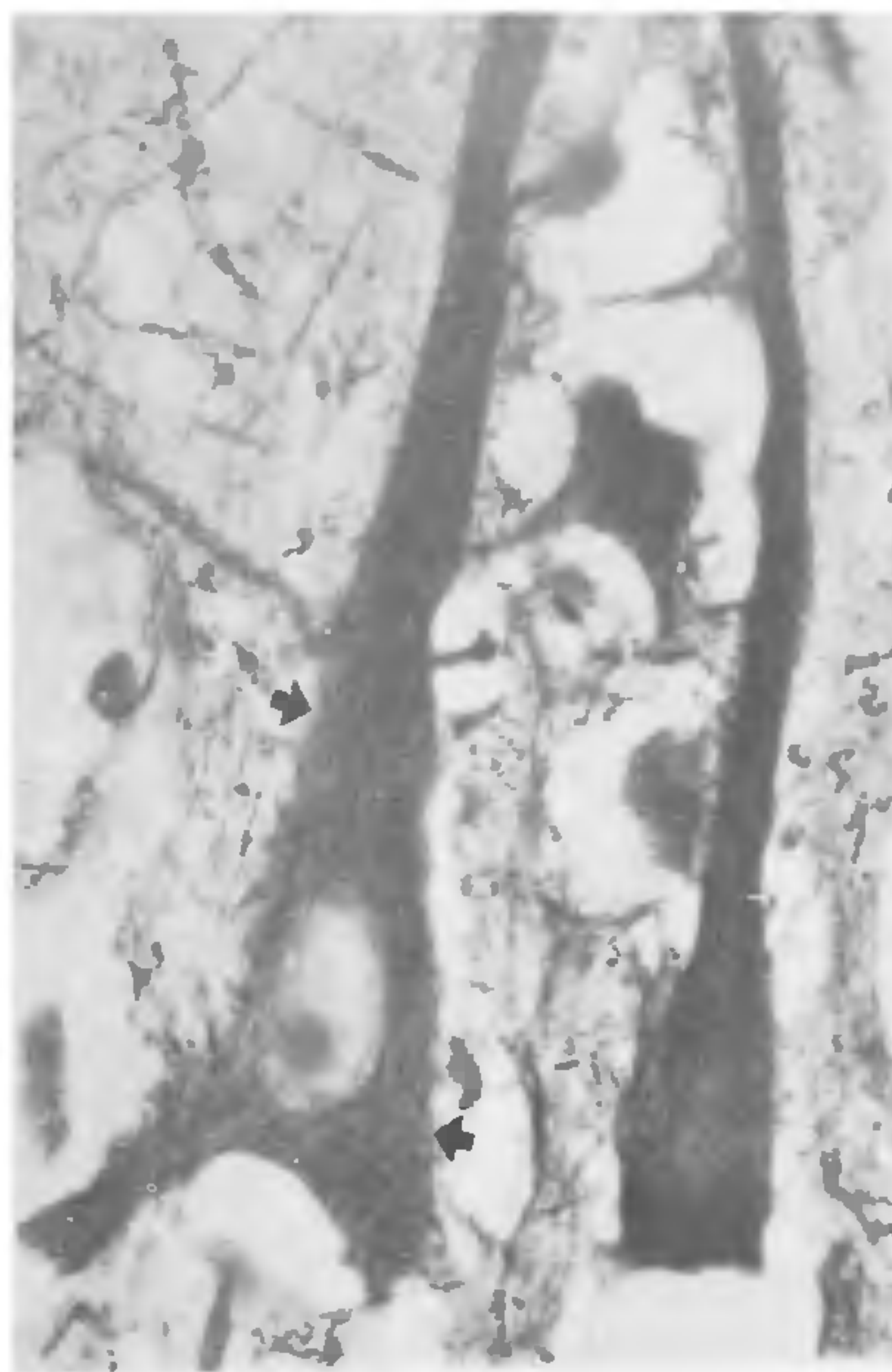


Figure 1b.

Figure 2. (Q-419), (G-B stained section, $\times 630$); 65-year-old patient with mild dementia; hippocampal cortex showing severe neuronal disorganization and degeneration. The picture shows vacuolated cytoplasm of the neuron on the left; and irregular argyrophilic material in a roughly circular area around a totally degenerated neuron, probably representing a neuritic plaque (NP).

Figure 3. (Q-167), (G-B stained section, $\times 630$); 65-year-old patient with mild dementia; cerebellar cortex showing moderate loss and degeneration of Purkinje cells. The picture shows a densely argyrophilic neuron (degenerating?) on the left. The central neuron is out of the plane of section, possibly degenerated and represented by thickened argyrophilic fibres (of surrounding basket cell?) and irregular argyrophilic material, possibly representing the early stage of an NP (between arrows).

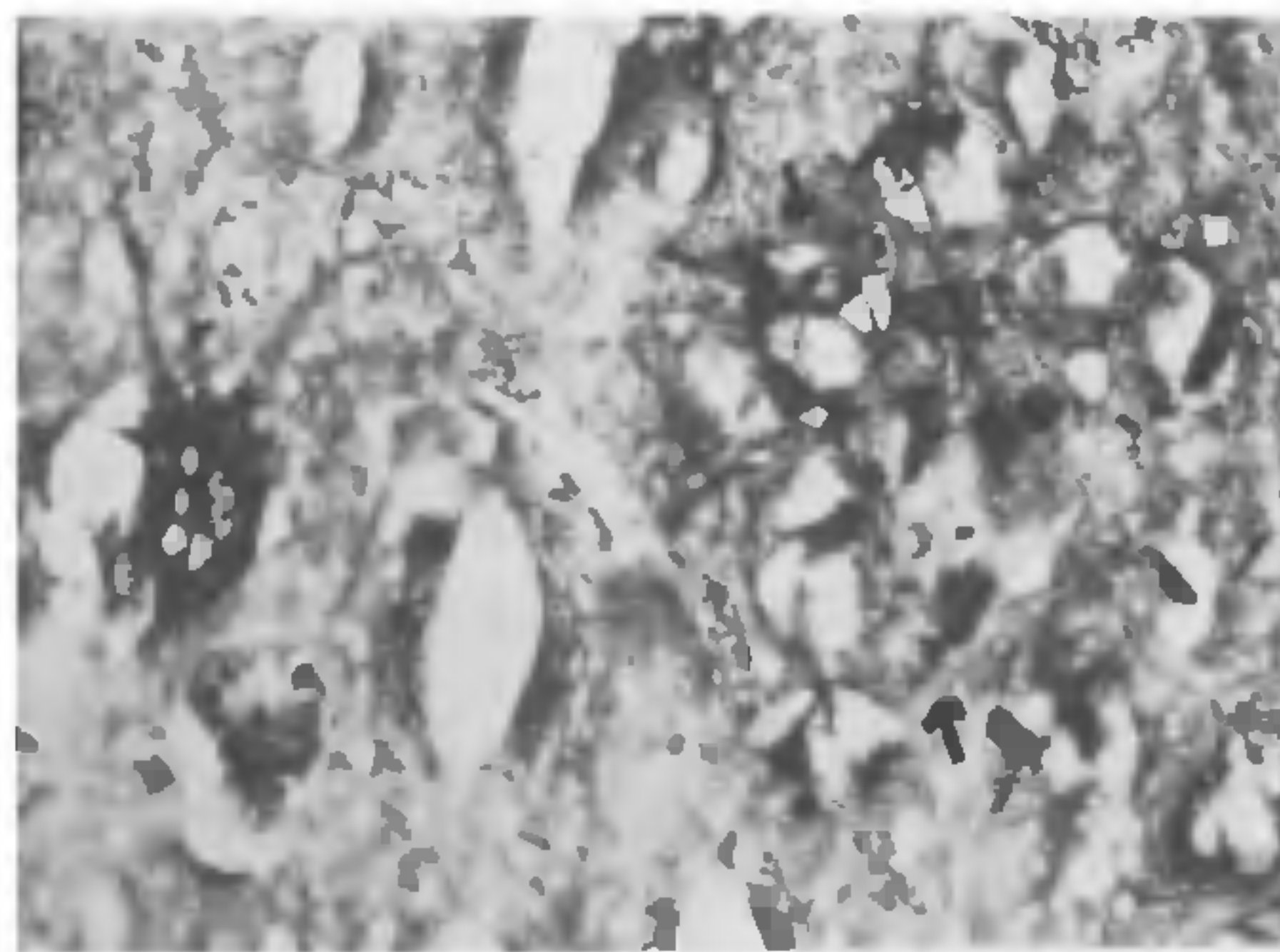


Figure 2.

*Bruins of Rhesus monkeys fed different preparations of fruits of *Cycas circinalis**

The false sago palm (*C. circinalis*) grows in many parts of the world, including India. The fruits (also called

'nuts') are eaten by the natives of some of the islands in the Eastern Pacific, notably Guam. Ingestion has been associated with a high incidence of amyotrophic lateral sclerosis (ALS) in these populations⁹ during the 1940s to 1960s. At later dates (during the seventies through eighties) associations were suggested for forms of

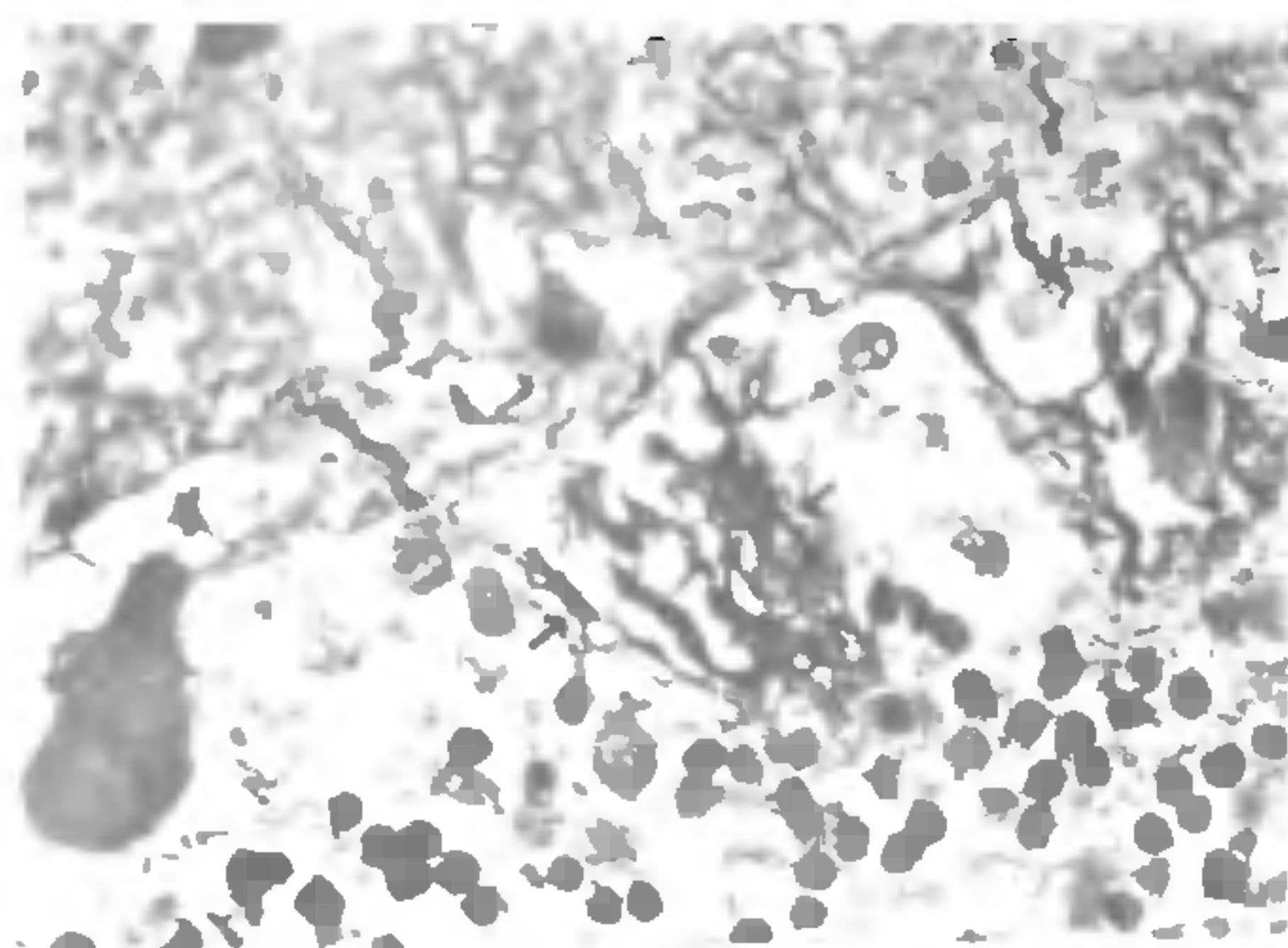


Figure 3

dementia with or without Parkinsonism^{10,11}. Cycad nuts are consumed in different ways: (a) uncooked, or (b) boiled, or (c) repeatedly soaked and washed over many days. Guamanians often follow the third procedure before preparing a thick gruel or tortillas.

By the sixties, a putative toxin isolated from the cycad nuts ('cycasin') was identified as methyl-azoxymethanol (MAM) glucoside. It was found to be a potent hepatotoxin, producing severe liver degeneration and occasionally even malignancy. It was further found that the 'aglycone' MAM was an extremely potent carcinogen producing a variety of tumours in different body tissues in various experimental animals. At our laboratories of the Neuropathology Unit, then at the J. J. Hospitals, we also confirmed the hepatotoxicity in monkeys and the carcinogenicity in newborn rats^{12,13}. Moreover, feeding Rhesus monkeys separately with one of the three different preparations of cycad nuts mentioned above, we were able to produce neuropathological change in animals 1–2 years old, particularly loss of motor neurons in the spinal cord and brain and thinning of the cortex (Figure 4; C-389, $\times 15$, PTAH),



Figure 4.

using the washed cycad preparation, which was almost devoid of cycasin^{14,15}. This suggested the presence of a different toxic agent (a neurotoxin) in the repeatedly washed cycad nut. This was later shown to be β -methylamino-alanine (β MAA)¹⁶.

One older monkey (C-857) fed this cycad nut preparation for two years (from age 9 to age 11 years) showed severe generalized weakness and hepatotoxicity (jaundice) and was sacrificed. The brain showed moderate diffuse loss of pyramidal neurons and nerve fibres, and a number of early stage argyrophilic plaques, without any congophilia. Rarely, the cerebral cortex and white matter showed a more developed central NP-like structure, accompanied or ringed by small less argyrophilic, possibly 'dystrophic' plaques (Figure 5a; G-B staining, $\times 200$). The cores of larger plaques were positive for the 160-kD neurofilament protein (Figure 5b; PAP-oxidase method, $\times 500$).

The neurotoxin β MAA is structurally similar to the neurotoxin β -oxalyl-amino-alanine (β OAA)¹¹, the compound probably responsible for the motor neurone and

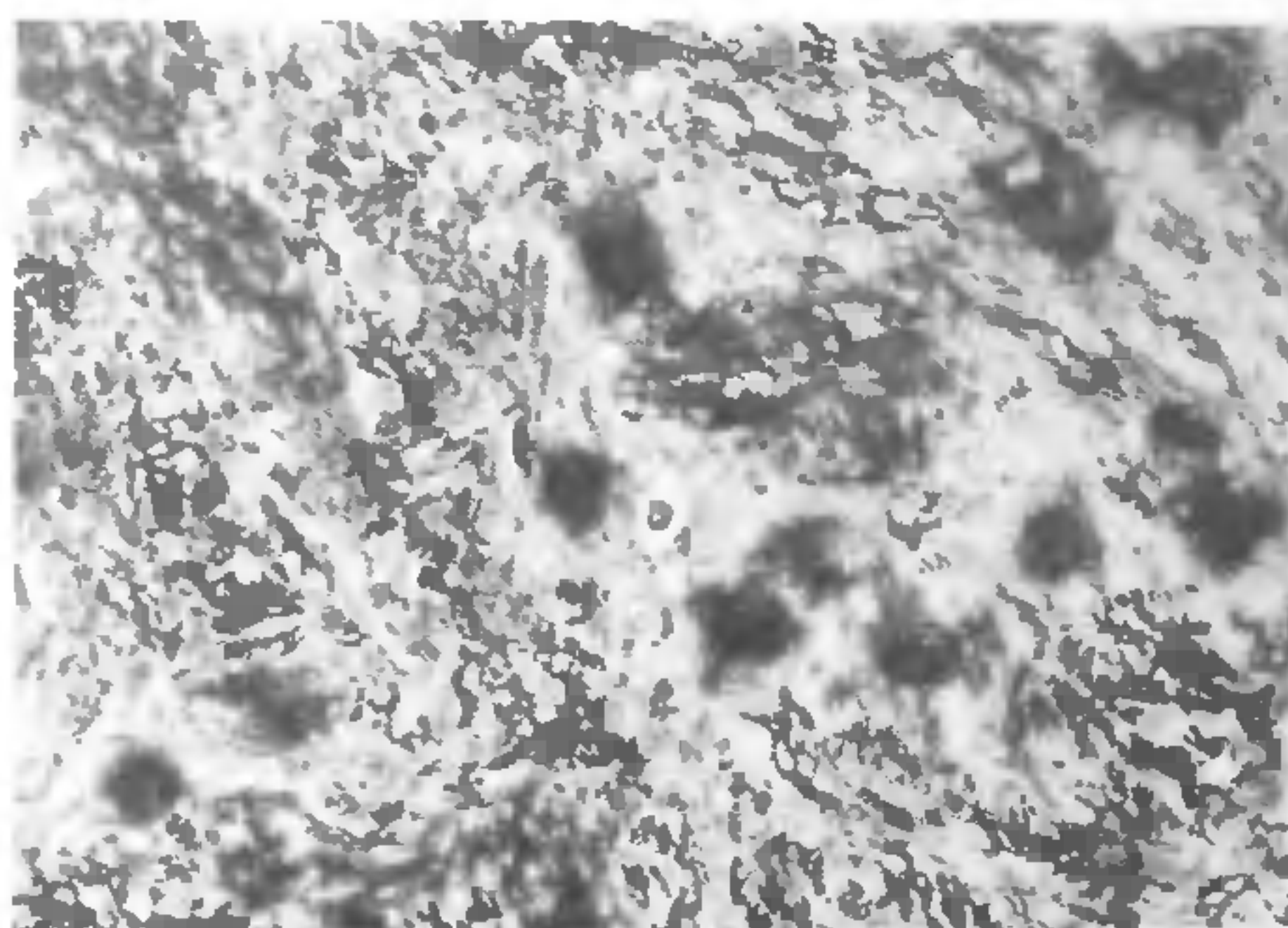


Figure 5 a.

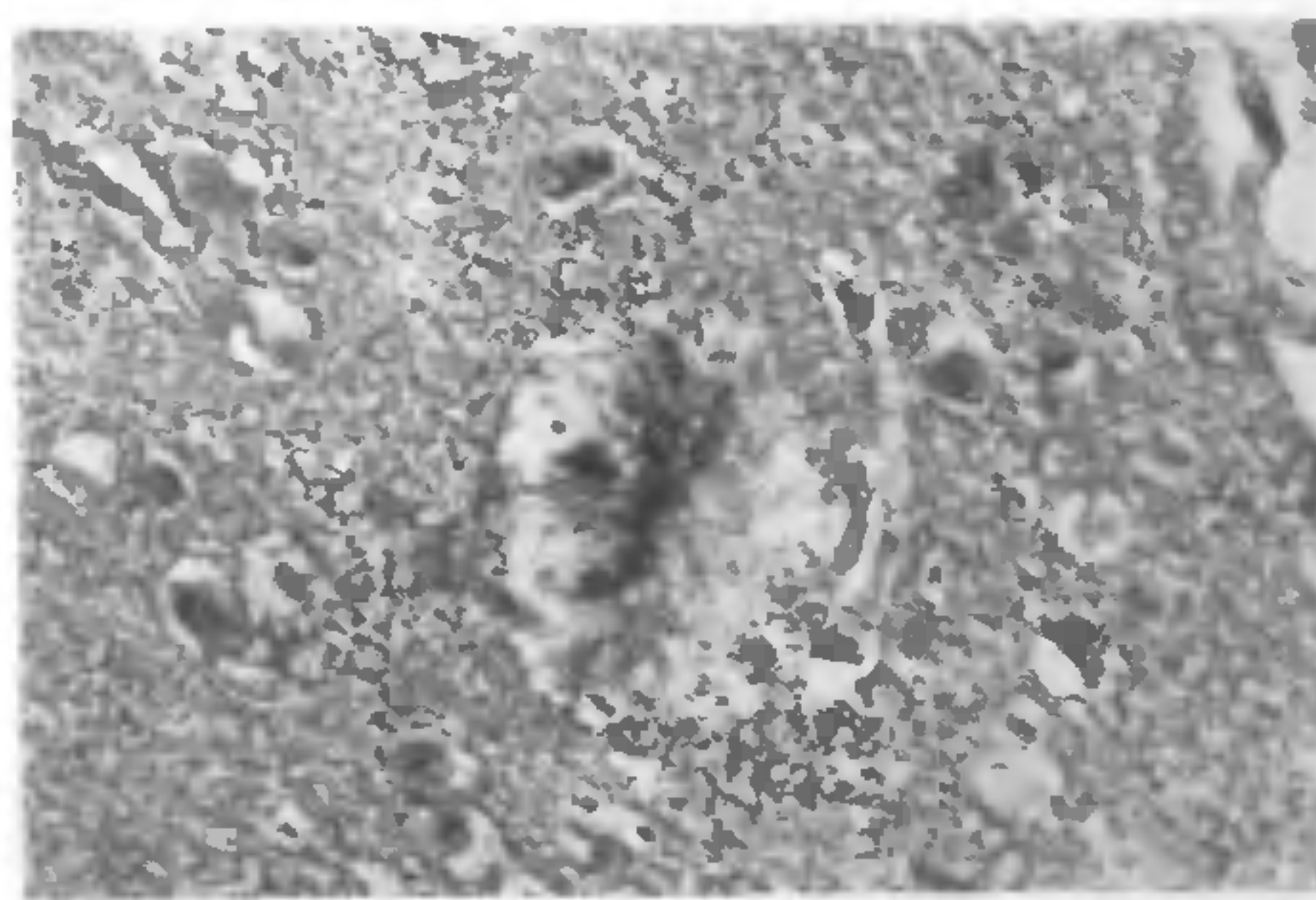


Figure 5 b.

motor fibre tract damage in people who chronically consume preparations of the chick pea, *Lathyrus sativus*, typically in times of drought as in India and some of the Mediterranean countries¹⁷⁻¹⁹.

Both cycasin and β MAA in the nuts of *C. circinalis* may produce other types of pathology. For instance, changes produced by the nitrile group of toxins might develop, such as those known to occur after administration of β -amino-propio-nitrile (β APN), or of $\beta\beta$ -imino-dipropionitrile ($\beta\beta$ -IDPN). The latter is known to produce swelling of axons and nerve cells by neurofilament accumulation²⁰. It was surprising and interesting to note the swelling of anterior horn cells, anterior nerve root fibres and rarely other motor nerve cells in one animal (C-772, about a year old), fed *boiled* cycad nut, for just two months. Figure 6a. 1 shows such swollen neurons in one anterior horn (H&E, $\times 145$). Despite the artefact of shrinkage due to formalin fixation, the neurons are large for this low magnification. Figure 6b. 1 (H&E, $\times 630$) shows swollen axons ringed by a thin

myelin sheath in the anterior root emerging from the same anterior horn. These anterior horn neurons and especially the swollen axons of the anterior nerve root fibres, showed clear positivity for 160 kD neurofilament protein on immunohistological staining with the PAP-oxidase method (Figures 6a. 2 and 6b. 2 colour, $\times 800$ approximately, each). The neurons of the cranial nerve nuclei of this animal were generally not affected. The one exception was a solitary argyrophilic nerve cell of the oculomotor nucleus in a cross-section of the mid-brain (Figure 6c, arrow: G-B, $\times 180$). At least a dozen other neurons of this nucleus are small and normal-looking (Figure 6c, small arrows). This monkey actually died of acute cycasin toxicity manifested by terminal severe jaundice and coma, the liver showing extensive degeneration and precancerous changes.

Structure of brain in spongiform encephalopathy and in CJD of long duration

Creutzfeldt-Jakob disease (CJD) is generally rapidly progressive, death occurring within 2 to 8 months after

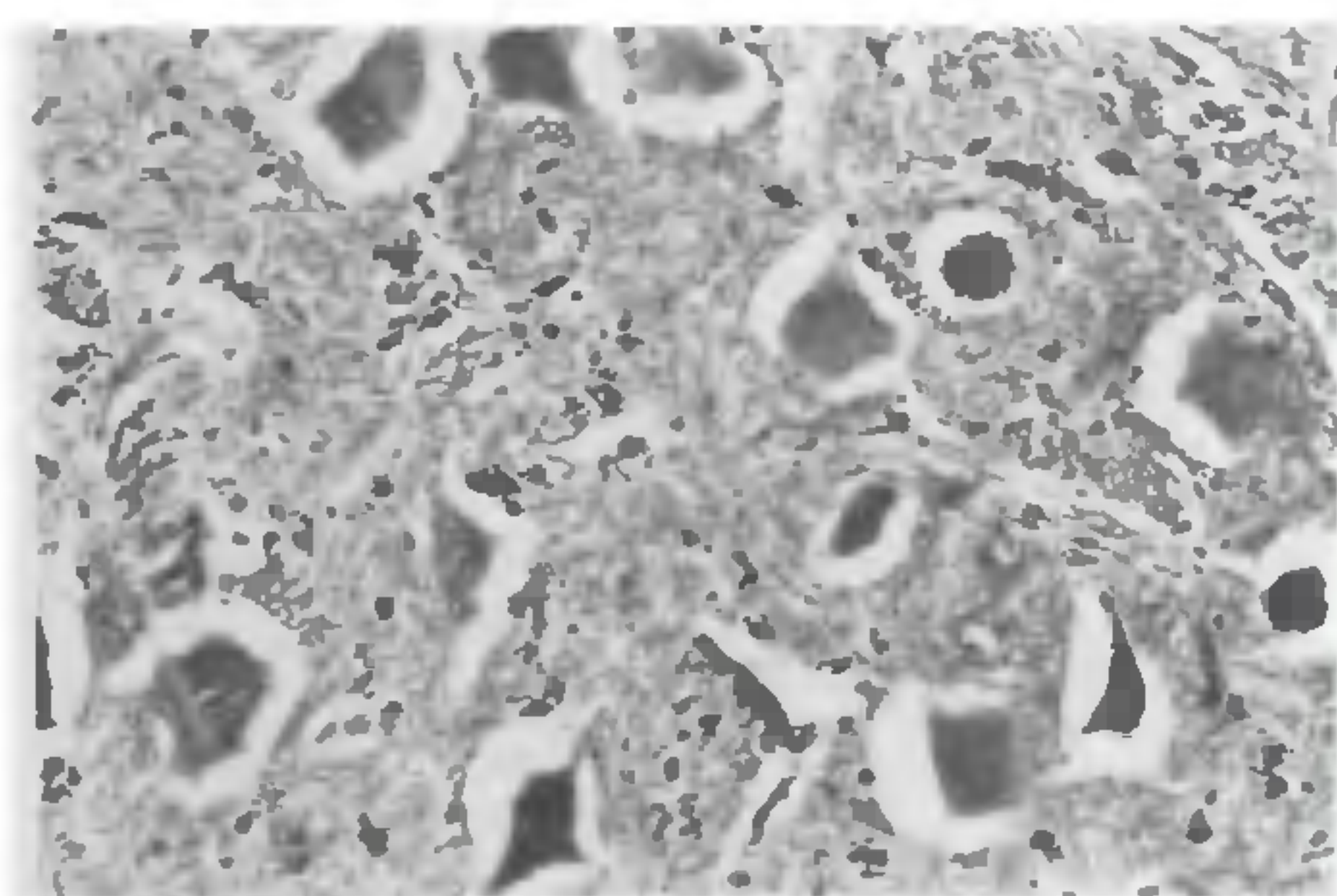


Figure 6 a.1.

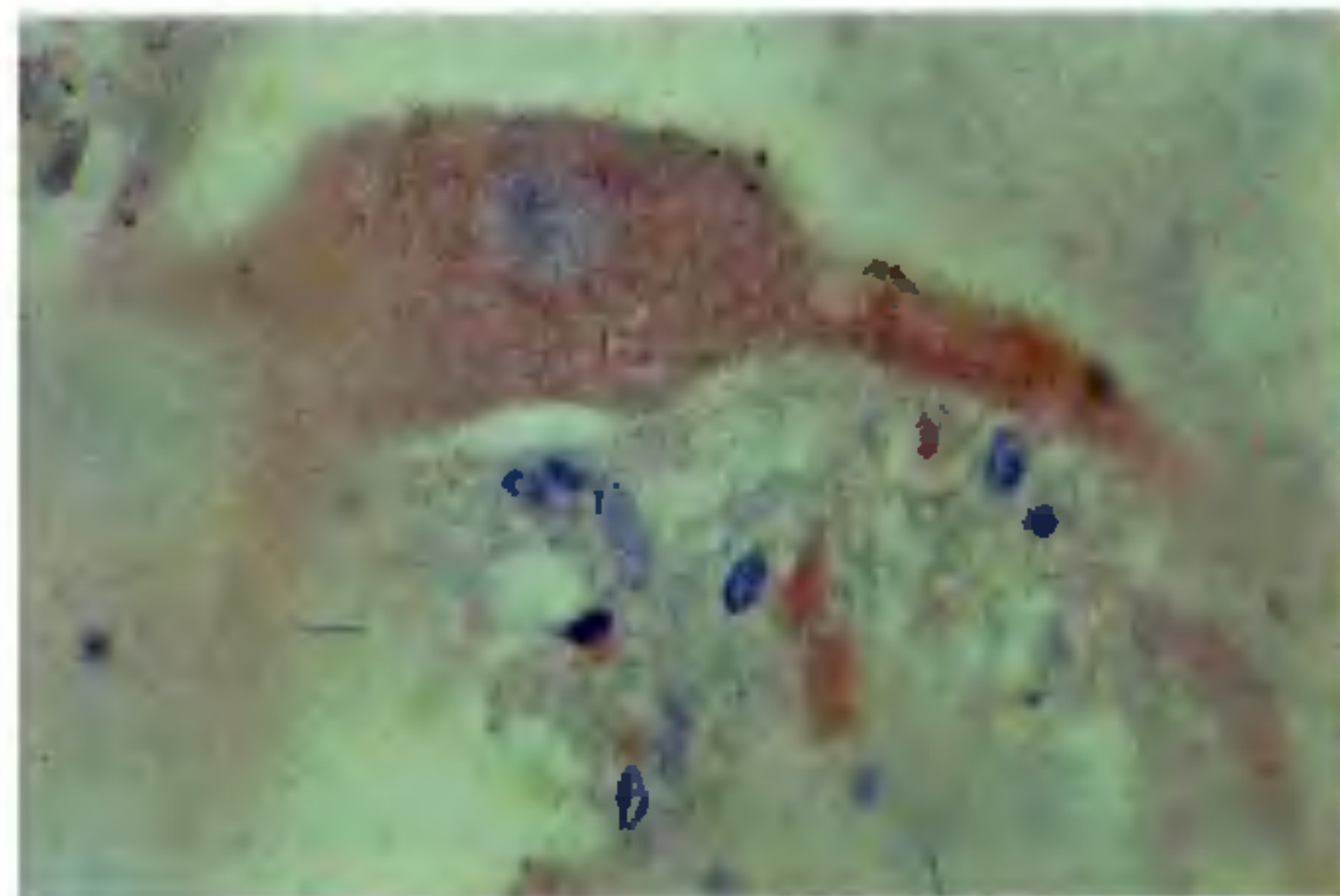


Figure 6 a.2.

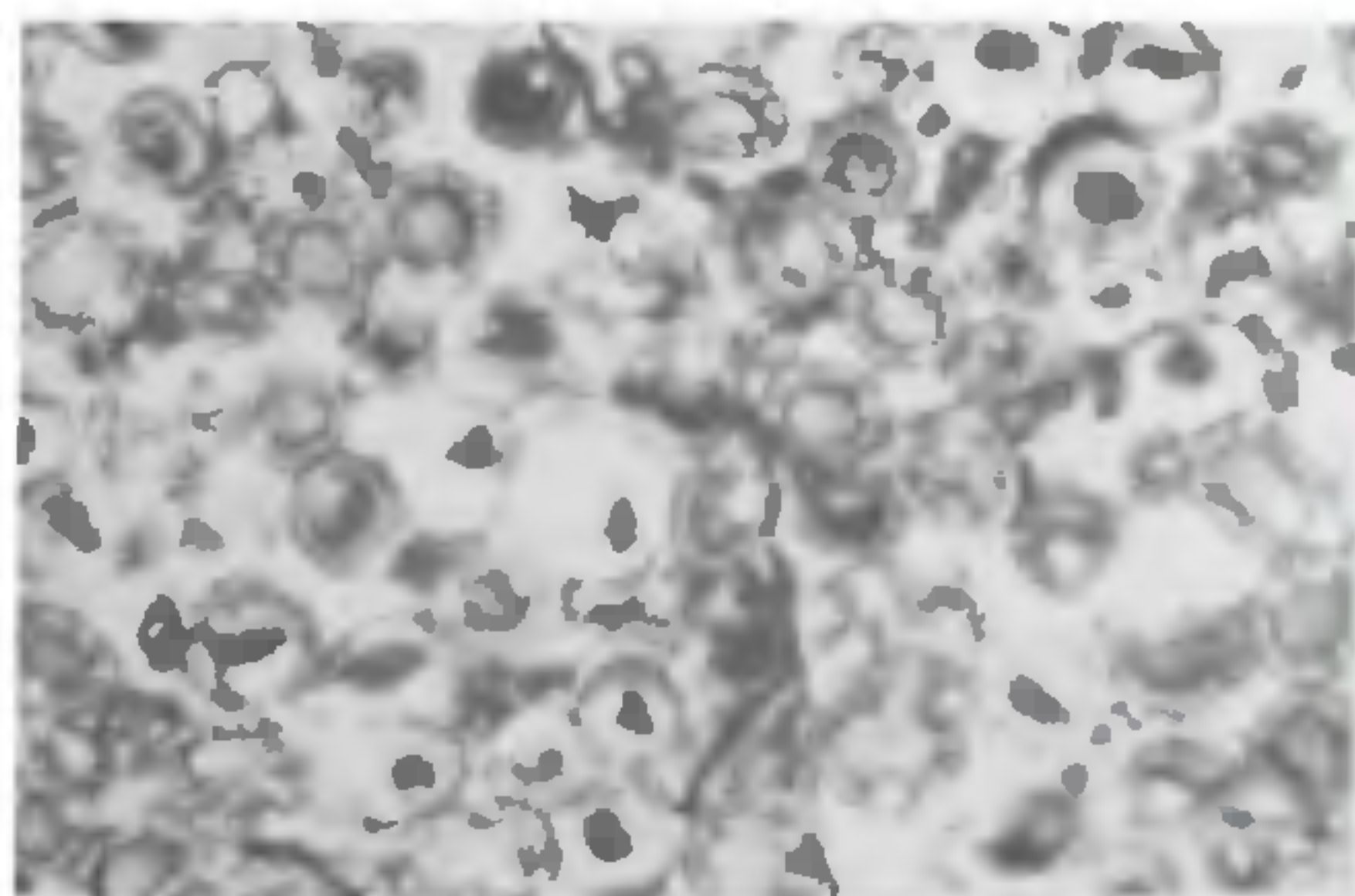


Figure 6 b.1

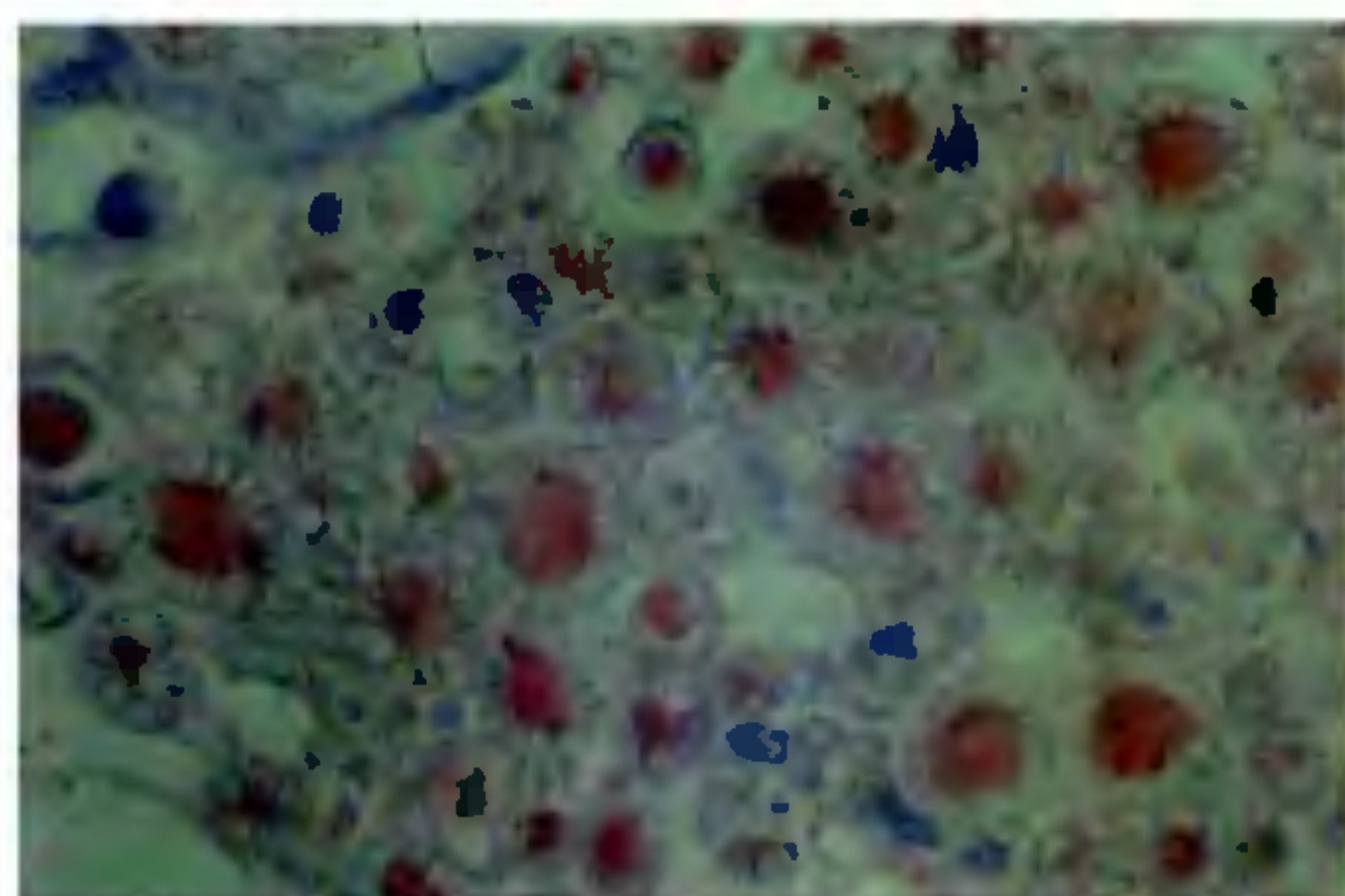


Figure 6 b.2

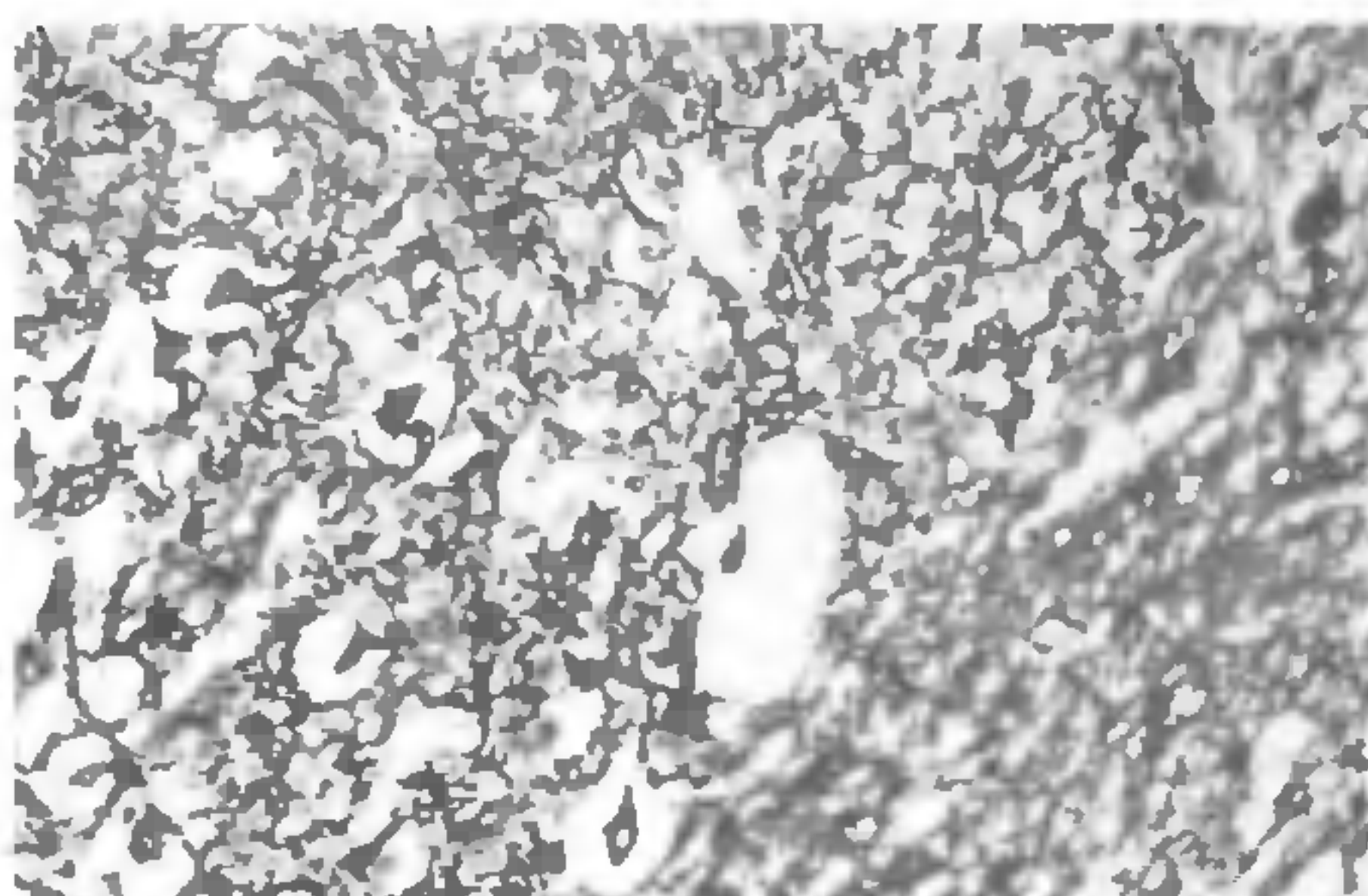


Figure 6 c.

the onset of neurological symptoms in the majority of cases. However, about 10% of patients survive for over 2 years. They exhibit dementia with pyramidal and occasionally extrapyramidal or cerebellar signs²¹. The clinical and pathological features of patients in the Western world were described, among others, by Chou *et al.*²², in Japan by Yamamoto *et al.*²³ and in India by Singhal and Dastur²⁴. We have recently described the pathology in 6 of our 9 cases of CJD in the Bombay region. Electron microscopic examination of the brain was performed in 5 of these 6 cases (ref. 2), including one case of long duration.

Beck *et al.*²⁵ noted that the neuropathology of human and experimental CJD was similar to that of scrapie, a naturally occurring transmissible disorder of sheep, and to experimentally induced scrapie in mice. It was found that the viral material was unusual in appearance, presenting as thin or slightly thick circular membranous profiles, in singles or in roughly concentric layers, in the cytoplasm of processes of nerve cells, and occasionally even in myelinated fibres (see figures 3-6 and 9 of our recent paper²). Usual structures such as 'nucleo-capsids' characteristic of the conventional RNA and DNA viruses, were not detected. Moreover, chemical extraction of the infectious material from the brains of mice infected with scrapie, yielded only protein and no nucleic acid whatsoever. Thus, a proteinaceous infectious agent was established. This infective material was therefore called 'prion', instead of virion, by Prusiner and his colleagues²⁶. Two other pioneering groups of investigators in this field have been those of Wisniewski and colleagues^{27,28}, who isolated 'scrapie-associated filaments' from the brains of scrapie mice, and of Gajdusek and colleagues²⁹.

Gajdusek made the seminal discovery that the neurological disease 'Kuru', then endemic among the natives of New Guinea, was caused by the ingestion of brains of the affected dead subjects. This was proven by inoculating such brains into chimpanzees; after

periods of up to several years, these great apes showed signs of a similar disorder. Together with his neuropathologist colleague, Gibbs, they observed spongiform changes, with or without amyloid plaques, both in the brains of affected man and ape³⁰. Thus, the whole new field of 'slow virus infections' was born. It was not till several decades after the original description of the rare cases of dementia in the twenties by Jakob and by Creutzfeldt, that their similarity to the neuropathology and etiology of Kuru, was recognized. The long duration cases of CJD show a greater resemblance to brains of Kuru patients, as they show Kuru-like plaques in addition to the spongiform change and 'prion' membranes. Some brains of CJD may also show 'axonal dystrophy' and formation of wisps or clusters of filamentous material. Such material could be a form of NFT made up of paired helical filaments, as was shown by Liberski *et al.*³¹ in one unusual case of CJD with plaques and also features of AD.

Our one illustrative case (K-129) was a woman aged 56 who survived for three years after the onset of dementia and myoclonus. Pyramidal and cerebellar signs were also present, similar to patients described by Masters *et al.*³². The cerebrum showed many areas of

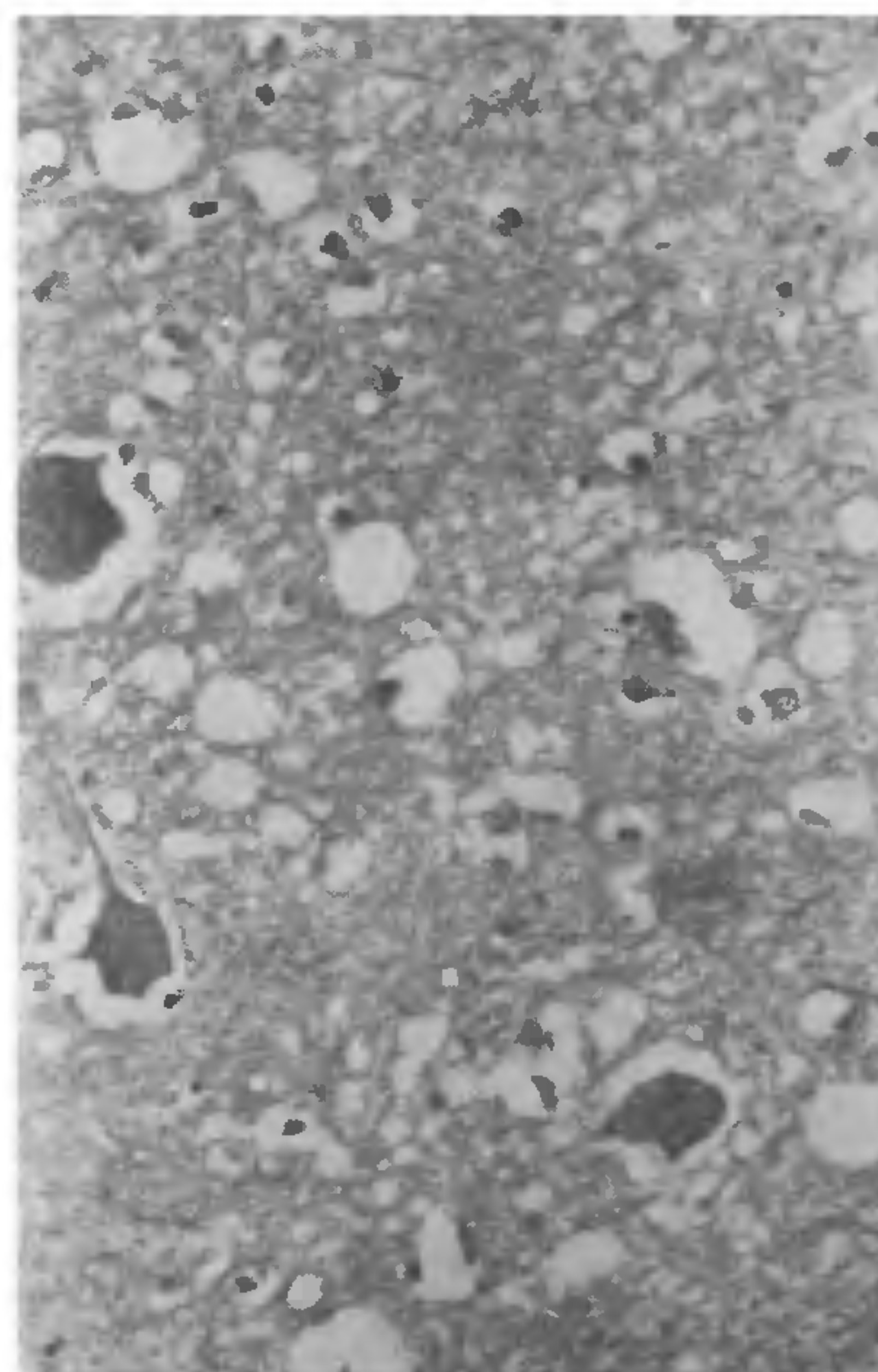


Figure 7 a.

spongiform change and depletion of neurons, as in Figure 7a (H&E, $\times 225$). Electron microscopy revealed thin smooth membrane profiles occupying some of the 'holes' seen on light microscopy. (Figure 7b is of such a thin osmicated araldite section, stained with uranyl acetate and lead citrate, $\times 22,700$). The persistence of these profiles in this and most of our other cases² suggested to us that infectious material could survive formalin fixative for a few to many years, despite the fact that other cytoplasmic constituents are 'damaged' (see Figure 7b showing dark material that includes one barely recognizable myelinated fibre.) Gajdusek and others have in fact detected infectivity of such formalin-fixed brains after many years.

Many microscopic plaques were seen in the cerebrum of this patient, typically exhibiting glial whorls such as those seen in Figure 8 (colour). This section stained positively for carbohydrate material by the PAS method; a dense PAS-positive deposit is seen in the upper part of the upper glial whorl in Figure 8 ($\times 250$ approx.). Most of the other nuclei in this area belong to proliferated astrocytes. Many of these plaques stained positively for the protein component of amyloid by the Congo red method. Such a glycoproteinaceous 'amyloid plaque' shows its greater central part to be homogeneous, but with peripheral radiating filaments (Figure 9a;

colour, $\times 630$). The formalin fixation had rendered unclear many fine structural details of all cellular and filamentous material. On higher magnification, however, even the dense centre of the amyloid plaque resolved into compactly arranged thin filaments with a faint cross-striational pattern (Figure 9b; processed as for Figure 7b, $\times 180,000$). A nonspecific but interesting change in this brain was the presence of several degenerating or 'dystrophic' cell processes, often amidst slightly better preserved very small myelinated fibres and profiles of smooth endoplasmic reticulum (Figure 10a, $\times 10,200$). Of further interest are the wisps or aggregates of filamentous material emerging from such degenerating 'neurites' (Figure 10a; F, F). Figure 10b ($\times 30,500$) shows a closer view of such a filamentous aggregate and also scattered single filaments, roughly of intermediate size, in its vicinity.

Cytoplasmic and neuropil changes (other than intranuclear virus) in SSPE

A viral encephalitis of children, called 'subacute progressive encephalitis' by Dawson in the thirties and 'sclerosing leuco-encephalitis' (SLE) by Van Bogaert in the forties was finally named 'subacute sclerosing panencepha-

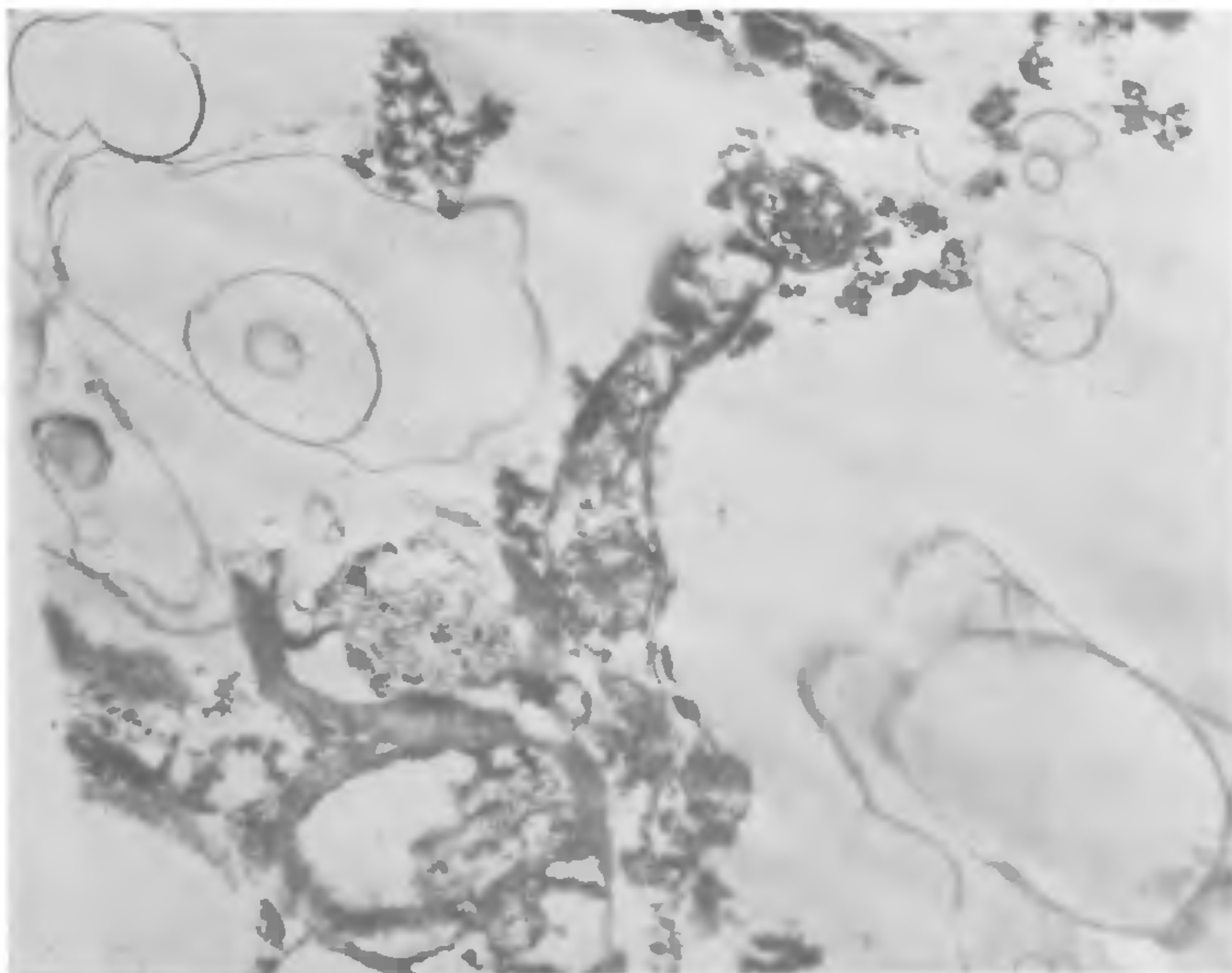


Figure 7b.

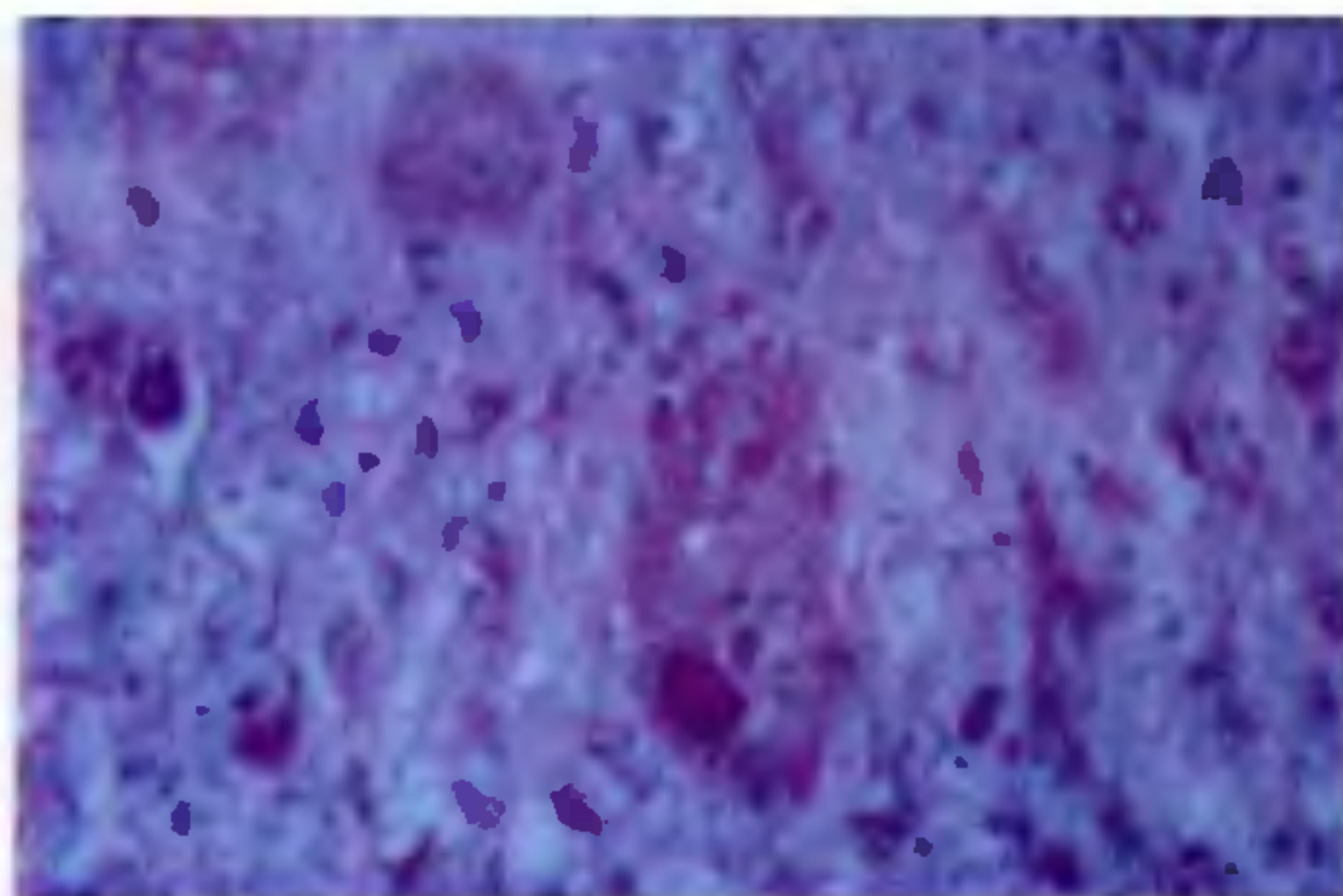


Figure 8.

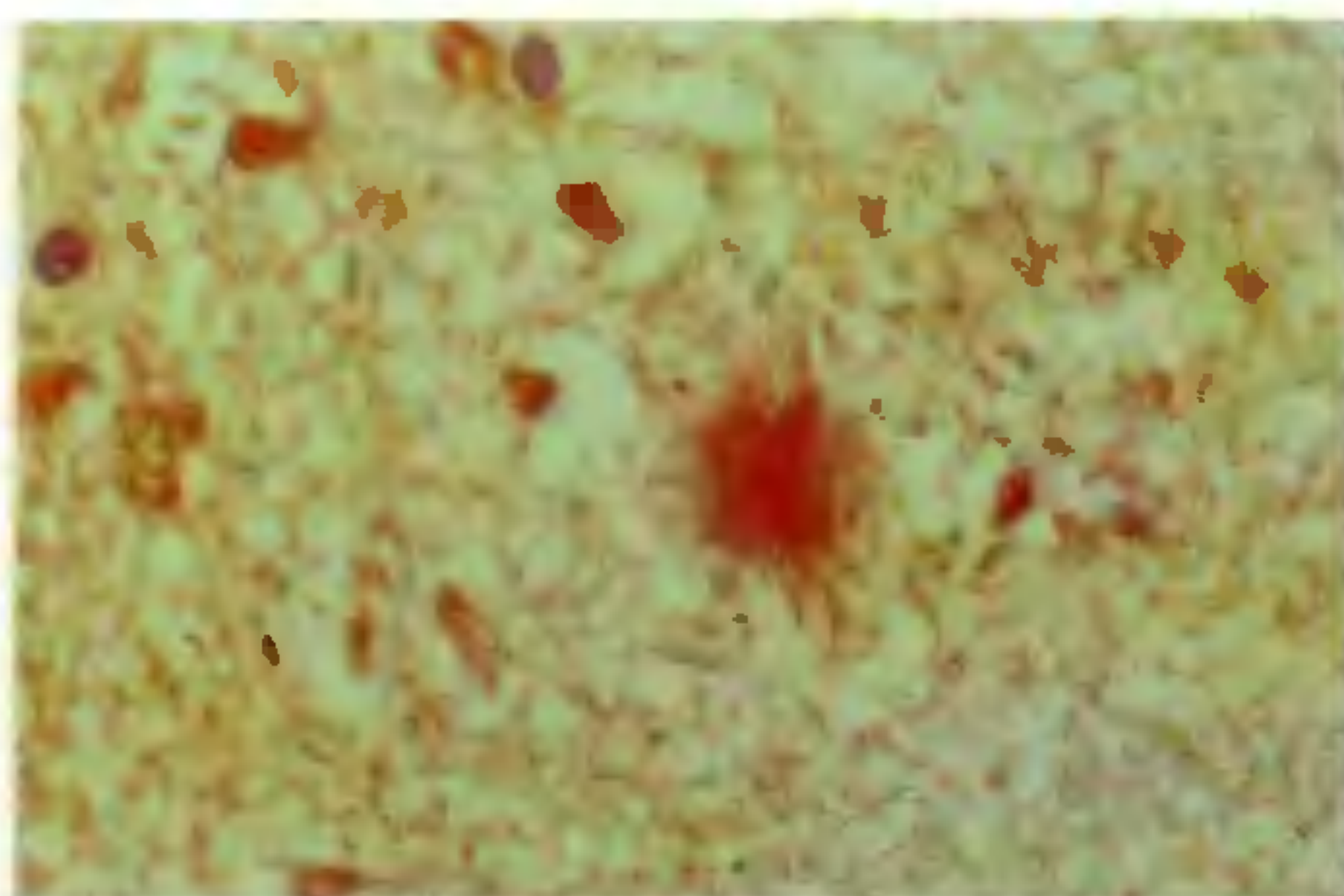


Figure 9 a.

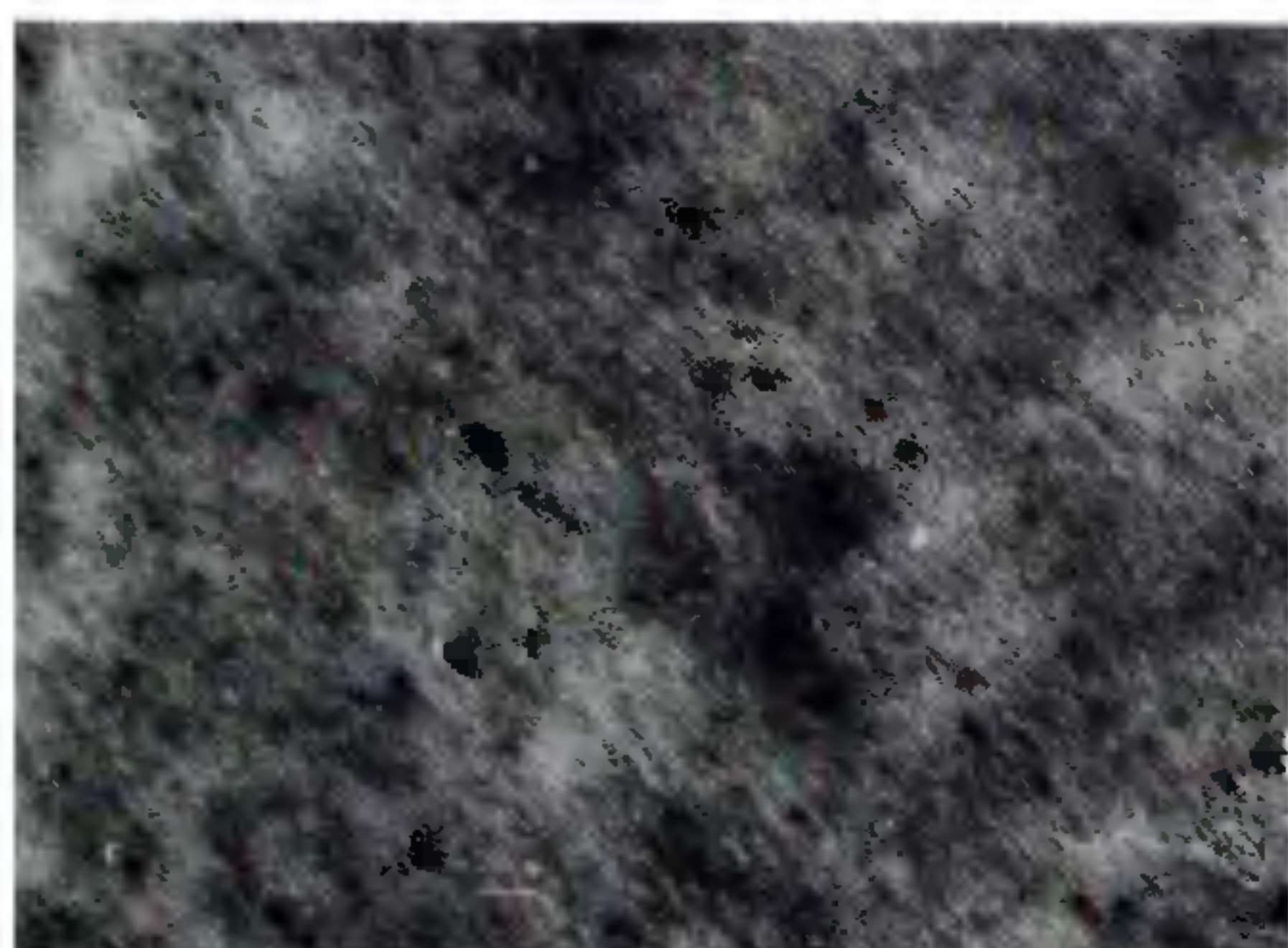


Figure 9 b.

litis' (SSPE) at a special meeting at the NIH (USA) in 1967. A year before that (in November, 1966), in his presidential address to the Neurological Society of India,

one of us (D. K. D.) had already named this condition 'subacute gliosing panencephalitis'³³ on the basis of both grey and white matter changes and astrocytic reaction as observed in over a dozen brain biopsy specimens, including both cortex and subjacent white matter. These were obtained in the 1962–66 period, mostly from the patients of our then neurologist colleague at the J. J. Hospitals—N. H. Wadia (see his contribution in this issue on clinical dementias in India).

SSPE was characterized, in most cases, by a preceding history of measles infection, by the presence of intranuclear viral inclusions, and more recently by the demonstration of clear tubular myxovirus of measles almost filling the nucleus of the affected neurons and glial cells. More diffusely outlined myxovirus material, also called 'nucleo-capsids' can be encountered in the cytoplasm of the same cells^{34,35}.

We have two such autopsied brains from cases of SSPE which have been studied by both light and electron microscopy and which showed intracytoplasmic and intranuclear inclusions and paramyxovirus of the type described above (Dastur and Manghani, unpublished data). Figure 11 (semi-thin osmicated araldite section stained with toluidine blue, $\times 700$) shows one very large swollen cell, probably a neuron with a large intranuclear inclusion (arrow), a very thin rim of residual nuclear heterochromatin and a smaller adjacent cell, probably a glial cell with a smaller nucleus, also bearing an inclusion (short arrow). Of greater relevance to us here, are our findings of filamentous material, generally densely aggregated, which we found in the neuropil and cell processes in the brain of this 13-year-old boy who had only a four month history of neurological signs and symptoms prior to his death (case of V. R. Joshi, then of Nair Hospital, Bombay).

Figure 12 is from an ultrathin section of the same brain showing no clear cell outlines, only the lipofuscin and distended mitochondrion in the right lower corner (split arrow) suggesting that this might have been a neuron. Of interest in this electron micrograph (processed as for Figure 10a; $\times 36,400$) are the faintly somiophilic wisps (small groups) of filaments (F) in the right mid-zone of the picture and the dense L-shaped filamentous band (FB) on the left side of the picture. The general disruption of the architecture here is further evidenced by the distended astrocyte processes (A) and the degenerating axons of the three myelinated fibres included in this picture. Figure 13a ($\times 31,300$) shows an almost degenerated, distended myelinated fibre full of single membrane lysosomal profiles, some of them reaching the stage of dense bodies (D), remaining mitochondria, and axon debris. Of interest is the oval area in the upper left corner of this picture bearing an aggregate of pale filaments (F).

Figure 13b ($\times 77,000$) is a closer look at another similar group of filaments in a disrupted cell process or

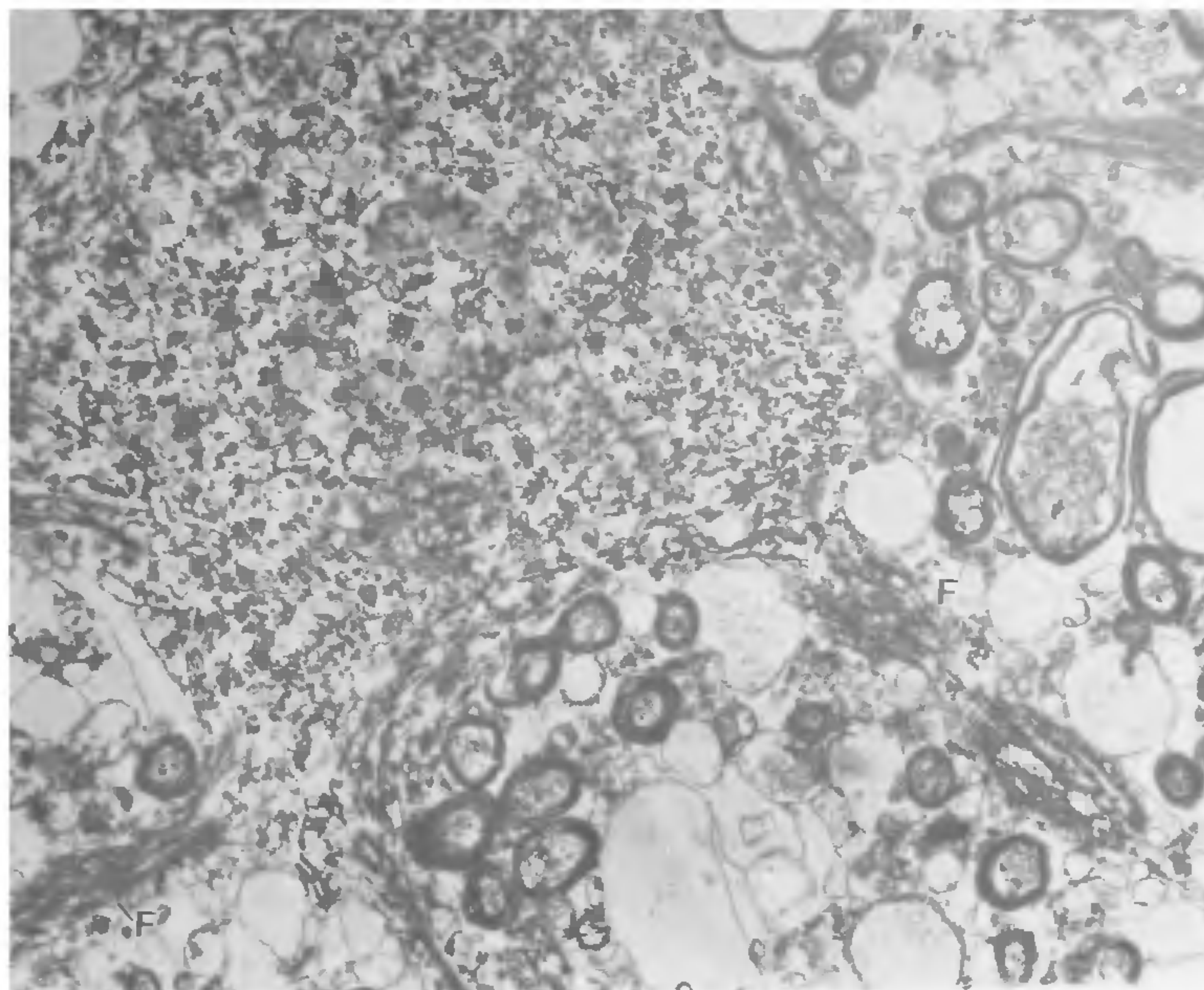


Figure 10 a.

a nerve fibre. These are 'paired helical filaments' (PF) forming the group of filaments in the right; while there are discrete unpaired, i.e., single filaments (SF) on the left. Each of these separate filaments shows a beaded structure, the beads being at periodic intervals. A. Hirano, who was sent this picture, agreed with our suggestion that this whole configuration can be called a 'Hirano body'. To the best of our knowledge, there is no published description to-date of the fine structure of this 'single-beaded filament'; nor the demonstration that two such filaments intertwine to form a 'paired helical filament'.

Cerebral circulatory and physiological data in aged subjects

This section on physiological and biochemical parameters of human ageing is introduced because, in the final analysis, we are concerned with the functional status of the brain. In particular, 'vasogenic' factors may be highly significant modulators of function during ageing. The classical Oslerian dictum that 'we are as old as our arteries' probably remains valid today. Selective brief accounts presented below are based on work

conducted in the fifties and sixties at the US NIH, Department of Brain Metabolism (directed by Louis Sokoloff and Seymour Kety). The findings, however, are probably applicable to populations in other countries, such as the urban population in India.

One of us (D. K. D.) was intimately concerned with the first such study, which showed that there were no significant differences in cerebral blood flow (CBF) and cerebral metabolism in terms of oxygen consumption (CMRO_2), between 'normal' elderly men over 65 years of age (mean age of 71 years, Group I) and a control population of young subjects of mean age 21 years. The elderly subjects in this group were, however, carefully selected, in that they were functioning adequately physically and mentally in their respective environments, and did not have any signs of cerebral or cardiovascular disease. When these unusually well-preserved elderly subjects were compared with those having signs of atherosclerosis (Group II), the latter were found to show a small but significant fall in the CBF and in the cerebral metabolism in terms of glucose consumption (CMRG), but there was no change in the CMRO_2 . It was only in elderly subjects with severe atherosclerosis and some mental changes (Group III) that all three parameters, viz. CBF, CMRO_2 and CMRG, fell signifi-

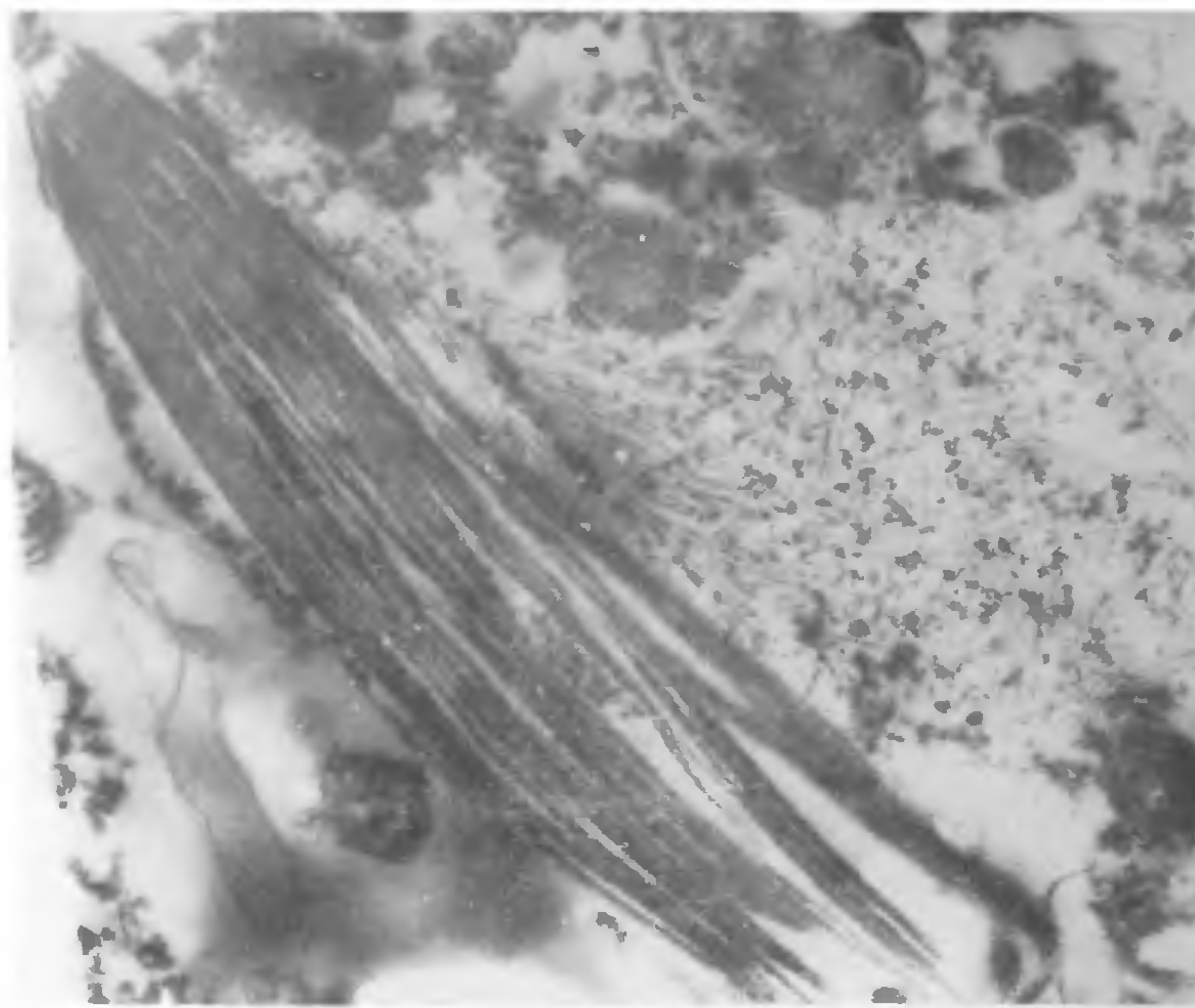


Figure 10 b.

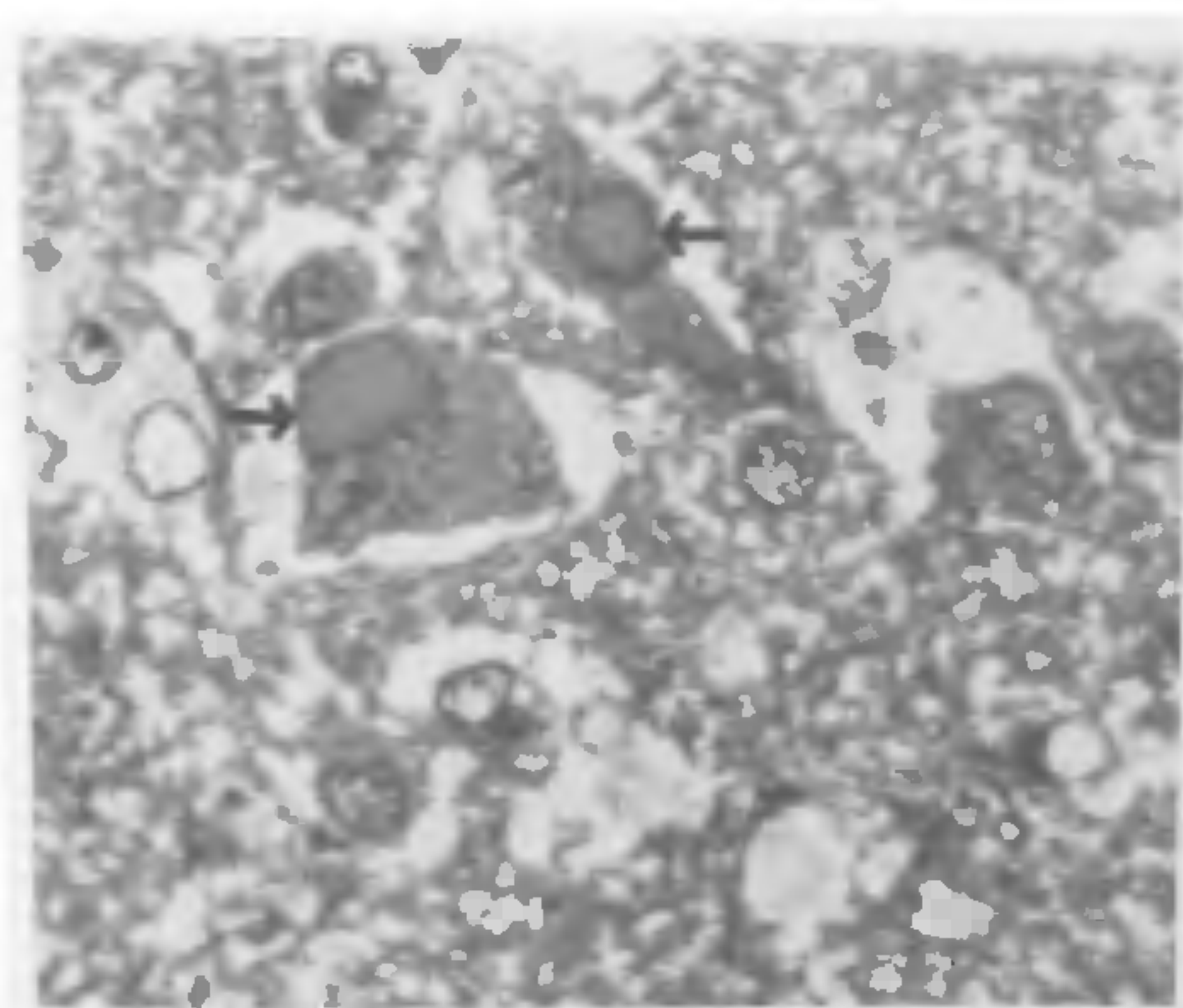


Figure 11.

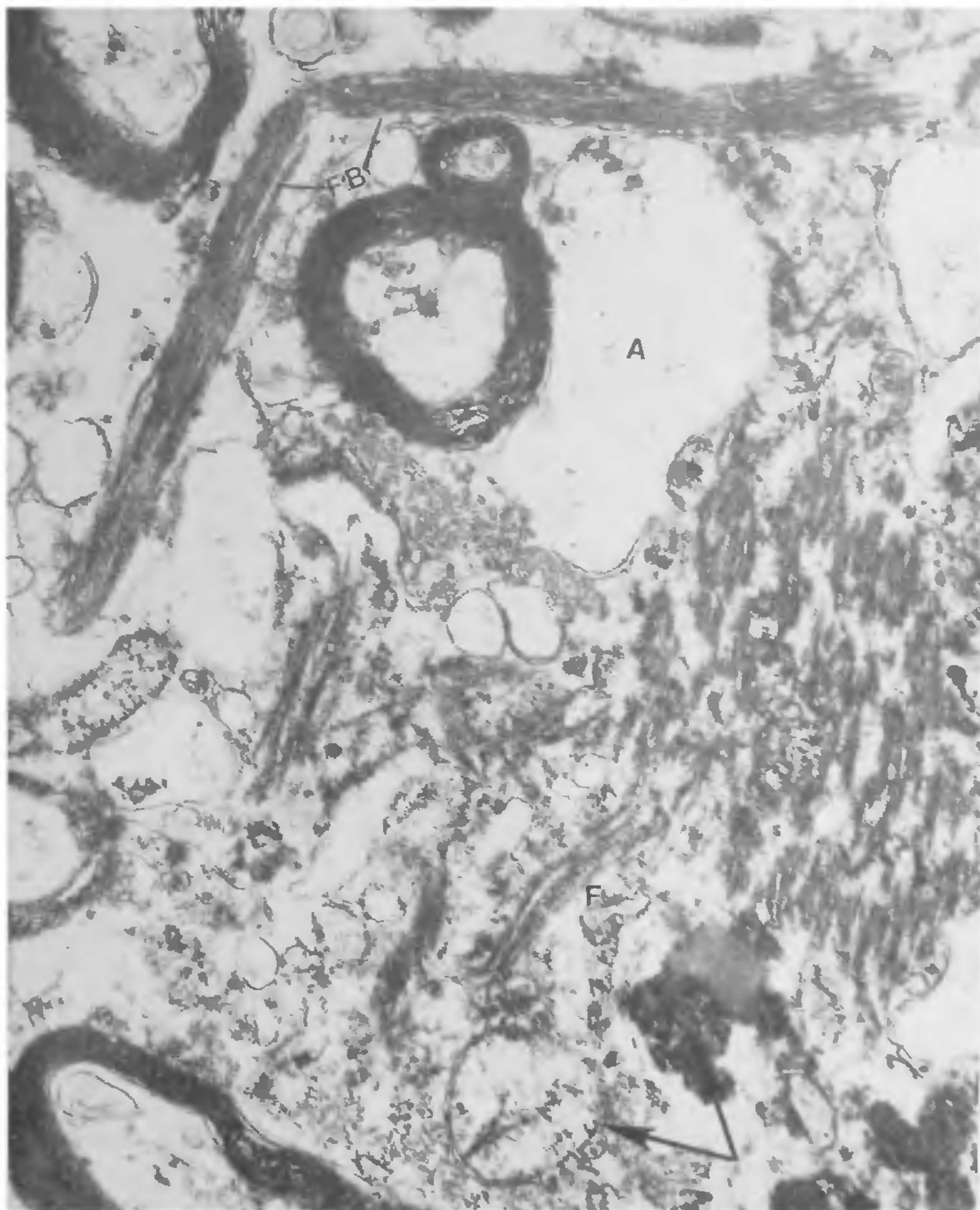
cantly³⁶. The detailed data on this, with statistical evaluations and correlations with psychological, psychiatric and cardiovascular parameters, were published in

the book *Human Aging*³⁷. There was a second similar study a decade later on some of the surviving elderly subjects³⁸, who now showed clear worsening of all the parameters of brain metabolism (CBF, CMRO₂ and CMRG), probably related to hypertension and increased cerebro-vascular resistance (CVR). The entire material was reviewed recently by Dastur⁸.

It would appear to us that the changes reported in patient Groups II and III in the above studies could play a role in the pathogenesis of various forms of dementia, possibly including AD.

Discussion

As mentioned under Observations, certain histopathologic and fine structural features characteristic of (a) plant toxicity such as due to *Cycas circinalis* or *Lathyrus sativus*, and (b) slow virus infections such as CJD and SSPE, may be encountered in brains of some patients with Alzheimer's disease also. For instance, dense argyrophilic material, generally resolving into filamentous structures, can be seen in neurons in Alzheimer's disease, and in ageing without Alzheimer's disease^{3,5-7,28,39}



Most of these authors have provided either immunohistological or fine structural evidence for this intraneuronal neurofibrillary material.

Similar argentophilic accumulations in neurons have been reported, though to a limited degree, in toxicity due to plant products such as β OAA in neuro-lathyrism⁴⁰ and β MAA or cycasin in neuro-cycadism⁴¹. Our own experience briefly recounted in a recent paper¹ and in the present communication, points to the occurrence of other related CNS changes in monkeys

fed, washed or boiled preparations of *Cycas circinalis*, viz. (i) intracerebral argyrophilic plaques in an older animal, fed the cycad for several years (NP-C-857); and (ii) swelling of anterior horn cells and anterior nerve root axons by 160 kD neurofilament protein in a younger animal fed for only a few months (NP-C-772). The former change might have been related more to the action of the neurotoxin β MAA, and the latter to the combined action of both cycasin and β MAA. The

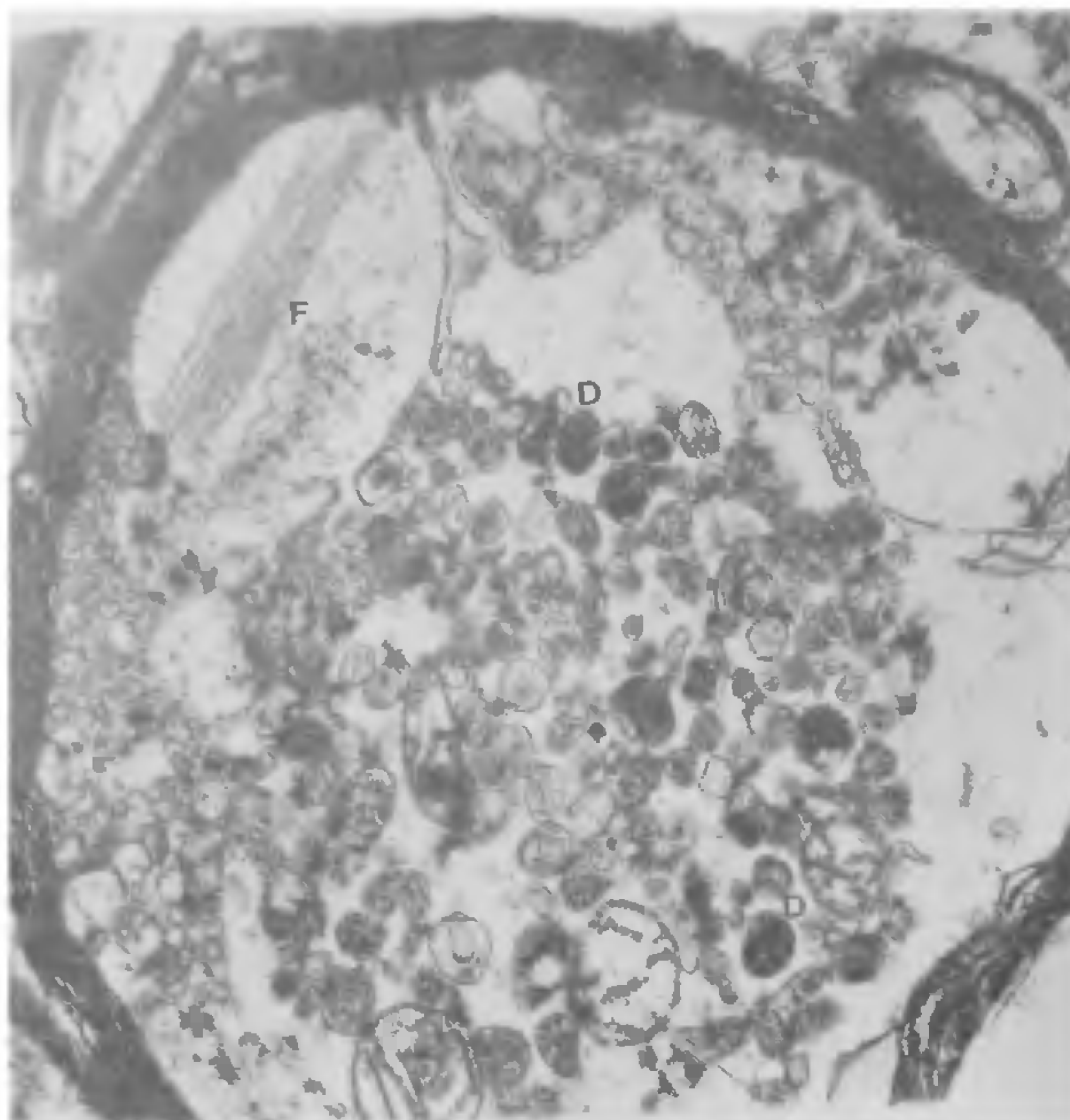


Figure 13 a.

potent hepatotoxicity of cycasin was manifested by both animals, but more by the latter, who died with severe jaundice, terminal convulsion and prostration after relatively small doses of the cycad nut. This second animal also gave histological evidence of severe hepatic degeneration, together with some precancerous changes. These two non-human primates in our experience are being purposely quoted here to illustrate the complexity of the so-called neurotoxic compound, β -methyl-amino-alanine (β MAA); and the so-called carcinogenic compound, methyl-azoxy-methanol-glycoside or cycasin, both residing in the nut of this false Sago palm (*C. circinalis*).

Spencer *et al.*⁴⁰ have also discussed the possible combined presence and dual neurotoxic role of these two compounds. They further quote the Japanese group of Shimizu and colleagues⁴¹, who reported that in goats fed cycasin alone, severe axonal swelling in anterior and lateral columns of the spinal cord, was produced. Thus, an effect like that produced by $\beta\beta$ -IDPN described earlier²⁰ and recently¹, as well as in the present paper, seems capable of manifesting on administration of cycasin. CNS changes have also been reported in post-

natal mice injected with cycasin⁴². Thus, the possibility arises that the etiology of ALS and/or PDC, at least in the populations of Western Pacific regions who are consuming or have consumed cycad nut preparations, might result from the combined action of the acknowledged neurotoxin β MAA as well as the less known neurotoxic action of the glycoside cycasin. As Spencer *et al.*⁴⁰ and Kurland⁴³ have stressed, the falling trend of ALS and the increasing development of PD or dementia alone, may be brought about by the lesser but longer consumption of the potentially toxic cycad nut in the case of neurocycadism. A similar situation might be operating in the slow development of neurolathyrism with lateral column degeneration and spasticity, after prolonged consumption of preparations of *L. sativus*, in populations of the Indian sub-continent and the Mediterranean region. As Spencer *et al.*⁴⁰ have rightly argued, in common with earlier investigators in India, such as Shourie⁴⁴, only subjects who are forced to eat preparations of this pulse in large quantities, for long periods in times of drought, are likely to develop the spastic paraplegia or quadriplegia.

A final point of interest in the pathogenesis of neuro-

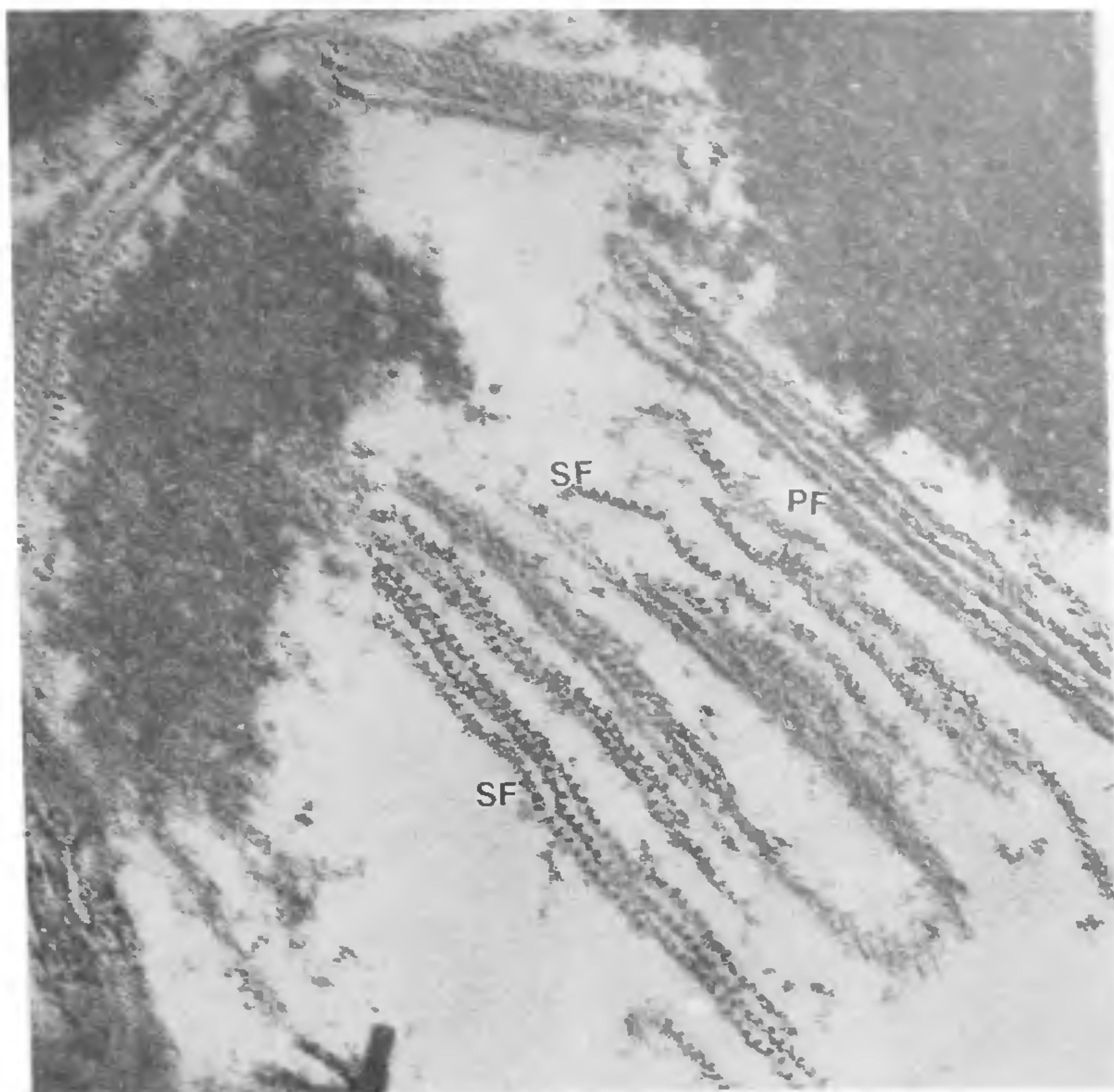


Figure 13 b.

cycadism is the recently proposed pathway of entry of the toxins of cycad nuts⁴³, viz. the olfactory mucosa, nerve and bulb, through inhalation of the 'dust' emerging from the nuts as they are being 'de-husked' prior to their cooking and consumption. Kurland⁴³ has reported 'severe neuronal degeneration in the olfactory bulb and loss of odour identification', especially 'in patients with PDC in Guam'.

Coming to a consideration of the morphological changes in brains of patients with AD and those with ageing with or without dementia and other conditions, one is reminded of the relatively frequent and non-specific change of formation of argyrophilic NFT. They have even been reported in 'progressive supra-nuclear palsy'⁴⁵, the condition which has recently been referred to by Gajdusek⁴⁶ in his review of the wide variety of disorders with 'amyloid plaques, tangles and vascular

deposits', with a possible uniform source and even a common genetic abnormality. However before considering even briefly the genetically common, i.e. DNA-related abnormality in AD and other conditions, one might consider the better understood morphological abnormality in affected neurons in various conditions.

This is the collection of intermediated-sized filaments in the perikarya of degenerating or swollen neurons in AD, as also in CJD, the Gerstmann-Straussler syndrome, in Down's syndrome, in ALS on Guam, or elsewhere, in Guamanian PDC, even in Guamanians without CNS disease, and in aged subjects. Gajdusek and his colleagues have studied this exhaustively, mainly through immunofluorescence, e.g. Brahmanyar *et al.*⁴⁷. Hirano and Inoue⁴⁸ demonstrated fine structurally in a New Yorker with ALS, that the intraneuronal material was in fact a mass of 10 nm neurofilaments. Gajdusek⁴⁹, in

his remarkable reviews on the subject, has often summed up the significance of this proteinaceous filament accumulation through his title, e.g. 'Interference with axonal transport of neurofilament as a mechanism of pathogenesis underlying Alzheimer's disease and many other degenerations of the CNS'. He then proposed, what has now been demonstrated to a great extent, that interference with axonal transport of the 10 nm neurofilament may lead to (1) pooling of the neurofilaments in the perikaryon and lysis of the neuron as in ALS and other motor neuron diseases; or (2) formation of NFT and NP, with neurofilament modified to form paired helical filaments, as in AD and ageing; and (3) a further degradation of the same neurofilament material, leading to amyloid plaque formation, as in AD and the larger more regular plaques of Kuru, CJD, related slow virus infections, and in Down's syndrome, and also in unrelated conditions such as dialysis dementia and pugilistic encephalopathy.

To this list, Gajdusek⁴⁹ also adds neurotoxicity due to $\beta\beta$ -IDPN which we have already considered as a neurofilament accumulating disorder, in a monkey fed on cycad (as described and discussed above). Among the lesser known of the slow virus infections, though more prevalent world-wide, we would add SSPE, where aggregates of neurofilaments, and rarely paired helical and single-beaded filaments, may form, as we have illustrated in this paper (Figure 13b).

Wisniewski *et al.*²⁸ and Gajdusek²⁹ were impressed by the familial occurrence of some cases of senile dementia of Alzheimer's type (SDAT), as also of some forms of CJD, especially the Gerstmann–Straussler syndrome (GSS) where 'kuru-like' plaques tend to develop, as in our one patient with CJD (K-129) illustrated earlier. They have also discussed the probable common genetics of SDAT, CJD and other transmissible encephalopathies, such as scrapie in sheep and mice, and the known inherited condition Down's syndrome. Wisniewski *et al.*²⁸, as well as Gajdusek and his colleagues^{29,50}, have stressed the role of genetic susceptibility in the development of these disorders, keeping in mind the frequent autosomal dominant mode of inheritance of the disease in all affected families, especially familial CJD²⁹. What was more interesting was the fact that plaque formation in SDAT, CJD or scrapie seemed to require the combination of an infectious agent and a genetically appropriate host. More recently Gajdusek⁵⁰ and Brown *et al.*⁵¹ have enhanced the notion 'that precursor proteins and the amyloids' might be the biochemical substrate of transmissible and non-transmissible dementias.

In fact, the viruses of scrapie, Kuru and CJD at least, are now being considered to be infectious polypeptides or amyloid-enhancing factors⁵¹. Furthermore, we are told that to understand this, one has to enter the arena of polymer chemistry and molecular genetics, especially

fibril crystallization of the infectious scrapie amyloid monomer (P_1, P_{27}), which is self-replicating and forms the scrapie–Kuru–CJD plaque^{50,51}.

Since plaques, amyloid fibrils and intermediate-sized filaments hold the morphological centre of the field in the neuropathology of AD, CJD, scrapie and other spongiform disorders, the beautifully illustrated fine structural and electron cytochemical study of Wisniewski *et al.*⁵² must be mentioned. These authors stress the central solid core and the radiating processes of the amyloid 'star', and its relationship with microglial macrophages which generally surround the 'star' and are even responsible for its formation. The surface of amyloid plaque and processes shows the enzyme product for the lysosomal enzyme nucleoside diphosphatase, more of which is seen in the digitations of the surrounding macrophage.

In their remarkably comprehensive and critical review of the structural, especially immunohistological, account of various types and stages of plaques in AD, Probst *et al.*⁵³ have traced the evolution of different constituents, with suitable markers from the (i) pre-amyloid 'amorphous' or incipient plaque, to (ii) the mature amyloid deposits, to (iii) the 'classic' plaque with argyrophilic dystrophic neurites. From (i) to (ii), the 6–10 nm amyloid fibrils increase considerably and Congo-red positivity develops. From (ii) to (iii), the argyrophilia (on appropriate silver impregnation) increases; paired helical filaments and tubulovesicular bodies appear; positive reactions to Tau, neurofilament proteins, ubiquitin and synaptophysin develop. At the same time, reactive microglia and/or astrocytes become prominent as one proceeds from (i) to (ii) or (iii). Like Wisniewski *et al.*⁵² and Probst *et al.*⁵³ consider the formation of the amyloid plaque to be in close proximity to microglia. Of interest to our current paper is their illustration in Figure 1 (ref. 53) of a 'ghost tangle' under silver impregnation in the brain of an AD patient, which is strikingly similar to the somewhat shrunken 'late stage' NFT's in the brain of an aged non-AD patient (Figure 1a).

In a personal communication, Probst⁵³ has sent us some electron micrographs of brains from cases with AD. One of these shows a 'dystrophic neurite from the rim of a classic plaque', and is strikingly similar to the dystrophic axon of a degenerating myelinated fibre in a case of CJD without a plaque (Figure 5b of Dastur *et al.*²); 'a dystrophic neurite' from the case of CJD with plaque, illustrated in the present paper (Figure 10a); and a 'dystrophic nerve cell process' from the brain of proven case of SSPE (Figure 13a of the present paper). In all these pictures, the degenerating neuritic process shows a collection of lysosomal lamellar or dense bodies, representing late stages of intralysosomal 'digestion', and degenerating mitochondria. This represents a further fine structural similarity between classic AD and

at least two human disorders with slow virus infections (CJD and SSPE). This last change of tissue breakdown with lysosomal activity, whether in neurons, axons, or neurites, 'dystrophic' or otherwise, is very non-specific; as in the formation of lipofuscin bodies seen, for instance, in Figures 10a, 12 and 13a in the present paper. We have been impressed by this almost universal lysosomal 'digestive activity', in the CNS, PNS, muscle, liver and other tissues, in a wide variety of disorders of infection, storage, toxicity, or cell breakdown due to any cause⁵⁴. However, in any disorder where filaments are formed, whether in singles or aggregated into wisps, tangles or fibrils, the challenging question is the mechanism or formation, or pathogenesis, of such abnormal material which leads to the abnormal cells.

The reason for introducing the short last section under Observations, on certain physiologic parameters of brain metabolism, was to keep in mind the contribution, whether occurring in middle age or older age, of reduced CBF, CMRO₂, CMRG, even in the presence of a primary neuronal disorder such as AD. A vasogenic factor operating through cerebro-vascular atherosclerosis, is bound to play a pathogenetic role in the worsening of brain junction with ageing. By the year 2000, even in India, according to population predictions, the number of aged people above 60 years will be about 65 million, i.e. a larger number than USA is expecting to have⁸; and they will form about 10% of the world's population, as quoted by Hoyer⁵⁵. The latter discusses how metabolic reductions with age may be tentatively accounted for by a physiologically occurring loss of neurons, dendrites and dendritic spines in distinct brain areas.

Hoyer reviews the older literature on geriatric patients and finds that with increasing age there is a falling trend in all the three parameters of brain circulation and metabolism. One of us (D. K. D.) would also concur with this, despite his earlier findings in the US in the sixties^{35,36}, because an *exceptionally healthy* population of subjects with a mean age of 71 years, constituted 'the normal aged' in that study.

Rogers *et al.*⁵⁶, in their 7-year prospective study on neurologically normal elderly volunteers (also of mean age about 71 years), found 3.3% developing AD and 5.5% developing multi-infarct dementia (MID), a vasogenic disorder. Nearly half of their volunteers had risk factors for atherothrombotic stroke. They concluded that 'decreased CBF precedes MID, but follows senile dementia of Alzheimer's type'.

Bell and Ball⁵⁷ used the different approach of histochemistry using alkaline phosphatase reaction, on thick sections (100 µm) of postmortem human visual cortex from normal aged subjects and those with Alzheimer's dementia. They found that capillary density was decreased to the same degree in both the groups. Amyloid-cored NP, were also present in comparable numbers in both the groups, but were more plentiful in

visual cortex with the highest vascularity, i.e. layers II–IV. They feel that the latter finding suggests a pathogenetic role for some blood-borne agent in Alzheimer's dementia, considering such plaques to be characteristic of this disorder.

Conclusions

It should be apparent that there is insufficient information to permit any firm answers to the question: 'Do environmental neurotoxins or novel infectious agents play a role in the genesis of Alzheimer's disease?' Suffice it to say that there is precedence, mainly based on fine structural, molecular and genetic evidence, for a primary pathogenetic role for both types of agents. Moreover, we should not rule out an important role for age-related alterations in blood vessels, i.e. a vasogenic factor operating concurrently with the neurogenic mechanism. Much more research will be needed to clarify the contributions of these different factors to varying types of dementia.

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