STUDIES ON BRAIN BIOPSIES OF PATIENTS WITH HUNTINGTON'S CHOREA

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ABSTRACT

Frontal cortex brain biopsies of four patients with Huntington's chorea were studied by histological, histochemical, and electron microscopic methods. The changes observed were evaluated in regard to the age of the patients and stage of the disease process. One of the most outstanding features was the large and generalized accumulation of lipofuscin in neurons and glial cells. This increase in lipofuscin was detectable in paraffin sections, but was most striking in frozen sections examined for fluorescence and in acid-phosphatase preparations. Preliminary extractions of lipofuscin demonstrated a higher content of the pigment in Huntington's chorea than in age-matched control brains. The glial lipofuscin was associated with very high acid-phosphatase activity, which was found to be localized in the dense component of the granule.

At the fine structural level, neurons evidenced several abnormalities including proliferation of Golgi membranes and Golgi-associated vesicles, disorganization of the rough endoplasmic reticulum with shedding of membrane-bound ribosomes; abnormally dense mitochondria with sparse cristae, occasionally containing crystalline fibrillar arrays within the matrix; abnormal nuclei with chromatin condensed in numerous dense granules and with increased porosity of the nuclear membrane. Increased numbers of microglial cells in the neuropil and efferent vessels, often containing remnants of neuronal cytoplasm with lipofuscin, suggest that active removal of irreversibly damaged neurons by phagocytosis is taking place. Astrocytes were proliferated and hypertrophic with a fair increase in glial filaments. They contained large quantities of lipopigment granules in their cell bodies and their processes. Neuronal death and axonal and synaptic degeneration account for the atrophy of the frontal cortex. The abnormalities found were present in all cases, and were of increasing severity with more prolonged disease.

INTRODUCTION

Huntington's chorea is a hereditary disorder characterized by onset of progressive choreiform movements and dementia usually between the third and fifth decades of life. A detailed clinical description and the first account of the hereditary nature of the disease were given by George Huntington in his report of 1872. The disease is inherited in an autosomal dominant fashion with complete penetrance. The metabolic basis of the disease is unknown. Classical
studies demonstrated atrophy of the neostriatum and cerebral cortex, mostly in the frontal lobes. Histological changes are not restricted to those areas, but they invariably bear the brunt of the damage. Current neuropathological information is based on a wealth of brain autopsy reports recently reviewed by Bruyn (1). The picture thus obtained is one corresponding to the state of the brain after 15, 20 or more years of illness. It has been difficult, so far, to relate this terminal stage morphology to possible pathogenetic mechanisms. One pallidal biopsy obtained during stereotaxic surgery was studied with the electron microscope in 1966 (2).

A biopsy study of the frontal cortex was undertaken to obtain information on the ultrastructural and enzymatic activity in this disorder.* This group of patients also provided the opportunity to evaluate changes taking place at somewhat varying and progressive stages of the disease.

CASE REPORTS†

Case 1 (G.C.): This patient was 24 years old at the time of the brain biopsy. His illness became apparent at age 13 with a psychotic episode diagnosed then as cataleptic schizophrenia. Later he developed choreic movements of the head, neck and upper extremities. These symptoms were followed by mutism and perseverative behavior in later years. His family is of Portuguese origin and there are six affected members in three generations (3).

Case 2 (N.H.): This woman exhibited the first symptoms of illness at age 32 when she became progressively withdrawn, forgetful and unable to cope with her daily activities. In the last 5-6 years she has had dystonia, choreic movements of the upper and lower extremities, and mild rigidity. Brain biopsy was performed at age 41. Five out of nine members of the preceding generation and one brother are affected with Huntington's disease.

Case 3 (S.F.): This patient started having irritability, memory deficit and abnormal movements at age 35. At the time of cortical biopsy (age 47) he had violent, continuous, choreiform movements involving the neck, trunk, limbs and fingers, moderate intellectual deterioration, dystonia and an inappropriate cheerful mood. A pronounced vigilance was present over the skin of the face, trunk and extremities. His mother, the maternal grandfather and a sister have had Huntington's disease.

Case 4 (P.R.): This patient was referred to us at age 65 after 16 years of recognized clinical illness. Symptoms have been slowly progressive since around age 45. At the time of this last admission he was severely demented, echolalic and bedridden, with contractures in semiflexion of both upper and lower extremities. He showed dystonic involuntary movements of the jaw, hands and tongue, and small choreic movements of his hands, arms and shoulder. He spoke few spontaneous dystonic words. There are four other members of the family with known chorea and dementia.

The four patients included in this study each had a well documented family history of Huntington's disease (4).

METHODS

Brain biopsies from the four patients were obtained by craniotomy according to criteria and techniques previously described (5). The sample was obtained from the second frontal

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† Preliminary electron microscopic studies on our first patient were reported at the International Congress of Neuropathology, Paris, August 1970 (3). Part of this work was presented at the Centennial Symposium on Huntington's Disease, Columbus, Ohio, March 28-29, 1972 (4).
convolution of the non-dominant hemisphere and contained gray matter and 2-3 mm of subcortical white matter. It was suitably divided and processed for histology, histochemistry and electron microscopy.

Histology: A small part of the sample was fixed in 10% formalin buffered with Na acetate, and subsequently embedded in paraffin. This tissue was sectioned and stained with hematoxylin-eosin, PAS, PTAH, sudan black B, Bodian, Heidenhain's hematoxylin and Luxol fast blue-cresyl violet stains.

The tissue for histochemical study was either fixed for 20 hours in cold formal calcium with 5% sucrose or for 2½ hours in cold 0.1 M cacodylate-buffered 3% glutaraldehyde, or it was fresh frozen. Frozen sections of fixed tissue were incubated to study the activities of phosphatases: adenosine triphosphatase, adenosine diphosphatase, thiamine pyrophosphatase and acid phosphatase (Gomori medium). The latter enzyme was also studied in fresh-frozen sections that had been cut in a cryostat and fixed 60 minutes in formal calcium. Oxidative enzymes were studied in formal-calcium fixed frozen sections and in fresh-frozen tissue sectioned in a cryostat and fixed for 10-15 minutes in formal calcium or acetone. The enzymes demonstrated were NADH-tetrazolium reductase, NADPH-tetrazolium reductase, a-glycerophosphate dehydrogenase and succinate dehydrogenase. The last two enzymes were studied with menadione and with phenazine methosulfate as intermediate electron acceptor. The methods for phosphatase and oxidative enzyme histochemistry are detailed elsewhere (6).

Fluorescence microscopy: A Zeiss photo-microscope with a UV light source and a dark field condenser was used. The examination was carried out with a UV source of 470 ms wave length. The Huntington's chorea biopsies and the control specimens were examined and photographed at the same time exposures and under identical conditions. The patients used as controls were: one frontal cortical biopsy of a 60 year old woman with a mild dementia and no tissue abnormalities, two specimens obtained at autopsy of elderly patients with no neurological disease (70 and 78 years of age), and sections of cortex of dogs with ceroid-lipofuscinosis.*

Electron microscopy: The biopsy tissue was separated into gray and white matter. Small pieces of both were fixed in 5% glutaraldehyde in 0.057 M phosphate buffer at pH 7.35, with 5% sucrose, for 2 hours and then post-fixed for 30 minutes in Dalton's fixative (7). Some fragments were fixed directly in Dalton's solution or in 2% osmiumtetetoxide in veronal buffer, pH 7.35 (8, 9) for 90 minutes. Dehydration was achieved by successive change in graded alcohols every 15 minutes. Propylene oxide-Epon mixture was used for infiltration at room temperature for 2-10 hours. Epon was polymerized at 45° C for 16 hours and at 70° C for 48 hours. Diamond knives and a Porter-Blum ultramicrotome were used for thin sectioning. The material was examined and photographed in a Siemens 1-A electron microscope operated at 80 KV. In addition, non-frozen sections of 3% glutaraldehyde-fixed tissue were incubated in Gomori's acid phosphatase medium with added sucrose, post-fixed in osmium and processed for electron microscopy.

RESULTS

Histological and Histochemical Observations

The frontal cortex in all four patients showed atrophy. This varied from moderate in the first three patients to marked in the oldest and most advanced case (case 4). The principal histological changes were neuronal loss, most severe in cortical layers V and VI, lipofuscinosis of the nerve cells, and proliferation of cortical astrocytes with massive accumulation of lipopigment in the glial cytoplasm. Satellitosis in the deep layers and low grade macrophage

* We are grateful to Dr. Koppang who kindly provided us this material from the Department of Pathology, Indiana University School of Medicine, Indianapolis, Indiana, U.S.A.
activity around capillaries were common secondary features in this biopsy material (Fig. 1a and 1b).

Neuronal and glial accumulation of lipofuscin was manifest in the P.A.S. and sudan black B preparations and by the histochemical reaction for acid phosphatase (Gomori's technique) (Fig. 2a, b and c). The acid phosphatase reaction product in the cortical astrocytes was in coarse granules forming large cytoplasmic aggregates, usually at one side of the nucleus (Fig. 2c). This deposition was very marked after 30 minutes of incubation with substrate, indicating a very fast rate and high level of enzyme activity in the astrocytes. Neuronal lipofuscin also showed acid phosphatase activity, although the rate and amount of the activity was less than in astroglial lipofuscin. Staining of small particles corresponding to normal sized lysosomes was also seen in neurons, including those with the larger lipofuscin granules, but they were fewer normal. The massive accumulation of lipopigment, rich in acid phosphatase, in the astrocytes throughout the cortex (Fig. 2a) was one of the most remarkable findings. This abnormality was consistently observed and constituted a feature common to all the cases in this series.

Adenosine triphosphatase activity, which is demonstrable in neuroglial membranes, was increased and was related to the richness of fibrous astrocytes. An unusually heavily knit meshwork of glial processes extended from the subpial region well into the second and third cortical layers. Higher than normal
Fig. 2. (a) Section of frontal cortex displaying intense acid phosphatase activity in astrocytes throughout the cortex. Neurons also exhibit acid phosphatase activity. Formal-calcium fixed tissue. Gomori method. X 100 (b) Cortical astrocytes and neurons are rich in PAS positive lipofuscin granules Period Acid Schiff preparation. X 250 (c) Strongly acid phosphatase-positive granules fill up the cytoplasm of three cortical astrocytes. The neuron contains abundant lipofuscin pigment, which is less reactive than that in the adjacent astrocytes. Gomori method. X 450
activity was also present at the cortico-medullary junction and deep cortical layers. The neuronal Golgi apparatus, visualized in thiamine pyrophosphatase reactions, was unremarkable by light microscopy.

Oxidative enzyme activity for NADH-tetrazolium reductase, NADPH-tetrazolium reductase, \( \alpha \)-glycerophosphate dehydrogenase and succinate dehydrogenase was moderately diminished in neurons and cortical astrocytes containing large amounts of lipofuscin. The background activity of the neuropil was normal. In contrast to cortical astrocytes, the white matter astrocytes had high NADH-tetrazolium reductase and \( \alpha \)-glycerophosphate dehydrogenase activities despite the high content of lipofuscin.

The findings in patient number 4, the oldest subject, differed somewhat from the other 3 cases. In addition to all the features described above, he presented the most severe cortical atrophy, and a few senile plaques were scattered in the neuropil. No neurofibrillary tangles were present. From the histochemical standpoint, the acid phosphatase activity of cortical glia was less striking despite their evident lipofuscin content. The number of cortical microglia, best visualized in adenosine diphosphatase and thiamine pyrophosphatase preparations, was considerably increased throughout the cortex. The senile plaques had the same enzymatic and staining characteristics as the plaques seen in other conditions (6).

Unstained paraffin sections and unstained and unfixed cryostat sections of the cortical biopsies were also studied with ultraviolet light to investigate the native fluorescence of the lipopigments found in neurons and glial cells. They were compared with a cortical biopsy of a similar age, with brains of elderly, non-neurological patients and with sections of cortex of dogs with neuronal ceroid-lipofuscinosis (10). Figure 3b shows a field in the middle layers of the cortex in Huntington's disease. Neurons and glial cells were packed with autofluorescent material which exhibited yellowish fluorescence when excited with UV light at 470 m\(\lambda\) wave length. The overall picture differed from normal human ageing brain (Fig. 3a) in the larger amount of pigment accumulated neurons and in the greater involvement of the glia, which accounted for a great deal of the fluorescence in the neuropil. The fluorescent properties of Huntington's chorea lipofuscin were different from the ceroid accumulated in the dog neurons. The latter exhibited white fluorescence when excited by the same UV source, and occurred predominantly in the neurons with no involvement of the neuropil and little fluorescence in non-neuronal cells.

**Ultrastructural Observations**

*Neurons:* Most cortical neurons exhibited abnormalities affecting both the nucleus and the cytoplasmic structures. Large accumulation of lipofuscin granules were frequent in the perikaryon, which sometimes resembled storage cells (Fig. 4) such as those seen in patients with lipidoses. This observation correlated well with the light microscopic changes described above. These neurons, in addition, presented nuclear chromatin which was condensed into numerous small aggregates. The nuclear membrane had an irregular, indented outline with excessive nuclear pores (Figs. 4 and 5).
Fig. 3. Autofluorescence of comparable frontal cortical fields in control: (a) 50 year old woman, with mild dementia and no pathological findings and, (b) a 47 year old patient with Huntington's Chorea, case 3. Autofluorescent pigment is more abundant in neurons, glia and neuropil in Huntington's Chorea. × 250

Other changes in neurons related to lysosomes, to Golgi-associated structures, to endoplasmic reticulum, and lastly, to mitochondria.

Dense bodies were increased in number around areas with large clusters of lipofuscin granules. Although most dense bodies appeared free of inclusions, several contained lamellae within the dense matrix. A rather puzzling phenome-
Fig. 4. Electron micrograph of an abnormal neuron. Nuclear chromatin is condensed in multiple small granules. The nuclear envelope presents innumerable pores throughout its entire perimeter. The perikaryon is distended by large amount of lipofuscin granules. Distension of RER seems might be related to the biopsy and fixation procedure. X 7000
Fig. 5. Another neuron with an abnormal nucleus. Note the dense granules in the nucleus and the indented nuclear membrane with numerous pores. The Golgi complexes are prominent. × 6,500.

Fig. 6. Portion of neuronal perikaryon near the Golgi apparatus. Abundant tubular profiles, smooth vesicles, coated vesicles and multivesicular bodies are present. There are also numerous small lysosomes. Dense aggregates, without a membrane, are seen between ribosomal clusters in the adjacent RER region. × 25,000
non was noticed in areas of the rough endoplasmic reticulum (RER) neighboring the Golgi region where there was dense granular material resembling cores of dense bodies. They were scattered between RER sacs and ribosomes, not in the lumen of RER sacs. Most were not enclosed by membrane, some were partially enclosed by a limiting membrane and others were fully enveloped, resulting in particles identical to small lysosomes (Fig. 6). Possibly the dense material represented tangential cuts of lysosomes.

The membranous sacs of the Golgi complexes, as well as of the RER, were dilated in blocks of tissue which were otherwise regarded as satisfactory from the standpoint of fixation. The smooth endoplasmic reticulum (SER) seemed increased in some nerve cell perikarya, especially by numerous vesicles of various types and tubular profiles (Fig. 6). Dendrites and some terminal axons also had increased numbers of SER tubular profiles (Fig. 7). Some sacs were close to and almost completely surrounded abnormal mitochondria in dendrites. In other cells, the orderly grouping of membranes of RER in Nissl bodies was lost and there was a preponderance of detached ribosomes and ribosomal clusters while the membranous component seemed rather scanty. This evaluation was difficult in cells with large aggregates of lipofuscin, filling cytoplasmic spaces, displacing and distorting the normal distribution of organelles within the cell. Membranous whorls made up of loose membranes were present in

**Fig. 7.** A terminal axon and its presynaptic ending contain tubular profiles and a few synaptic vesicles. An adjacent neuron shows membranous whorls in the cytoplasm under the cell membrane. X 25,000
neuronal cytoplasm (Fig. 7) but true sequestration of organelles in autophagic vacuoles was not seen.

Greatly enlarged mitochondria with very few cristae and the matrix occupied by dense granular material were seen in dendrites and in glial cells (Figs. 8 and 9). Neurites had occasional mitochondria filled with fibrillary material arranged in small fascicles. These mitochondria were surrounded by smooth ER (Figs. 10a and 10b). In cross section the filaments appeared arranged in a crystalline pattern (Fig. 10b). Other planes of section disclosed several bundles with a slightly curvilinear course. Some cristae were preserved and present between these fascicles.

Astrocytes: Proliferation of the astroglia was characteristic of this pathological material. Glial cell processes rich in glycogen and with bundles of 60-70Å glial filaments were increased in the neuropil and especially in the molecular layer and deep cortex. All the astrocytic cell bodies and many glial processes contained clusters of coarse, large granules of lipopigment (Fig. 11). The glial lipofuscin differed from neuronal lipofuscin in that it was more varied in appearance (Figs. 11 and 12). There was a dense component of variable electron density. Vacuoles were more abundant than in neuronal lipofuscin; some of them contained homogenous material of very low electron density. It was interesting to note that the patient with the longest course of the disease had lower acid phosphatase activity in glial lipofuscin, both at the light microscopic and electron microscopic levels. Glial lipofuscin in this patient was predominantly of a vacuolated type (Fig. 12). The electron dense component was present, but not as abundant as in the other cases. This correlated well with the fact that this enzyme activity was found in the dark granular component of lipofuscin (Fig. 13). Astrocytic mitochondria in all patients were usually large with a dense granular matrix (Fig. 12). Increased numbers of centrioles were seen in astrocytes.

Neuropil: Scattered degenerating axons were present in all cases. They had dense axoplasm filled with numerous vacuoles (Fig. 8). The myelin sheath was still present around them. These are, most likely, axons undergoing secondary changes due to neuronal cell damage. Abnormal presynaptic endings were also seen throughout the neuropil. They were more abundant in the neuropil of the deep cortex where neuronal damage and loss were greatest. The frequency of this change was related to the length of the illness and the severity of the degenerative process. The abnormal endings were crowded with synaptic vesicles and were denser than usual. They contained dense bodies limited by a membrane, and also whorls made up of loosely packed concentric layers of membrane (Figs. 14 and 15). The elements of the synaptic junction had a normal structure and no abnormalities were noticed in the post-synaptic membrane or sub-synaptic web (Fig. 15, arrows). The post-synaptic dendritic process sometimes contained an excess of SER membranes (Fig. 15, long arrow). An excessive number of astrocytic processes filled up the interstices amongst the various cell elements of the neuropil.

Other cells and blood vessels: Microgial cells were characterized by an
FIG. 3. Low power field of the neorou. Cells containing enlarged, dense mitochondria with few cristae are seen in the lower half of this field (arrows). Three axons undergoing degeneration are present (arrow heads). × 12,000
Fig. 9. Enlarged mitochondrion, with dense granular matrix and very few cristae. × 32,000

Fig. 10. (a) and (b) Abnormal mitochondrion with unusual fibrillar fascicles in the matrix. The fascicles form paracrystalline arrays in cross section (b) and have a slightly curvilinear course (a). Few cristae are present between the fascicles. × 24,000

elongated nucleus with dense chromatin, usually distributed in coarse aggregates at the periphery, and by a scanty, very dark cytoplasm. They were in both the neuropil and the subcortical white matter. These cells contained various amounts of osmiophilic granules. Macrophages engulfing large fragments of cells with lipofuscin were occasionally seen in the neuropil (Fig. 16), and more commonly in the perivascular spaces (Fig. 17). The phagocytosed material in Figures 16 and 17 appears to be part of neuronal cytoplasm, judging from the type of lipofuscin granules. A membrane surrounds the engulfed material, which in turn is entirely surrounded by microglial cytoplasm. Similar findings were seen in other areas of the biopsies. This process of
phagocytosis of neurons filled with lipofuscin was most intense in patient 4, but present in all others. Excessive numbers of satellite cells surrounded nerve cell perikarya in the deep layers of the cortex. Many of these cells we interpreted as of probable microglial origin (Fig. 18). Accumulation of lipofuscin was seen in
Fig. 12. The pigment granules in astrocytes have varied ultrastructural features. In this cell, electron lucent and pale vacuoles are more abundant than the dense portion of the granules. Note the cytoplasm rich in fibrils and glycogen, and very dense mitochondria. × 5,000

Fig. 13. Electron micrograph of glial cell cytoplasm showing localization of black deposits representing acid phosphatase activity in the dense granular component of lipofuscin. Gomori method. × 8000
cells adjacent to the basement membrane of vessels. Perithelial and endothelial cells also contained masses of lipofuscin and, sometimes, huge lipid aggregates with numerous vacuoles (Fig. 19).

DISCUSSION

1. Clinico-Pathological Correlation

Our four patients had classical clinical and genealogical histories of Huntington's chorea. The cortical samples were taken from comparable areas of the frontal lobes. It was found that there was a direct correlation between the length of clinical illness and the severity of the pathological changes, especially in regard to the degree of cortical atrophy. The histopathological findings were otherwise quite consistent in all four biopsies. In only one case (F.R.), were additional pathological changes found. This was a 65 year old man with an illness of 18 years duration, who also appeared physically very aged. He had moderate numbers of senile plaques in the frontal cortex. Neurofibrillary tangles such as those seen in Alzheimer's dementia were absent in sections scanned by silver impregnation and with the electron microscope. In every other regard, this patient's cortex was similar to those of the other three cases and provided the most advanced example of Huntington's disease. Association of senile plaques with other pathological processes in the presenile period has been reported
Fig. 16. Two microglial cells in the neuropil of the deep cortex. One of them contains abundant lipofuscin of probable neuronal origin. × 5,000
Fig. 17. Macrophage in a perivascular space. It contains large lipofuscin granules. × 5,000

(11). Their occurrence in low numbers in the frontal cortex of elderly non-demented people, has been noted by Tomlinson et al. (12). It is impossible to ascertain here whether the presence of plaques is fortuitous and coincidental, or whether their production has been favoured by the ongoing degenerative process of neurons and neurites which is taking place in the cortex.
Fig. 18. One neuron surrounded by satellite cells. The origin of satellite cells is difficult to establish; in this micrograph, two are probably oligodendrocytes and the third may be microglial. × 3,200

Fig. 19. Cortical capillary. Lipid granules are present in perithelial cells. Large lipid deposits are also present in perivascular cell processes, sometimes identifiable as of glial origin. × 10,500
II. Light Microscopic Studies

A re-evaluation of the classical histological picture was possible, aided by a correlation with histochemical and ultrastructural findings. One of the questions that we have tried to answer is the following: Is there a common pattern of pathological changes which enables us to recognize the disease with the light microscope? In our material, three main features became quite apparent. Although each feature by itself does not constitute a specific abnormality, the consistency of all these, considered together, provided a common profile of the changes observed in the cortex of patients with Huntington's chorea.

The first feature was the increase in lipopigment in neurons and astrocytes. This accumulation was estimated as considerably higher than expected for age. Preliminary data obtained after extraction and purification of lipofuscin from Huntington's chorea frozen brain cortex confirmed this observation and showed yields several times higher than those from ageing brains (13). The acid phosphatase activity of cortical astrocyte lipopigment was strikingly high and universal, making this deposition a phenomenon quite different from that generally observed in other dementias with gliosis, or in normal ageing brains. This histochemical finding had a remarkable constancy and singularity. Fluorescent microscopy was helpful in further demonstrating the distribution of autofluorescent lipopigment within the cortex. The possible significance of this finding will be discussed in a later section of this paper.

The second feature was neuronal loss. Death and removal of neurons seems to be an ongoing process throughout the course of the disease, as manifested by progressive atrophy of the cortex. Another feature which supports the idea of slow, scattered degeneration of nerve cells is the presence of brain macrophages in all biopsies, regardless of the evolutionary period of the case. Fully transformed macrophages were common in perivascular spaces; infiltration of the neuropil and satellitosis by microglial cells were quite frequent in areas of maximal damage. Small dark cells were abundant around capillaries, and probably corresponded to activated microglia. In no case were there cuffs of hematogenous cells, although rare lymphocytes were seen with the electron microscope.

The third feature was proliferation of astrocytes. Classical techniques for staining astrocytes, such as Holzer stain or PTAH (phosphotungstic acid hematoxylin) revealed transformation of gray matter astrocytes into fibrous astrocytes with increased numbers of fibrillary processes, as well as a total increase in the population of these cells throughout the cortex. Our results in the adenosine triphosphatase and electron microscope preparations confirmed the fibrillary gliosis. Cortical gliosis, however, has usually been of a lesser degree than the gliosis in the caudate or putamen. Most previous studies have interpreted gliosis as a reactive response secondary to the loss of nerve cells. The constancy and intensity of the phenomenon suggested to Bruyn the possibility of a primary glial disorder (1). Our histochemical acid phosphatase findings, along with the electron microscopic observations, revealed diffuse and intense
involvement of the astroglia by intracellular pigment deposition. This observation raised the possibility that a more generalized metabolic disorder might be affecting both neurons and glia and that this metabolic abnormality was expressed, ultimately, in increased formation of lipopigment. This hypothesis is further discussed below in relation to the electron microscopic findings.

III. Electron Microscopic Studies

The changes established by means of the electron microscope indicated various abnormalities in brain cells, particularly in the neurons. That these changes were, indeed, of an irreversible nature was signaled by the presence of macrophages, phagocytosing and removing fragments of dead nerve cells. Neurons evidenced major abnormalities in regard to: a) the intracellular membranes; b) the formation of lipofuscin at an excessive rate; c) enlarged, granular mitochondria; and d) nuclear changes.

All membrane systems of the neuron were disturbed. The nuclear envelope was interrupted by numerous pores with very little of the normal envelope left. The cisterns forming part of the Golgi apparatus were dilated and there seemed to be an increase in the number of SER membranes relative to those of RER. Tubules and vesicles were abundant in cytoplasm of the cell body, dendrites, axons and endings. Redundant membranes apparently not related to poor fixation and forming whorls were not uncommon. They were connected with the outer cell membrane and usually found in the superficial part of the perikaryon under the cell membrane. Other loose membranous arrays were found in the nerve endings and dendrites. Although these changes can be analyzed and discussed separately, one of our conclusions is that in Huntington’s Disease there seems to be a general disturbance of the cell reflected first and most profoundly in a derangement of all membrane structures that form the cell itself rather than a disturbance affecting one functional cell compartment or organelle. It seems warranted to suggest that the normal equilibrium between the degradation and rebuilding processes of membranes has been lost, and as a result, there is increased lipofuscin formation.

Lipofuscin is a complex polymer, formed of lysosomal hydrolases, cations, phospholipids and non-polar lipid polymers, glycolipids and insoluble residues. It is an autofluorescent pigment (14). Lipofuscin deposits are regarded as residual bodies representing the insoluble auto-oxidized residuum of material accumulated during a process of autophagocytosis (15). Some cells, like the neurons, cannot discharge such material and are subject to progressive deposition and accumulation. It has been established experimentally that in conditions leading to liberation of free radicals, like ionizing or ultraviolet irradiation, or in deficiency of antioxidants such as vitamin E (16, 17), lipid peroxidation is enhanced leading to damage of unsaturated membrane lipids, degradation of membranes and formation of lipofuscin.

Enlarged mitochondria with diminished numbers of cristae and a granular matrix of increased electron density have been produced in liver cells of experi-
mental animals submitted to a vitamin E deficient diet (18), also in a combined
deficiency of both vitamin E and factor 3-selenium (19), and in dietary defi-
ciency of essential fatty acids (20). Administration of a copper-chelating agent
such as cuprizone, which is a powerful inhibitor of amine oxidase, resulted in
the formation of giant mitochondria in mouse hepatocytes but not in brain cells
(21). Other diverse pathological stimuli acting upon nervous tissue can cause
mitochondrial swelling (22–26). Unlike the changes described in this report,
swelling of the mitochondria in the latter experimental conditions was accom-
panied by dissolution of the cristae and an enlarged pale matrix. Rats fed a
vitamin E-deficient diet developed dystrophic axons which were rich in abnor-
mal mitochondria and dense bodies (27). Mitochondrial enlargement and pleo-
morphism were commonly observed. Dissolution of the cristae and the appear-
ance of abnormal intramitochondrial deposits of glycogen granules in some
instances, and of an amorphous finely granular, dense material in others, were
conspicuous findings. Both autophagic and non-autophagic dissolution of mito-
chondria appeared characteristic of this condition of chronic neuronal injury.
Similar changes have been observed also in dystrophic axons of the aged rats.
Vitamin E deficiency may favor these changes, but cannot be regarded as the
only factor that can cause such changes. It is known that mitochondrial mem-
branes are quite different from other biological membranes in regard to their
function and chemical composition. The constituents responsible for electron
transport and oxidative phosphorylation occur only in the inner mitochondrial
membrane. Lipids are involved in the structural integrity of the mitochondrion.
More than 90% of mitochondrial lipids are phospholipids, which are signifi-
cantly high in unsaturated fatty acyl chains (28). Uncoupling of oxidative
phosphorylation and conspicuous ultrastructural changes of the mitochondria
have been observed experimentally with a diet deficient in essential fatty acids
(20). The abundance of unsaturated fatty acid chains would make mitochon-
dria especially vulnerable to damage in any circumstance leading to increase in
intracellular oxidation.

Seattered mitochondria in these biopsies were filled with fasseicles of filaments
in paracrystalline formations. The few remaining cristae and the double outer
membrane were seemingly intact. Filamentous inclusions have been observed in
the mitochondria of glial cells and neurons of the normal rat striatum, but they
were characteristically located in the intracristal space (29). Intramitochon-
drial filamentous helical structures have been found in pathological tissues such
as liver cells after a protein-deficient diet (30), or after prolonged ethanol
ingestion (31) and in myopathies (32, 33). None of these resembles the fila-
mentous inclusions described here. In our material, the abnormal mitochondria
had greatly decreased inner membrane surface which would probably imply
abnormal function.

Since a generalized alteration of membrane structures is present in Hunt-
tington’s disease, including the abnormalities in mitochondria, it is conceivable that
a basic alteration may be present in the structural proteins or proto-lipid
moieties of membranes, which would be thus unable to maintain a physiological
rate of turnover and would be subjected to accelerated degradation and, ultimately, necrosis of the cell.

One question needs to be considered in regard to the abnormalities discussed above. Are these manifestations of the basic metabolic abnormality in Huntington's chorea, or could they possibly be caused by the action of other agents such as long term drug treatment to which some of these patients have been submitted? The answer to this question is necessarily limited by the nature of our material. It would be necessary to compare biopsies of similar areas in patients treated with similar drugs in pathological conditions different from hereditary chorea, and also in patients who have never received pharmacological therapy.

It is known that phenobarbital (34) and reserpine (35) administration stimulate the synthesis of endoplasmic membrane in the liver, leading to increase in SER and RER cisternae, with large proliferations and redundant whorls. Concomitant with this, is a parallel increase in hepatic membrane enzymes. In our biopsy material, the changes are not predominantly in the direction of membrane overgrowth, except perhaps for a relative increase in smooth endoplasmic membranes, but towards a disintegration and increased residual body formation. It is possible that drug action may have influenced the pathological changes here observed, but the abnormalities noted in this material were different from those observed in experimental intoxication with drugs, in regard to degree, as well as in the distribution of the pathological involvement.

The neuronal nuclei in Huntington's chorea were also the site of numerous abnormalities, such as clumping of chromatin in small aggregates and increased numbers of nuclear pores. These changes may be reflecting an important disturbance of the processes of protein synthesis. Although chromatin aggregation is a non-specific, rather prompt response to diverse forms of injury to cells (36), it may be worth indicating that nuclear changes such as we observed have not been noted in other dementias such as Alzheimer's, Pick's, or Creutzfeldt-Jakob's diseases, which were obtained and fixed under identical laboratory conditions. Clumping and condensation of the chromatin somewhat similar to that observed here, but even more profound, has been seen in neurons of patients with slow virus infection, such as subacute sclerosing panencephalitis (37, 38). From this comparison, we can draw a parallel to further emphasize the possible meaning of these nuclear changes as related to deranged metabolism in severely disturbed cells. There was no electron microscopic evidence to indicate the presence of a virus in the brains of our patients with chorea.

The role of the glia in the pathogenesis of this disorder is not well understood. Light microscopists have generally assumed that astrocytosis was reactive and secondary to neuronal degeneration. Our findings demonstrate active participation of astroglia in lipofuscin deposition associated with high activity of acid phosphatase. It is possible that astrocytes may be participating in catabolic degradation of abnormal products resulting from neuronal breakdown. An alternative possibility is that astroglia are involved in a more general metabolic disorder leading to excessive formation of lipofuscin by both neurons and glial cells. While neurons, in these cases, seem to reach a state of irreversible damage
and die, astrocytes continue to proliferate and divide. Glial end feet around the vessel wall were filled with lipopigment granules, suggesting that astrocytes participate in mobilizing pigment towards the vessel. Accumulation of lipofuscin granules in astrocytes has been seen in human (11) and experimental Creutzfeldt-Jakob disease in chimpanzees (39). Neither the extent or degree of accumulation was as severe as that found in our cases.

Our findings confirm the observations by Tellez-Nagel and Wisniewski (Arch. Neurol. 29, 324–327, 1973) who describe straight 150° wide tubules in neurofibrillary tangles of another case of PSP and who also emphasize that this change is distinct from other manifestations of human or experimental, neurofibrillary pathology.

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