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THE PATHOLOGY OF AN INHERITED LYSOSOMAL STORAGE DISORDER OF CALVES

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ABSTRACT

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The clinical symptoms, gross and histopathological, as well as the ultrastructural appearance of a condition in cross-bred Aberdeen Angus calves resembling the inherited lysosomal storage disease, α-mannosidosis, are reported.

The neurones and perithelial cells in the brain and the reticuloendothelial cells in the lymph nodes and spleen were extensively vacuolated. The vacuoles were filled with a sparse, flocculated to granular material within which membranous structures were frequently seen. No specific substance could be identified within the vacuoles, either histochemically or ultrastructurally. Besides the vacuolation, cystic tubular structures were seen in the kidneys. The lining epithelial cells of the thyroid follicles were vacuolated while some of the follicles contained no colloid.

Résumé

PATHOLOGIE D'UN TROUBLE HÉRÉDITAIRE DU STOCKAGE LYSOSOMIAL CHEZ LE VEAU

On décrit les symptômes cliniques, les lésions anatomo-pathologiques et histo-pathologiques ainsi que l'apparence ultrastructurelle d'une condition qui a été observée chez des veaux hybrides Aberdeen-Angus et qui ressemble au trouble héréditaire du stockage lysosomial, l' α-mannosidose.

Les neurones et cellules périthéliales du cerveau ainsi que les cellules réticuloendothéliales des ganglions lymphatiques et de la rate étaient largement vacuolaires. Les vacuoles étaient remplies d'un matériel épars, floculé à granuleux, à l'intérieur duquel on pouvait souvent voir des structures membraneuses. On n'a pu identifier aucune substance spécifique à l'intérieur des vacuoles, que ce soit histochimiquement ou par étude de l'ultrastructure. Outre la vacuolisation, on a observé dans les reins des structures cystiques tubulaires. Les cellules épithéliales des follicules thyroïdiens étaient vacuolaires tandis que certains des follicules ne contenaient pas du colloïde.

INTRODUCTION

Much work has been done on the pathogenesis of lysosomal storage diseases in man, but comparatively little on domestic animals.

Blakemore & Palmer (1971) stated that these metabolic disorders are usually linked with lysosomal enzymatic deficiencies which result in the accumulation of storage material in various cells; also that a deficiency of a single enzyme may provoke a chain reaction resulting in the storage of various substances. According to these authors, cerebral lipodoses or "amaurotic familial idiocies" differ from leucodystrophies in that in the former the lesions are primarily in the neurones, while in the latter the white matter is mainly involved with demyelinization and degenerative changes. Various authors have also described perivascular accumulation of globoid cells containing myelin breakdown products in the leucodystrophies.

Some of the lysosomal storage diseases in domestic animals resemble metabolic disorders in man; for example, in sheep, lipidosis of the reticuloendothelial cells in the liver is very similar to Gaucher's disease (Laws & Saal, 1968); in dogs, cerebral lipidosis or lipodystrophy reveals similarities to Tay-Sachs disease (McGrath, Kelley & Steinberg, 1968; Ribelin & Kintner, 1956); globoid cell leucodystrophy, also in dogs, is comparable to Krabbe's disease in man (Fletcher, Kurtz & Low, 1966; Hirth & Nielsen, 1967; Kurtz & Fletcher, 1970; Suzuki, Austin, Armstrong, Suzuki, Schenker & Fletcher, 1970; Frankhauser, Luginbühl & Hartley, 1963) and in calves, GM_1 gangliosidosis corresponds to the same condition in children (Donnelly, Sheahan & Kelly, 1973).

Whittem & Walker (1957) reported the first cases of "pseudolipidosis" in Aberdeen Angus calves, but they were unable to demonstrate any defects in the lipid metabolism in these animals. Hocking, Jolly & Batt (1972) detected an α-mannosidase deficiency in these calves and Jolly (1974) suggested the term "mannosidosis" to replace the misleading name "pseudolipidosis".

The object of this paper is to report on a condition in cross-bred calves which pathologically resembles mannosidosis. As far as could be ascertained, this is the first report of this condition in the Republic of South Africa.

HISTORY AND CLINICAL SYMPTOMS

The calves examined came from a herd comprising a pure-bred Simmentaler bull and cross-bred cows, originating from Afrikaner, Shorthorn, Gallaway, Aberdeen Angus, Hereford and Friesland stock. The bull was bred with 34 of his daughters. From these matings 8 abnormal calves were born. The owner reported that affected calves resulted only when the bull was mated with his daughters born out of cross-bred Aberdeen Angus/Shorthorn and Aberdeen Angus/Simmentaler cows. However, one of the affected calves examined was sired by this bull out of a grade Afrikaner cow.

All the affected calves were born alive but were unable to rise, were apathetic, and showed head tremors. There were no visual defects in any of the animals. By assisting one of these calves to drink, the farmer managed to keep it alive for nearly 2 months.

MATERIALS AND METHODS

Formalin-fixed organ specimens of a calf which was unable to rise after birth were received for histopathological examination and a tentative diagnosis of lipidosis or mannosidosis was made. A further 4 live calves, submitted from the same herd, were euthanized with pentabarbitone sodium at 4–6 days of age and autopsied. Various tissues from these calves were collected in 10% buffered formalin for processing and embedding in paraffin wax. Sections were cut at 3–6 μm and stained with haematoxylin and eosin (HE). In addition, various staining techniques were applied to various tissues (Table 1).

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TABLE 1 Staining techniques applied to various tissues

Stained for	Reagent	Tissue
1. Mucopolysaccharides.....	Periodic acid Schiff (P.A.S.) (Pearse, 1961)...	Brain, spinal cord, kidneys, pancreas, spleen, lymph nodes, thyroid
2. Unsaturated lipids.....	Performic acid Schiff (PFAS) (Thompson, 1966)	Brain, spinal cord, kidneys, pancreas, spleen, lymph nodes, thyroid
3. Acid mucopolysaccharides....	Alcian blue (Anon, 1968).....	Brain, spinal cord, kidneys, pancreas, spleen, lymph nodes, thyroid
4. Myelin.....	Luxol fast blue (LFB) (Margolis & Pickett, 1956)	Brain, spinal cord
5. Lipids.....	Oil red O (ORO) (Pearse, 1961) Frozen sections	Brain, spinal cord, kidneys, pancreas, spleen, lymph nodes
6. Phospholipids.....	Menschik's Nile blue sulfate (Anon, 1968)....	Brain, spinal cord, kidneys, pancreas, spleen, lymph node, thyroid

Segments (1–2 mm³) of brain, lymph node and spleen from 2 of these calves were collected in 4% phosphate buffered glutaraldehyde (pH 7,25) for electron microscopy, post-fixed in 2% buffered osmium tetroxide, dehydrated in a graded series of ethyl alcohols and embedded in Epon 812.

For the purpose of orientation, sections 1–2 μm thick were cut and stained with toluidine blue. The staining of these ultra-thin sections with 1% aqueous uranyl acetate at 60 °C was followed by treatment with Reynolds' lead citrate at room temperature for examination with the electron microscope.

Pathology

Gross Pathology

The periorbital region and forehead in 3 of the calves were unduly pronounced and appeared thickened. As a result of this the eyes appeared smaller than normal (Fig. 1).

FIG. 1 Calf with thickening of the periorbital region and forehead. The eyes appear smaller than normal

A moderate congestion and oedema of the brain in all the calves resulted in a narrowing of the gyri. Compression of the cerebellum inside the calvarium, scattered submeningeal petechial haemorrhages and severe congestion of the cerebellum were seen in one of the animals. In another animal a slight internal hydrocephalus was present.

In 3 of the calves the cortex and, to a lesser extent, the medulla of individual kidney lobules were almost obliterated by small cysts, 1–2 mm in diameter. The kidneys and liver were slightly swollen and yellowish-brown in colour.

Histopathology

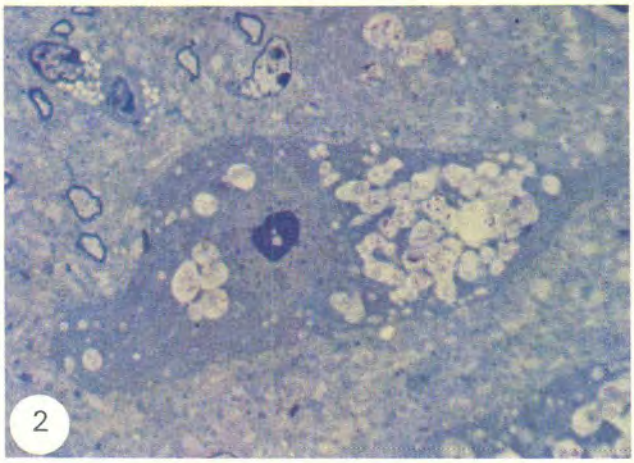
Light microscopy

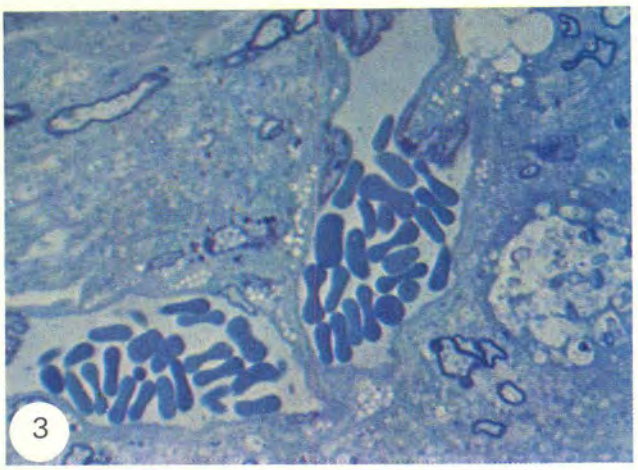
Central nervous system (CNS). The neurones and perithelial cells (pericytes) were those mainly affected and they revealed a variety of vacuolar changes in their cytoplasm.

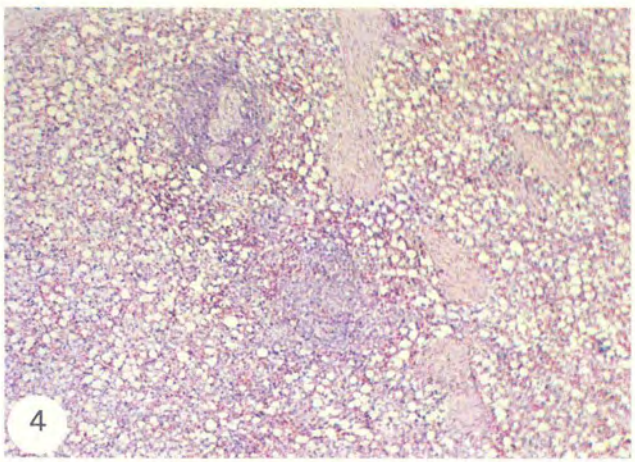
Although affected nerve cells occurred throughout the brain, the more severe changes were seen in the motor neurones of the spinal cord and in the neurones of the thalamus, medulla, internal capsule, cerebral cortex and hippocampus. The smaller and medium-sized neurones in the lateral geniculate showed more severe vacuolation than the bigger neurones in the thalamic region. The Purkinje, basket and Golgi cells in the cerebellum were mildly affected. In addition, some of the Purkinje cells had a dark-blue, elongated and shrunken appearance. The cytoplasm of these neurones stained less intense in HE sections and appeared foamy. Vacuolation of the cytoplasm of neurones was a consistent finding in all the calves and practically every neurone in the above-mentioned areas was affected (Fig. 2). These vacuoles varied in number, size and shape, some of them having ill-defined edges and many containing membranous structures (Fig. 2). Some neurones showed 2–3 large vacuoles displacing the nucleus, while in others the entire cytoplasm was filled with small vacuoles, especially in the motor neurones of the spinal cord. The nuclei of the affected neurones appeared to be normal as a rule but, in markedly vacuolated neurones, karyolysis and pyknosis were evident.

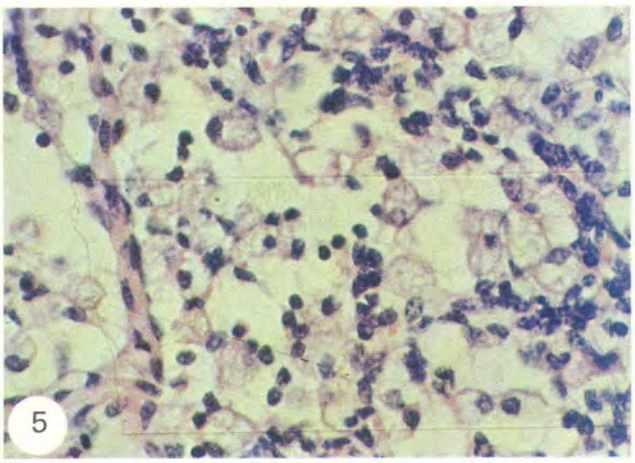
The perivascular spaces were frequently dilated and contained a light pink amorphous material. Cytoplasmic vacuoles, similar to those found in the neurones, were seen in the perithelial cells (Fig. 3).

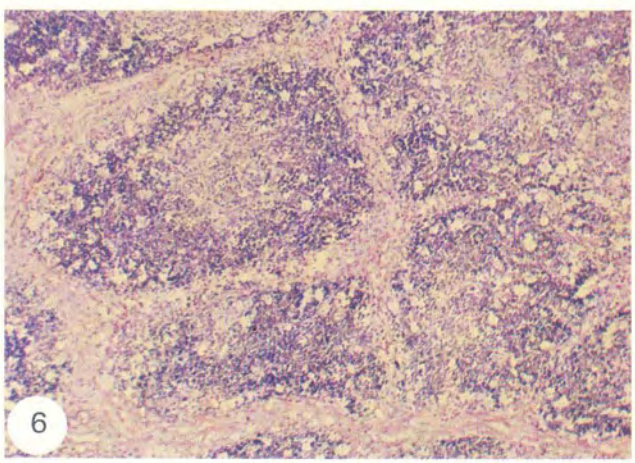
Hyalin bodies (spheroids) occurred in the white and grey matter in all 5 cases and were particularly prominent in one animal. Inflammatory changes, such as gliosis, mineralization and perivascular mononuclear cell infiltrations, were noticed in the midbrain of one calf only. There was no indication of demyelinization in any of the cases examined. Small, scattered haemorrhages throughout the substance of the brain, but mainly in the molecular layer of the cerebellum, were seen in one of the calves. Various peripheral nerves were also examined, but these appeared normal.

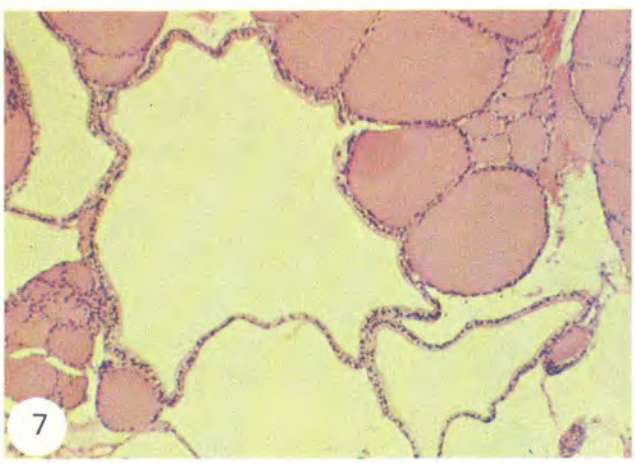


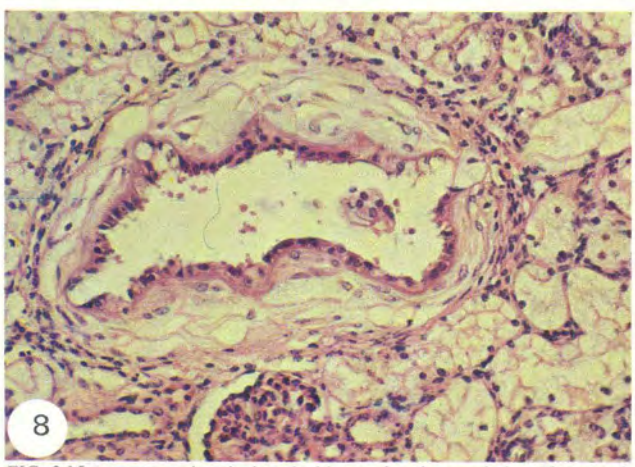












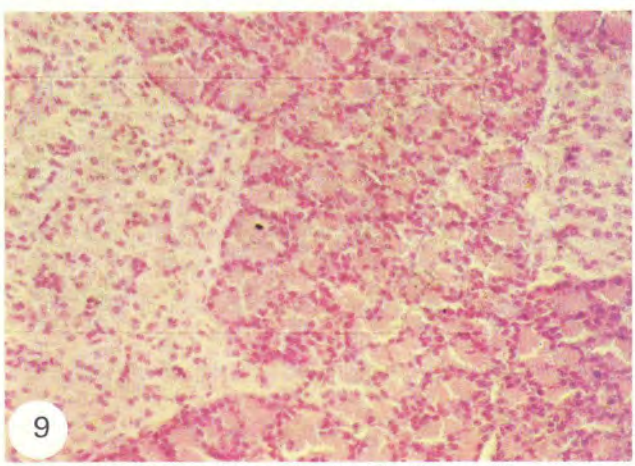
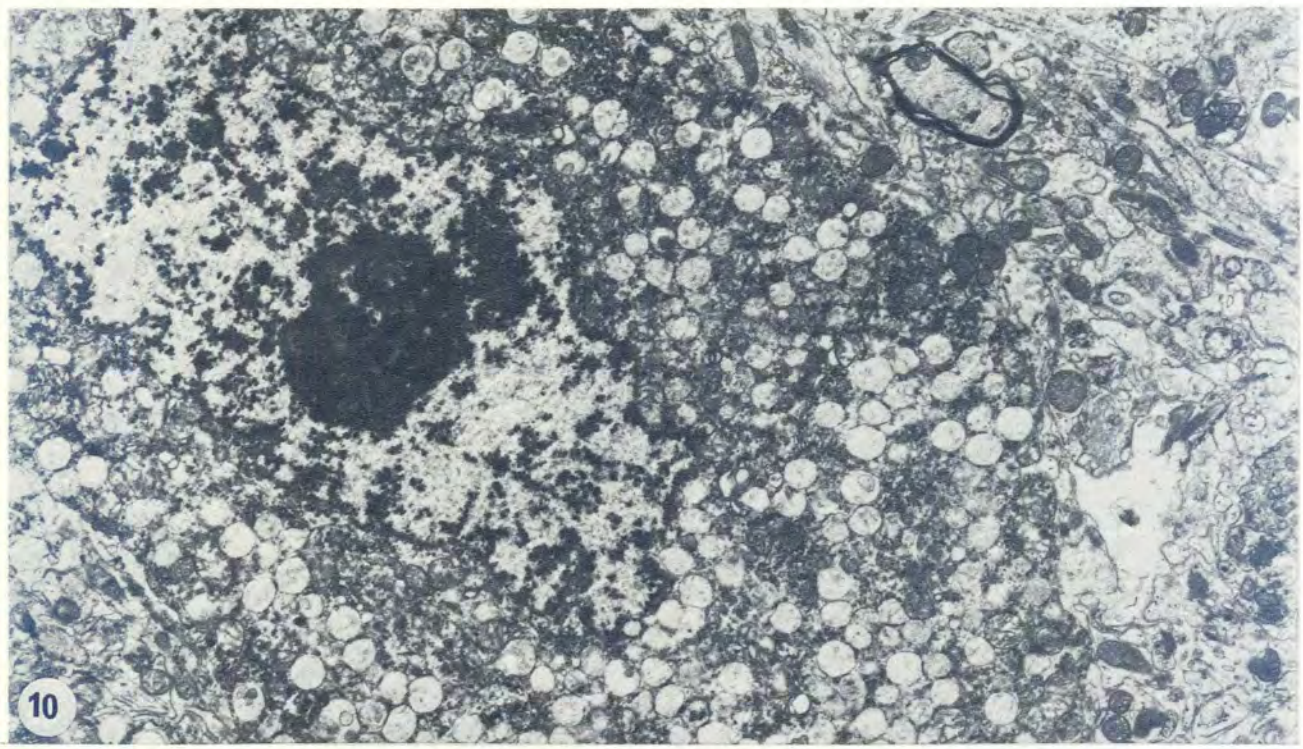
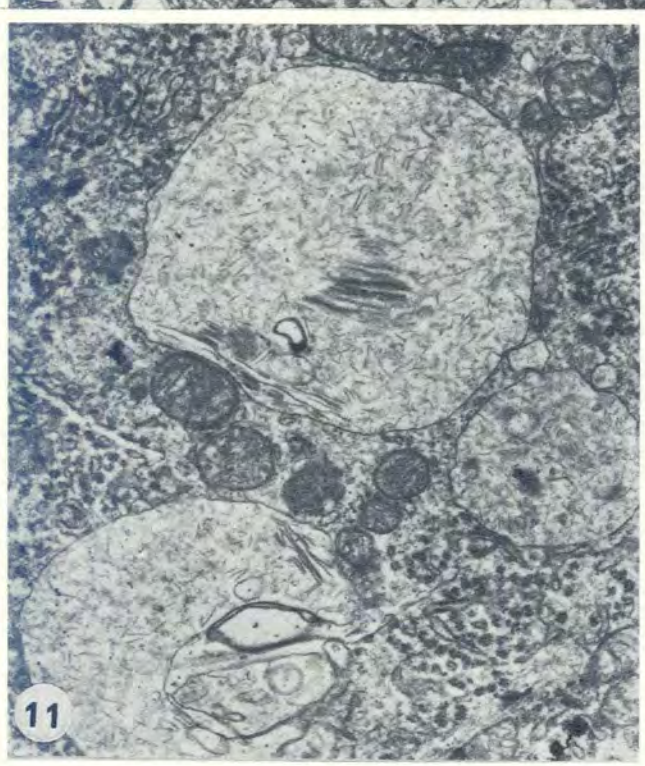


FIG. 2 Motor neurone in spinal cord with cytoplasmic vacuolation. Note membranous structures in vacuoles. Epon section. Toluidine blue. ×1 200
FIG. 3 Brain capillary with vacuolation of the perithelial cells. Epon section. Toluidine blue. ×1 200
FIG. 4 Spleen with many big vacuoles in the reticuloendothelial cells. HE×75
FIG. 5 Medullary sinuses of lymph node showing vacuolation of the reticuloendothelial cells. HE×500
FIG. 6 Distinct vacuoles in the cortex and medulla of the thymus. HE×75
FIG. 7 Thyroid with dilated empty follicles and vacuolated epithelial cells. HE×200
FIG. 8 Kidney showing big tubular structures lined with a cuboidal epithelium and surrounded by a loose connective tissue. Note the many vacuoles in the convoluted tubular epithelium. HE×500
FIG. 9 Focal areas of pale staining pancreatic acinar cells. HE×500





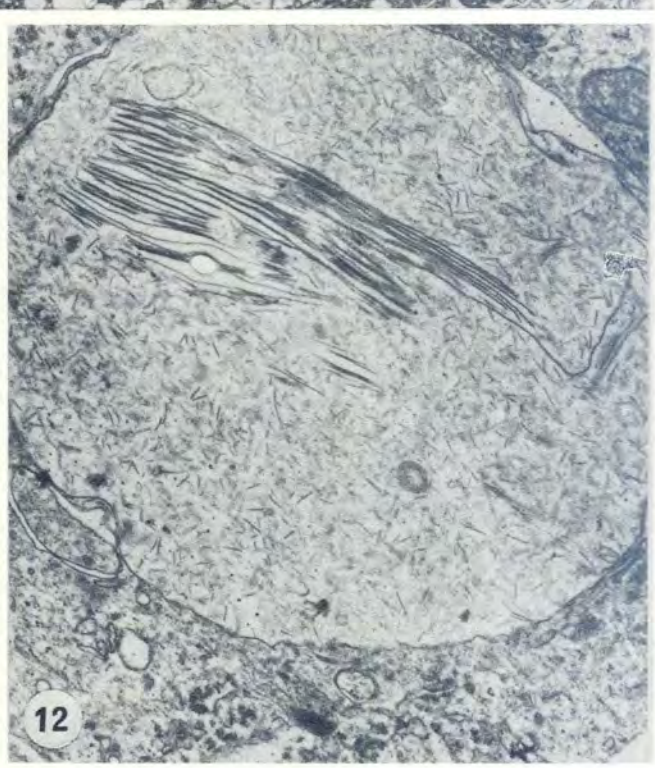
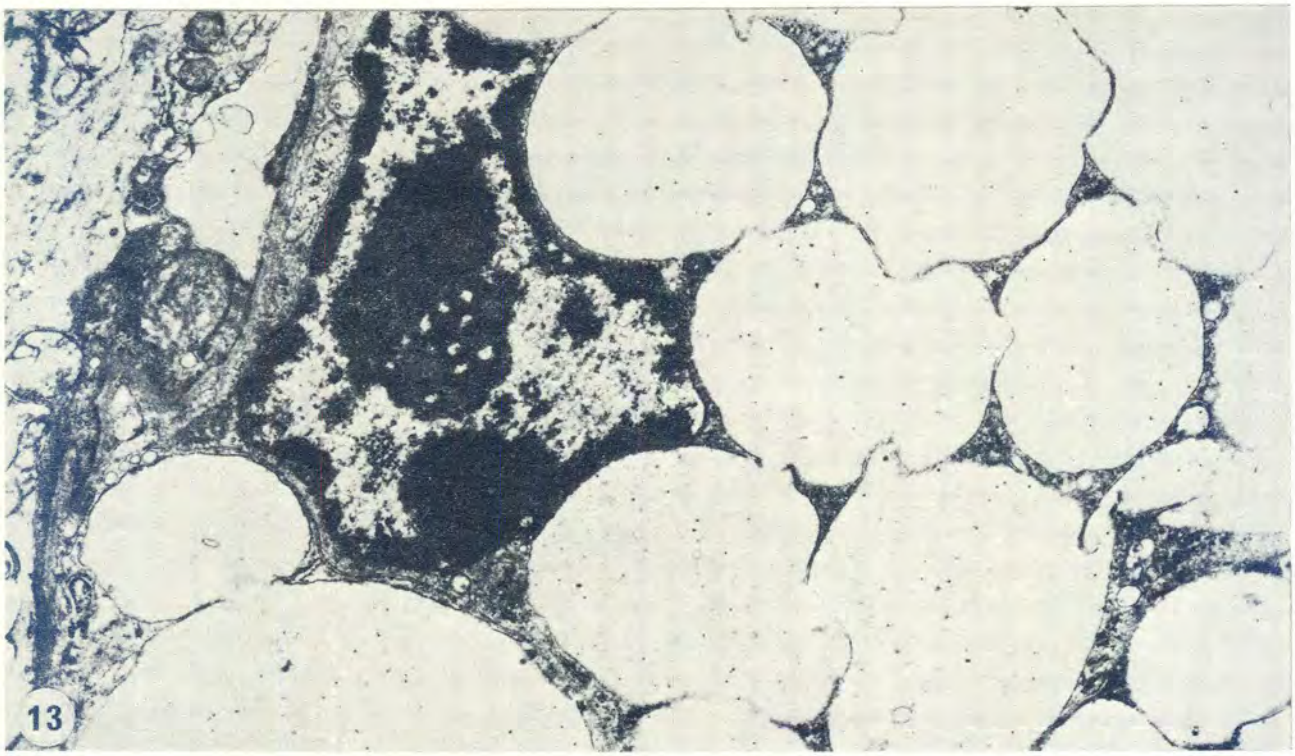


FIG. 10 Neurone with numerous vacuoles interspersed between the cytoplasmic organelles. ×7 800
FIG. 11 & 12 Neurone with vacuoles containing membranous structures and a fibrillar material. ×1 650



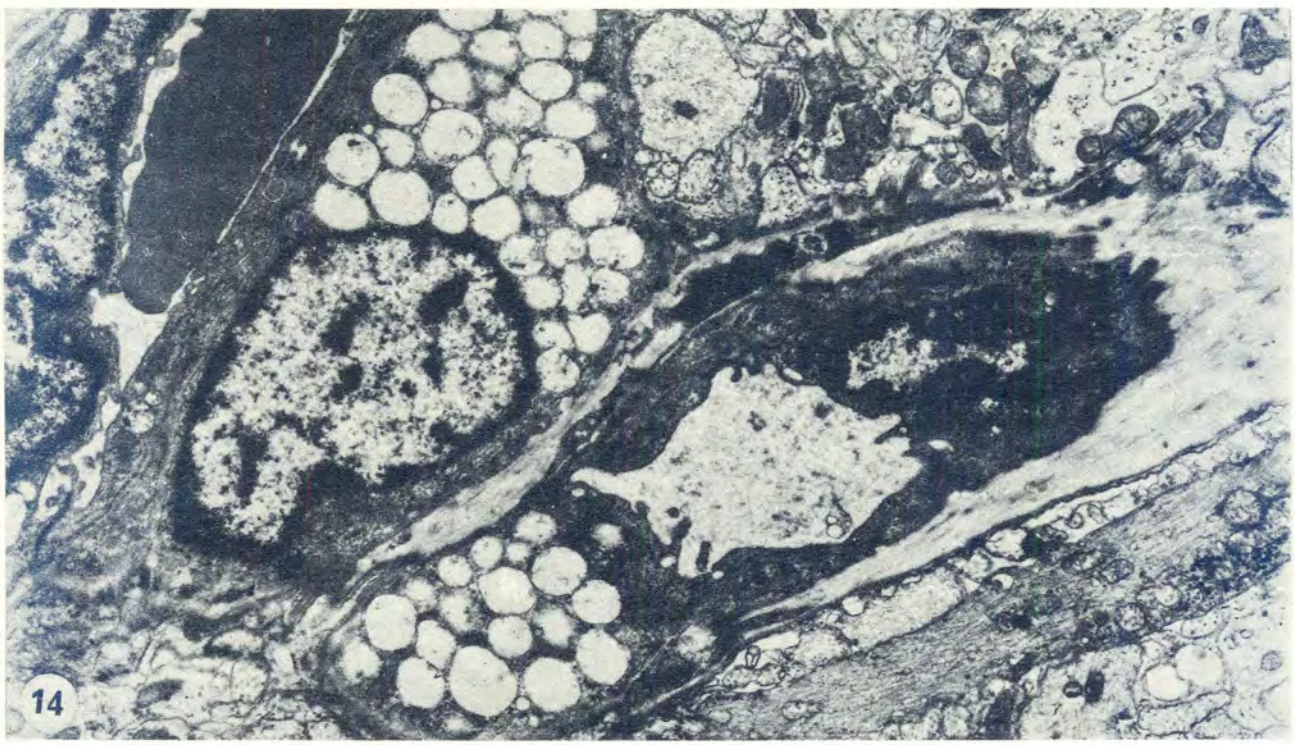
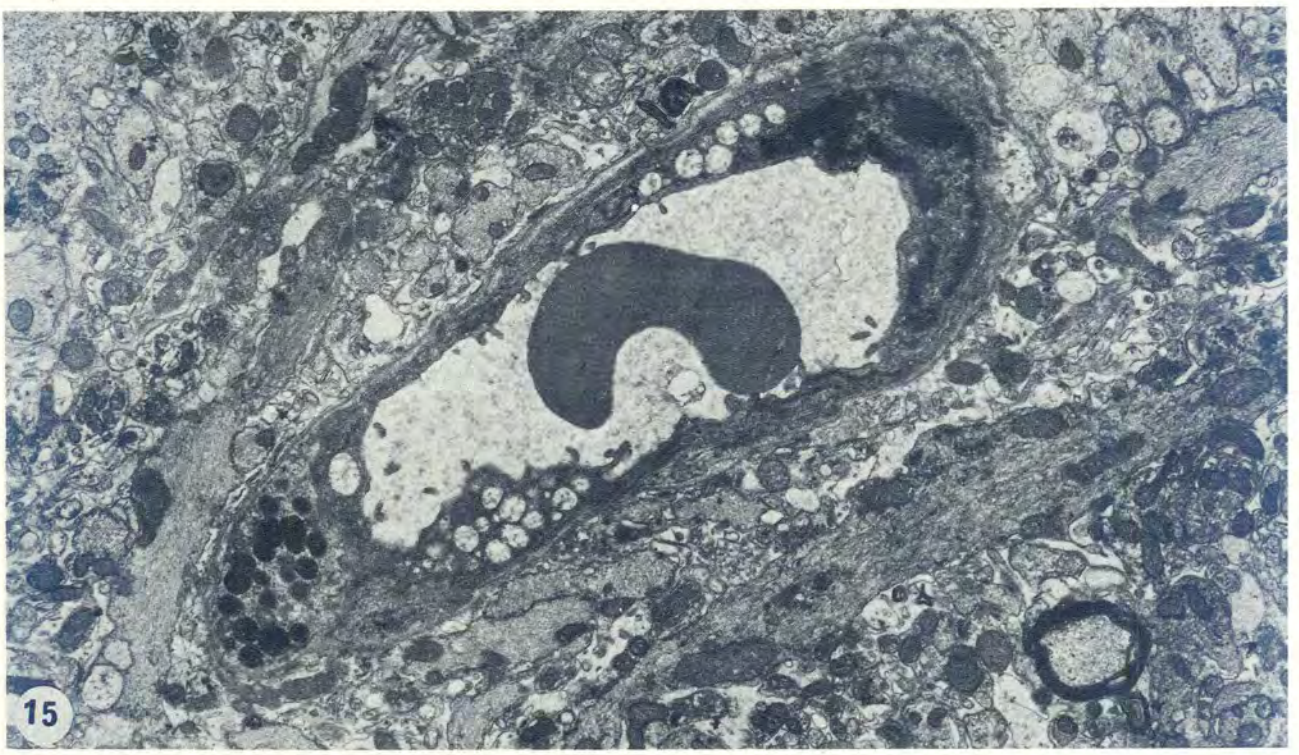


FIG. 13 Perithelial cell with bigger vacuoles indenting the nucleus. ×30 000
FIG. 14 Marked vacuolation of the perithelial cells in a brain capillary. ×8 500



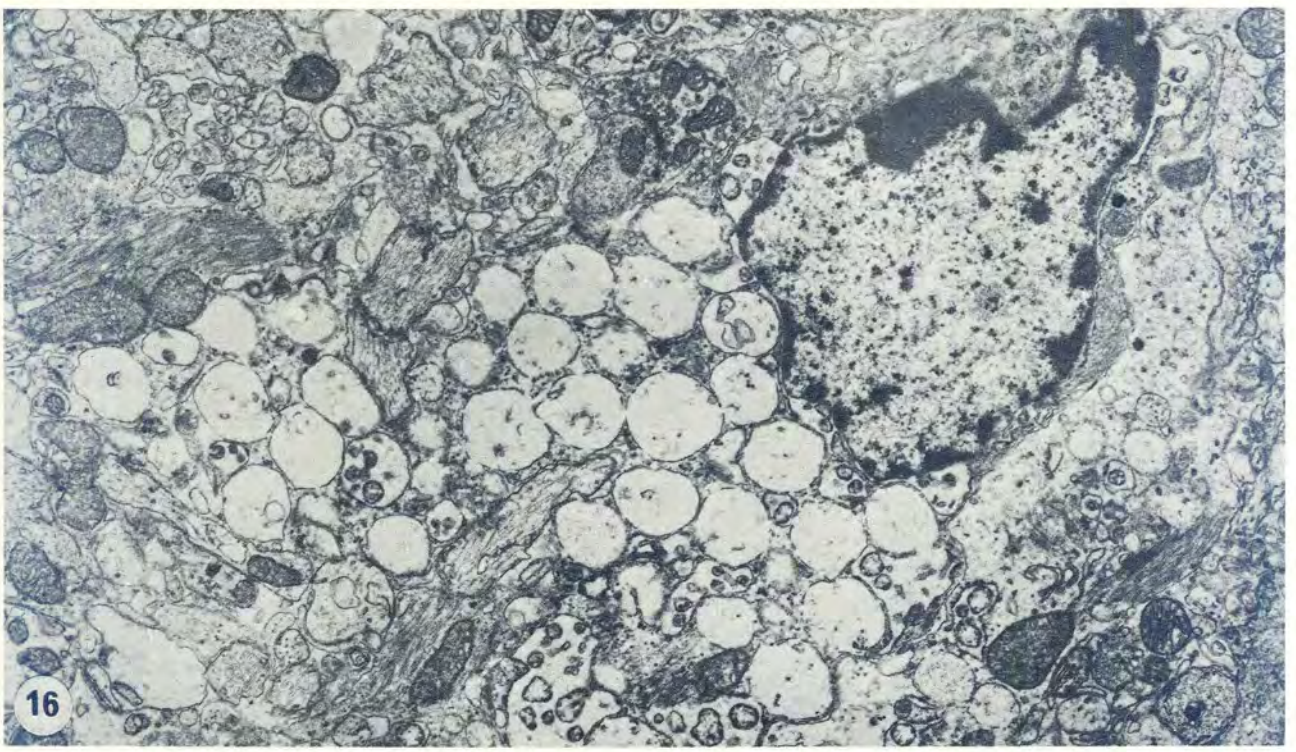
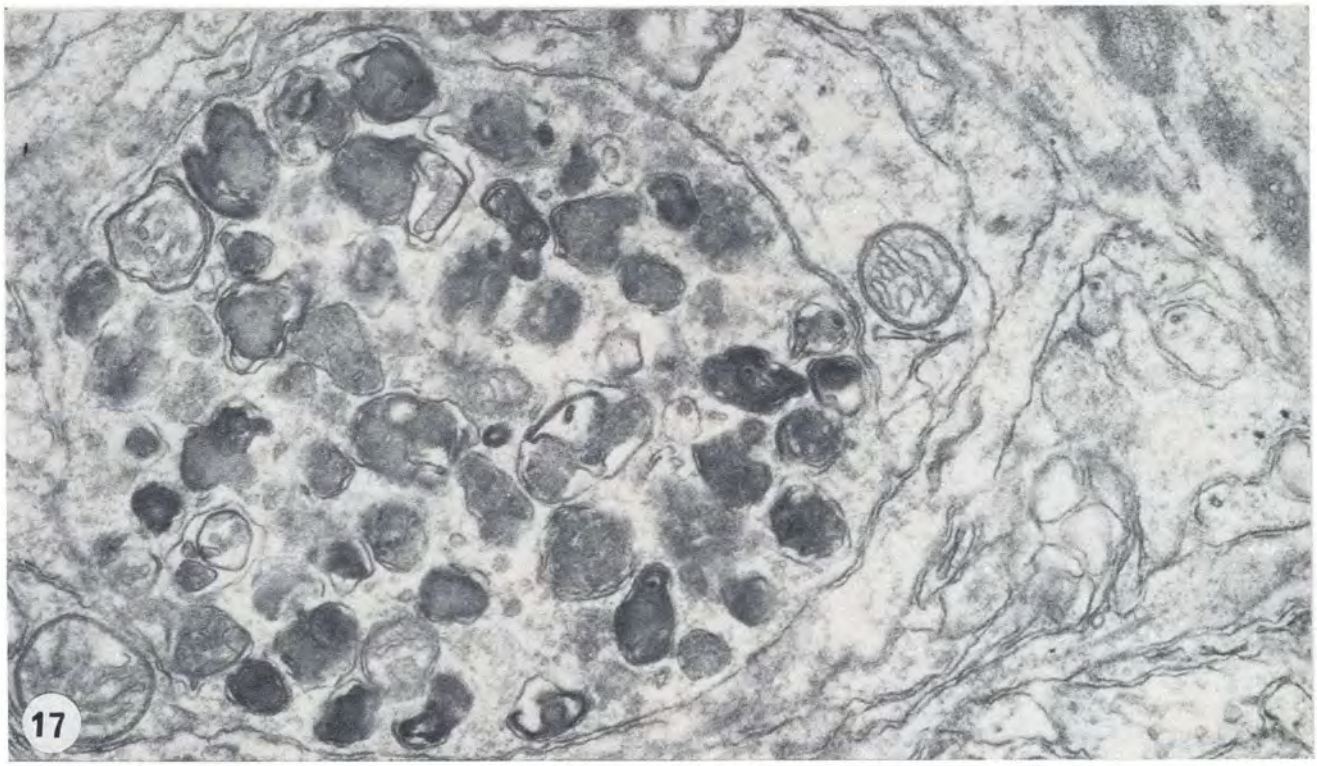


FIG. 15 Brain capillary. Note vacuoles in endothelial cells. ×7 800
FIG. 16 Vacuolation of a glial cell. ×3 000



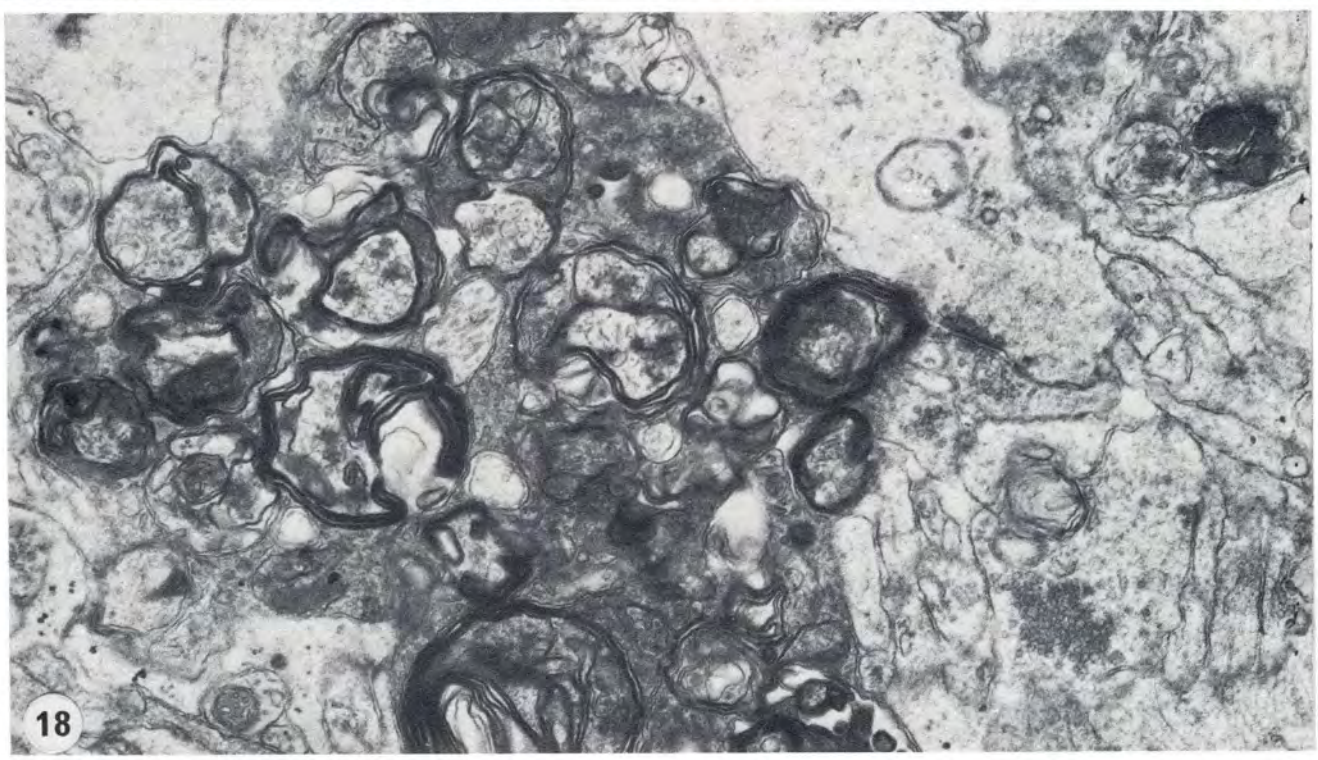


FIG. 17 Spheroid located in a dendrite or axon of an unmyelinated nerve fibre. Note electron-dense bodies, neurofilaments and neurotubules. ×41 000

FIG. 18 Spheroid with electron-dense and membranous bodies. ×20 400

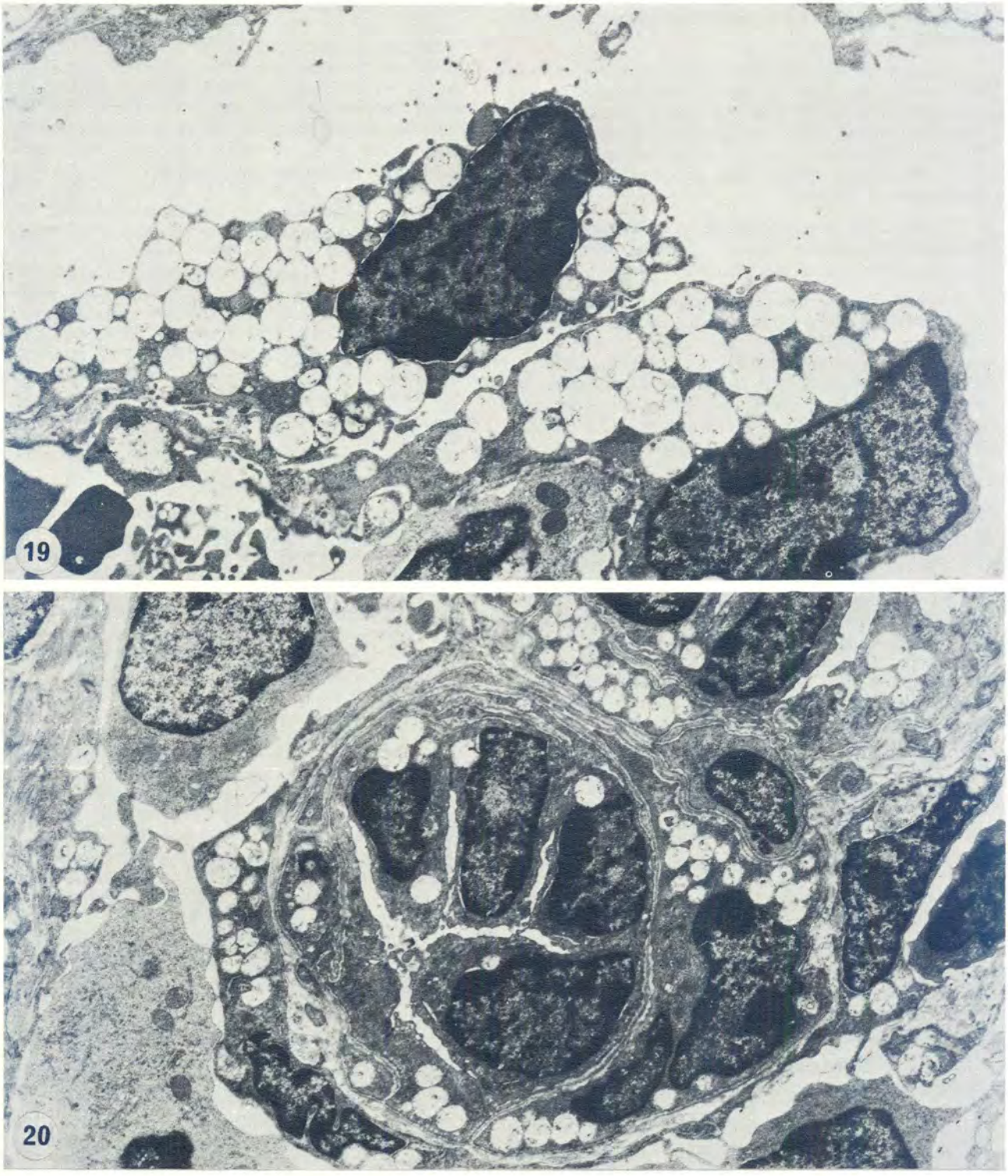


FIG. 19 Marked vacuolation of sinusoidal cells in the spleen. ×6 600
FIG. 20 Capillary in the spleen with vacuolation of the perithelial and endothelial cells. ×6 600

Additional staining methods (Table 1) applied to the brain yielded negative results.

Spleen. The cytoplasm of the reticuloendothelial (RE) cells in the red pulp was vacuolated (Fig. 4). A moderate lymphoid hyperplasia was evident in all the animals.

Lymph node. The changes in the lymph nodes were very similar to those in the spleen. All the lymph nodes were affected and the most severe lesions occurred in the RE cells situated in the medulla (Fig. 5). The subcapsular and intermediate sinuses in the cortex were dilated and frequently contained desquamated RE cells.

Thymus. The vacuolation of cells in the thymus were less pronounced. Vacuoles, however, were located in cells in the cortex and medulla (Fig. 6).

Thyroid. The thyroid gland was markedly abnormal. Although some of the follicles appeared normal, the lining epithelium of the majority was high, columnar and vacuolated (Fig. 7). Individual follicles scattered throughout the gland contained either no colloid, or a colloid which was foamy and light pink in colour (Fig. 7). The epithelium of a few of the dilated, empty follicles formed folds projecting into the lumen.

Kidneys. Vacuoles, varying in size and number, were observed mainly in the convoluted tubular epithelium in the cortex. A remarkable finding in 4 out of the 5 cases was the big tubular structures situated in the cortex (Fig. 8). These structures, which varied in shape and were mostly irregularly round, were lined with a cuboidal to low columnar epithelium with clear outlines which usually consisted of a single cell layer. In some of them the epithelial lining comprised 2–3 cell layers and occasionally the lumen was obliterated by epithelial cells. Some of these cells were desquamated and found free in the lumen. The abnormal tubules were usually surrounded by loose strands of connective tissue (Fig. 8).

Scattered tubuli in the medulla as well as some of the Bowman's capsules were markedly dilated. Where the latter was affected, the glomeruli appeared to be smaller than normal.

A focal, disseminated, lymphocytic nephritis was encountered in the only calf that also had inflammatory lesions in the brain.

Pancreas. In 2 of the calves, fairly large focal areas in the pancreas were seen where the cytoplasm of the acinar cells were light pink instead of a pinkish-red (Fig. 9). Individual small vacuoles were noticed in many of these cells.

Other Organs. Slight vacuolation was the only change seen in the adrenal cortex and medulla. The liver showed a slight fatty degeneration of the parenchymal cells and vacuolation of isolated Kupffer cells. The Peyer's patches in the small intestine were hyperplastic and dispersed vacuoles occurred in the RE cells between the lymphoid tissue. No lesions were seen in the eyes, myocardium, skeletal muscles or salivary glands.

Electron microscopy

Brain. The cytoplasm of neurones and perithelial cells were extensively vacuolated while the endothelial cells were affected to a lesser degree. Some neurones contained only a few clear vacuoles; in the majority, however, numerous vacuoles were interspersed between the cytoplasmic organelles. These appeared normal and well-preserved (Fig. 10). The vacuoles were located throughout the cytoplasm and bore no definite relationship to the Golgi-complexes. The vacuoles differed in size, shape and content, were usually oval or round to irregular, and contained a sparse, flocculated to granular material within which membranous structures were frequently seen (Fig. 10 & 11). Vacuoles filled with a fibrillar material were rarely encountered (Fig. 11 & 12). In the perithelial cells the vacuoles appeared to be even more abundant and larger and, in some instances, they almost completely displaced the cytoplasm (Fig. 13 & 14). The vacuoles, bound by a thin membrane, often formed protrusions into adjacent vacuoles, while rupture of the membranes between 2 adjacent vacuoles resulted in the formation of even larger vacuoles. These frequently indented the nuclei of the perithelial cells (Fig. 13). The capillary endothelial cells regularly disclosed a few small vacuoles filled with a flocculated material (Fig. 14 & 15). It appeared that some of the glial cells were also affected (Fig. 16). The electron microscopic examination of the spheroids indicated that they were probably located in the dendrites (Fig. 17). They contained many electron-dense or membranous bodies, while mitochondria, neurofilaments and neurotubules could also be demonstrated in them (Fig. 17 & 18).

Spleen and lymph node. The lining RE cells of the sinusoids in the spleen and the lymph node sinuses were extensively vacuolated (Fig. 19) and corresponded to those seen in the brain. In addition, the perithelial cells, recognized by their close proximity to the capillaries and their encircling basement membrane, exhibited numerous vacuoles (Fig. 20). The capillary endothelial cells which protruded into the lumen were moderately vacuolated (Fig. 20).

Discussion

Mannosidosis is a rare lysosomal storage disease of Aberdeen Angus cattle and, according to Jolly (1971), approximately 100 cases only have been confirmed in Australia and New Zealand. There is no record of its incidence in any other country. The cases reported in this country differ from those previously recorded in that they occurred in cross-bred Aberdeen Angus cattle and that symptoms were evident at birth. Whittem & Walker (1957) and Jolly (1971), however, reported that the age of the affected animals examined by them varied from less than 1 day to almost 2 years. Whittem & Walker (1957) also mentioned that the early symptoms, namely, swaying of the hindquarters, fine head tremors and symptoms of cerebellar ataxia, became apparent only after exercise. Jolly (1971) noted that the affected animals were in a poorer condition than the others in the herd. He also recorded a mild internal hydrocephalus in the majority of cases examined by him, while Whittem & Walker (1957) found this lesion in one case only. In the present study this condition was also found only in a single animal.

A very interesting gross lesion, not previously reported in mannosidosis, was the presence of multiple small cysts in the kidneys which almost replaced the whole cortex and medulla of individual lobules in 3 out of the 4 calves. Microscopy showed many severely dilated tubuli as well as peculiar tubular structures lined with a cuboidal to low columnar epithelium. These structures were also frequently surrounded by loose connective tissue.

A wide range of tissues exhibited vacuolation of various cells, especially of the brain, spleen, lymph nodes and thyroid gland. The genesis of the vacuoles in storage diseases remains unexplained. According to Hers & Van Hoof (1973), material to be degraded by the lysosomes can enter by either autophagy, heterophagy (endocytosis) or crinophagy. Ultrastructural studies done by Spicer, Garvin, Wohltmann & Simson (1974) on patients with Hurler and Hunter syndromes, 2 of the mucopolysaccharide storage diseases, found evidence that some of the vacuoles, especially those in the neurones of the CNS, originated through autophagy. The laminar structures they were able to demonstrate in these vacuoles suggested derivation from cell membranes. They also found indications that the vacuolar content originated through endocytosis of extracellular mucosubstance. Jolly & Thompson (1977), who studied the pathogenesis of the lesions in exocrine cells in cases of mannosidosis with the electron microscope, suggested that these vacuoles were probably formed through crinophagy. They could demonstrate zymogen granules within lysosomal storage vacuoles indicating fusion of the secretion granules with the lysosomes.

In the present study, the cells with high endocytic functions, such as the RE cells in the spleen and lymph nodes and the perithelial and endothelial cells in the brain, were found to be markedly affected. However, the vacuoles in the neurones very often contained membrane structures indicating an autophagic origin.

It was not possible to demonstrate fat or any other specific substance in these storage vacuoles with either the light or electron microscope during this study or those done by Whittem & Walker (1957) and Jolly (1971). Hocking, Jolly & Batt (1972) extracted mannoseglucosamine oligosaccharide from the lymph nodes of these calves. They argued that this substance could easily be leached out of the vacuoles during the preparation of the tissues for microscopy, making it impossible to show up the contents of these vacuoles.

The neuronal and perithelial vacuolation found in this study corresponded with that described by Jolly (1971). Whittem & Walker (1957) saw similar changes in the neurones but made no mention of vacuoles in the perithelial cells. Our investigation revealed the presence of vacuoles also in the endothelial cells in the brain, spleen and lymph nodes.

Round, eosinophilic bodies (spheroids) were noted in the grey and white matter, mainly of the midbrain. Both Whittem & Walker (1957) and Jolly (1971) described identical bodies in the brains of the calves studied by them. Jolly (1971) demonstrated that ultrastructurally these spheroids were local accumulations of electron-dense bodies in myelinated and unmyelinated nerve fibres and possibly in dendrites. According to him, the electron-dense bodies contained semi-membranous material, amorphous material or varying mixtures of both. He also described mitochondria, neurofilaments and neurotubules between these dense bodies in the spheroids. The ultrastructure of the spheroids as seen in our study corresponded with that reported by Jolly (1971), but it was sometimes difficult to determine their exact location. Jolly (1971) speculated that these electron-dense bodies could probably have originated from degenerated mitochondria, neurofibrils or other membranous organelles.

Vacuolation of the RE and endothelial cells in the spleen and lymph nodes, not mentioned by the previous authors, was a constant feature in all the animals examined during our investigation. The changes in the kidney, thyroid and thymus have not been previously reported in calves with mannosidosis.

Acknowledgements

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