Neuronal Ceroid-lipofuscin Storage in Siamese Cats

P. D. Green and P. B. Little*

ABSTRACT

The clinico-pathological findings of a nervous disease in two mature Siamese cats are described. In one animal intermittent convulsions and mania over a period of three weeks terminated in death, while in the other irritability and hind leg weakness were the main clinical features. Neuronal cytoplasmic inclusions composed of a complex lipid material were detected microscopically. The ultrastructure and staining reactions of these inclusions in one cat were similar to the curvilinear bodies described in neurovisceral storage disease in humans and in a neuronal lipodystrophy of cattle. The deposit was considered likely to be related to ceroid-lipofuscin. No differences in brain lipid quantity or quality could be detected when compared to two control cats.

RÉSUMÉ

Les auteurs rapportent les observations cliniques et pathologiques qu'ils ont notées chez deux chats Siamois adultes atteints d'une maladie nerveuse. Un de ces chats manifesta des convulsions et de la manie, au cours des trois semaines qui précédèrent sa mort. Par contre, les principaux signes cliniques observés chez l'autre se traduisirent par de l'irritabilité et de la faiblesse des membres postérieurs. L'examen microscopique révéla, dans le cytoplasme de certains neurones, la présence d'inclusions composées d'une substance lipidique complexe. L'ultra-structure et les affinités tinctoriales des inclusions de l'un des deux chats ressemblaient à celles des corps curvilignes décrits dans la maladie de l'emmagasinage lipidique neuro-viscéral des humains et dans la lipodystrophie des neurones des bovins. Ce dépôt donnait l'impression d'être apparenté à la lipofuschine ou à la céroïde. Une étude comparative du cerveau des deux chats malades et de celui de deux témoins ne révéla aucune différence, quant à la quantité ou à la qualité des lipides du cerveau.

INTRODUCTION

Disease characterized by the accumulation of lipid material in nervous tissue, reticulo-endothelial tissue or both have been reported in a number of different syndromes in man (1, 4, 5, 8, 11. 21) and in various animals (6, 12, 13, 16, 18, 19, 20). Chrisp *et al* (6) described such a disease in a nine month old Siamese cat which resembled Niemann-Pick disease of humans with cytoplasmic vacuolation of neurons, astroglial cells and reticulo-endothelial cells in various organs. A similar disease in a five month old domestic cat in which membranous cytoplasmic inclusions were demonstrated has been reported by Percy *et al* (17). An increase in sphingomyelin content of affected tissues was demonstrated in the latter case. Baker *et al* (2) and Farrell *et al* (9) have investigated progressive motor disability in Siamese cats and found excessive levels of GM1 ganglioside and a deficiency of B-galactosidase activity in the brain. The purpose of this paper is to describe the clinico-pathological findings of a central nervous system ceroid-lipofuscin storage disease in a mature Siamese cat examined in detail and in another cat on which less material was available. The addition of this condition to the literature now brings the total of neural and neurovisceral lipid storage diseases in cats to three: Type II GM1 gangliosidosis, neurovisceral sphingomyelin storage and neuronal ceroid-lipofuscin storage. Notably the

*Department of Pathology, Ontario Veterinary College, University of Guelph, Guelph, Ontario.

Present address of Dr. Green: Provincial Veterinary Laboratory, Department of Agriculture and Rural Development, Fredericton, New Brunswick.

Submitted March 15, 1972.

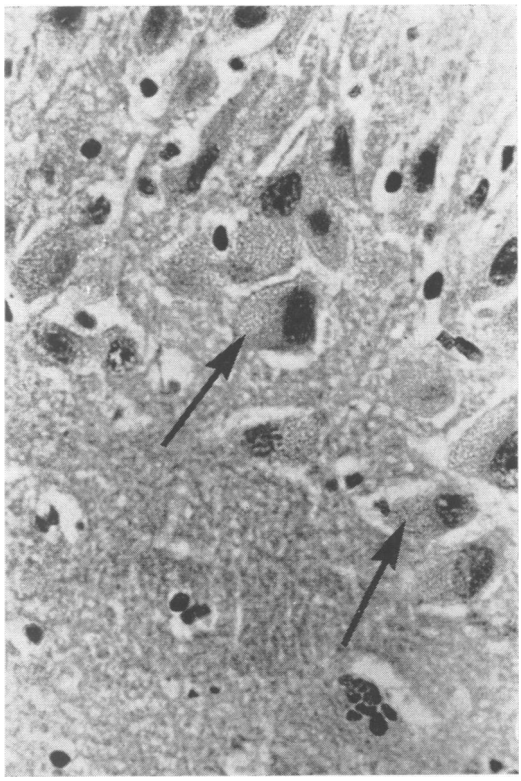

Fig. 1. Pyramidal neurons in the hippocampus of a Siamese cat with neuronal ceroid-lipofuscin storage. Note the refractile cytoplasmic inclusions. H & E. X375.

latter condition is seen at approximately two years of age while the other two types are seen in kittens six months of age or less.

CLINICAL FINDINGS

A 22 month old castrated male Siamese cat was presented to the referring veterinarian on July 15, 1970 with a recent history of becoming excited while watching television and with convulsions being observed by the owners. Examination at this time uncovered no obvious abnormalities and the animal was returned to the owner. Three days later the cat was again presented with a similar history and upon examination the animal was hyperaesthetic. The animal was quarantined and the following day mania and photophobia were obvious. The patient returned to normal within 48 hours and on July 29 it was vaccinated for rabies and treated parenterally with a vitamin B complex preparation. The cat was returned to the owner and appeared normal until August 4, when it was again presented in a convulsive state and salivating. The convulsions rapidly progressed to coma and terminated with the death of the animal in a few hours. A necropsy was performed 18 hours after death.

MATERIALS AND METHODS

Tissues taken at post mortem were fixed in 10% formalin, processed routinely and paraffin embedded. Approximately one-half of the brain was submitted for Rabies virus examination.[1]

Routine hematoxylin and eosin stained

[1]Animal Pathology Laboratory, C.D.A., Hull, P.Q.

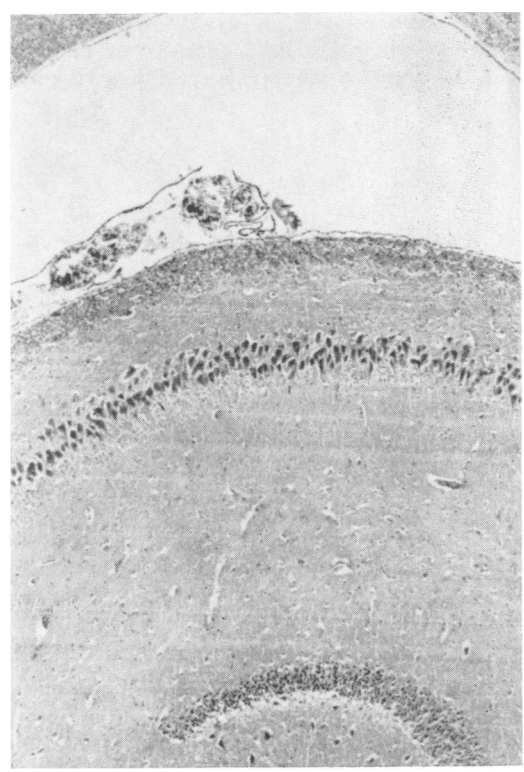

Fig. 2. Hippocampus of a Siamese cat with neuronal ceroid-lipofuscin storage. Note the widespread dark staining of the stored lipid in neurons by the Luxol Fast Blue stain. L.F.B. X75.

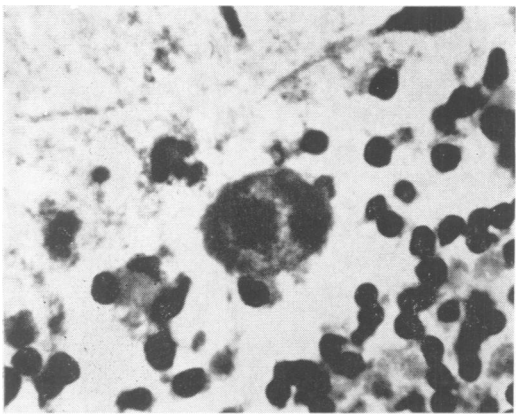

Fig. 3. A Purkinje cell from a cat with neuronal ceroid-lipofuscin storage. Note the coarse deposits to the right of the nucleous. L.F.B. X1000.

sections were examined, as well as sections from paraffin embedded tissue prepared with the following stains: Periodic acid — Schiff (PAS), Luxol Fast Blue (LFB), Sudan Black B, Oil Red O, Phosphotungstic acid hematoxylin (PTAH), Bodian Nerve Fibre Stain, Holzer Glial Fibre Stain, Crystal Violet, Loyez Stain for Myelin, Romansky type Stain, and Landings Method for Lipids (7, 14, 15). Frozen sections were also stained with Sudan Black B before and after extraction with hot chloroform-methanol solutions to examine for the stability of the deposits to lipid solvents.

Small 2 mm square blocks of formalin fixed tissue from the outer layer of the cerebellum were submitted for electron microscopic examination after approximately six months storage in 10% formalin. The tissue was cut into 1 mm square blocks, fixed 48 hours in 3% isotonic glutaraldehyde and then transferred to Sorenson's phosphate buffer (pH 7.2) with 2% sucrose for 12 hours. Post fixation was in 1% osium tetroxide in Sorenson's phosphate buffer (pH 7.2) for two hours with dehydration through acetone and subsequent embedding in Epon 812. Ultrathin sections were cut on a Porter Blum ultramicrotome and stained with uranyl acetate and lead citrate and examined in a Phillips 200 electron microscope.

Samples of brain were taken at post mortem from the frontal cortex and were stored for seven months at -20° C, then at -70° C for one month prior to analysis. Duplicate samples of brain were taken from two control cats of one year of age and of indeterminate breed. These samples were submitted to the Hospital for Sick Children, Toronto, Ontario, Canada for lipid analysis. Extraction was with chloroform methanol (10). The washed lower phase was concentrated and applied as a one centimeter streak to a silica gel G thin-layer plate. The chromatograms were run in chloroform: methanol: water 14:6:1 v/v/v/. The spots were located by spraying lightly with 50% sulfuric acid and charring at 120° C. The lightly charred spots were scraped individually into test tubes and the phospholipid content assayed according to a slightly modified Bartlett procedure (3). The upper phases from the Folch extraction were concentrated and dialyzed and the resulting gangliosides separated by thin-layer chromatography in chloroform: methanol: 2N ammonia 55:40:8 v/v/v. Insufficient material was available to attempt enzymatic analysis.

Fig. 4. An electromicrograph of one of the intracytoplasmic ceroid-lipofuscin bodies located in a Purkinje cell. Note the curved fibrillar interwoven structure and the lack of a boundary membrane. X22,400.

RESULTS

Gross Necropsy Findings

No lesions were noted on gross examination.

Histopathological Findings

On examination of hematoxylin and eosin sections a mild but diffuse microgliosis involving the medulla and cerebral cortex was observed. Meningeal vessels were engorged and a slight enlargement of the nuclei and occasional vacuolation of the cytoplasm of the endothelial cells of the vessels throughout the brain was noted. The Purkinje cells and the hippocampal and cortical neurons contained multiple cytoplasmic inclusions of various sizes which were round to irregular in shape, refractile, homogeneous and slightly more eosinophilic than the surrounding cytoplasm (Figs. 1 and 2). All inclusions in these areas were located in the marginal zone of the cell and did not cause nuclear displacement, even when present in large numbers. Enlargement of the neuronal perikaryon was slight. Distortion of cellular outline was present only in the Purkinje cells of the cerebellum. Some vacuolation of white matter was noted. However, no unequivocal evidence of demyelination was observed.

Congestion of the vessels in the medulla and at the cortico-medullary junction was present in the kidneys. Vacuolation of the tubular epithelium similar to that observed in normal feline kidneys was observed. The epithelial cells of the visceral layer of Bowman's capsule had enlarged and prominent nuclei. Moderately PAS positive material, in strands and clumps, occupied the glomerular basement membranes. Hepatocyes in the periacinar areas contained small clear cytoplasmic vacuoles. No lesions were identified in sections of the pancreas, spleen or intestines.

Fig. 5. An electronmicrograph of a portion of a neuronal intracytoplasmic ceroid-lipofuscin body. Note the multilamellar unit membrane-type structure. X180,000.

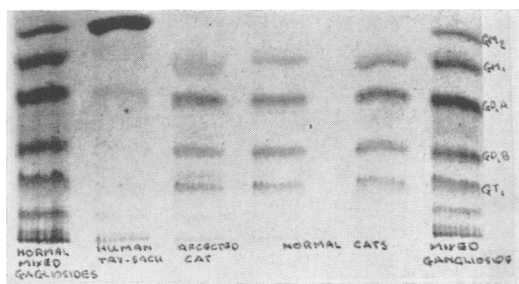

Fig. 6. Photograph of the chromatographic separation of upper phase brain lipids from a Siamese cat with neuronal ceroid-lipofuscin storage disease, two normal control cats, and a child with Tay-Sachs disease.

TABLE I. Histochemical Reactions of Intraneuronal Inclusions in two Siamese Cats with Ceroid-lipofuscin Storage Disease

Staining Method	Reaction
H & E	red
Luxol Fast Blue	dark blue
Oil Red O	red
PTAH	orange
Crystal Violet	pale blue
Bodian	unstained
Romansky	purple
Holzer	unstained
Loyez	orange-red
Landings	blue-green
P.A.S.	red
Sudan Black B.	black

Histochemical Findings

The appearance of the cytoplasmic neuronal inclusions with the various stains is summarized in Table I. Luxol Fast blue gave the most intense reaction and allowed some evaluation of the morphology of the structures and their position within the

cell. The neuronal inclusions varied in appearance from a fine diffuse granular dusting of the cytoplasm to large coarse granules which entirely filled the cytoplasm (Figure 3). Accumulation of luxolophilic material in small amounts was also noted in some astrocytes with no pattern of distribution discernible.

The results of chloroform: methanol extraction of frozen brain sections showed that there was significant retention of stored lipid material following these rigorous attempts to dissolve the substance.

Biochemical Analysis

The phospholipid analyses from the brains of the affected and two normal cats did not differ in percentage distribution and the ganglioside patterns were identical (Figs. 6 and 7).

Rabies Virus Examination

Examination by the fluorescent antibody technique and mouse inoculation was negative for the presence of rabies virus.

Electron Microscopic Examination

Numerous irregular shaped collections of fibrillar material were present in the cytoplasm of the Purkinje cells (Fig. 4). No enclosing membrane was present and examination at 180,000 x magnification showed the fibrillar masses to be composed of structures similar to those of unit membranes with a beading of electron dense particles on the surfaces (Fig. 5). The arrangement of these structures in the inclusions was in an interwoven pattern with straight and curved elements crowded closely together.

DISCUSSION

The previous reports of lipid storage diseases in Siamese cats reported the presence of material in organs other than the nervous system and on the basis of the histological, histochemical and biochemical results compared the findings with those of Niemann-Pick disease in humans (6, 17).

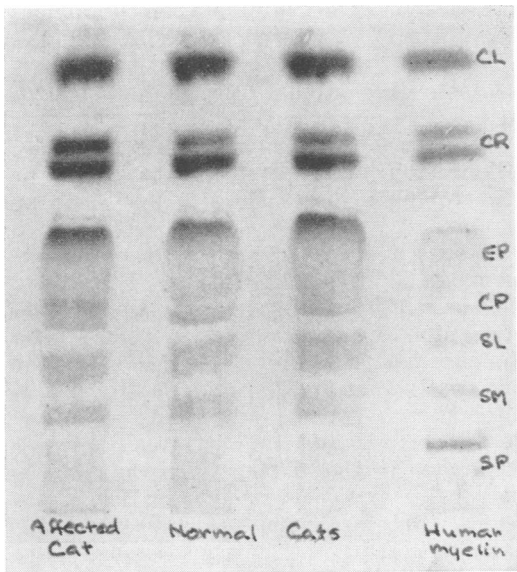

Fig. 7. Photograph of the chromatographic separation of lower phase brain lipids from a Siamese cat with neuronal ceroid-lipofuscin storage disease, two normal cats and normal human myelin. CL — Cholesterol, CR — Cerebrosides (two bands), EP — Ethanolamine phosphoglycerides, CP — Choline phosphoglycerides, SL — Sulphatide, SM — Sphingomyelin, SP — Serine phosphoglycerides.

The Type II GM1 gangliosidosis is characterized however by neural involvement alone and neurons are grossly distended by the stored lipid (2, 9). In the animal described in this report the material similarly was not detectable in organs other than the nervous system and was stable and identifiable after routine processing.

The electron microscopic findings of multilaminated curved cytoplasmic bodies are similar to those found in neurovisceral storage disease (NVSD) in humans (1, 5, 8, 11) and also those described in cattle (19) as curvilinear bodies. In our case the cytoplasmic bodies were not membrane bound, a point of similarity to the case reported in a six year old child (8) but at variance with observations of similar bodies in the neuronal and visceral cells of children with juvenile amaurotic idiocy (5, 11).

No abnormal quantity or quality of lipid could be demonstrated by biochemical analysis in this case and in the light of the microscopic findings this is difficult to explain.

While in this case and in the cases of late infantile and juvenile amaurotic idiocy the stored lipid has not yet been identified, it seems from the histochemical reactions to indicate a complex lipid which is only partially extractable with hot lipid solvents.

The lack of evidence of storage of abnormal lipids in this case perhaps is a reflection of the stability of the neuronal deposits to conventional lipid extraction procedures.

It seems quite possible, as suggested by others (21) that the stored material is a very complex lipid related to lipofuscin or ceroid.

A search of the records of the Department of Pathology, Ontario Veterinary College, University of Guelph, revealed one other similar case in a two year old female Siamese cat. This animal had a history of progressive irritability and loss of sight, although no ocular abnormality could be detected clinically. Hind leg weakness was also noted in the history when the cat was necropsied at two years of age. The histological and histochemical findings on reexamination of the nervous tissues were identical to the case reported here. No evidence of a similar material was demonstrated in the visceral organs. Insufficient tissue was present for electron microscopic examination.

Lipid storage diseases are rare in the human population where chances of detection and diagnosis are good. It is probable that some cases in animals go undetected. This group of diseases in man is considered in most cases to be inherited as an autosomal recessive trait and in the one case where investigation of pedigree was reported a similar pattern may be seen in Siamese cats (2). Cats related to the ones described in this report could not be located but most certainly other examples of this condition exist. The extensive inbreeding which may occur in some lines of pure bred cats could potentially lead to an increased incidence of this and other lipid storage diseases. The value of such animals or their relatives in establishing affected animal colonies lies in their utilization for studies to delineate the basic biochemical disorder(s) leading to the neuronal accumulations. Such animal models could probably be made available by the tracing of genetic lines and the establishment of a planned breeding program.

ACKNOWLEDGMENTS

The authors wish to acknowledge the excellent collaboration given by Dr. J. A. Lowden, Sick Childrens' Hospital, Toronto and the technical assistance provided by Dr. Philip R. Sweeny and Mr. Michael J. Baker-Pearce. We are also indebted to Dr. Noel Smith, Oakville, Ontario for referral of the case and pertinent clinical data.

REFERENCES

1. **ANDREWS, J. M., V. SORENSON, P. A. CANCILLA, H. M. PRICE and J. H. MENKES.** Late infantile neurovisceral storage disease with curvilinear bodies. Neurology **21**: 207-217. 1971.
2. **BAKER, H. J., J. R. LINDSAY, G. M. McKHANN and D. F. FARRELL.** Neuronal GM-1 gangliosidosis in a Siamese cat with B-galactosidase deficiency. Science **174**: 838-839. 1971.
3. **BARTLETT, G. R.** Phosporus assay in column chromatography. J. biol. Chem. **234**: 466-468. 1959.
4. **BLACKWOOD, W., W. H. McMENEMEY, A. MEYER and R. M. NORMAN.** Greenfield's Neuropathology. 2nd Edition. London: Edward Arnold. 1963.
5. **CARPENTER, S., G. KARPATI and F. ANDERMANN.** Specific involvement of muscle, nerve, and skin in late infantile and juvenile amaurotic idiocy, Neurology. **22**: 170-186. 1972.
6. **CHRISP, C. E., D. H. RINGLER, G. D. ABRAMS, N. S. RADIN and A. BRANKERT.** Lipid storage disease in a Siamese cat. J. Am. vet. med. Ass. **156**: 616-622. 1970.
7. **CULLING, C. F. A.** Handbook of Histopathological Technique. Butterworth and Company Ltd. 1957.
8. **DUFFY, P. E., M. KORNFELD and K. SUZUKI.** Neurovisceral storage disease with curvilinear bodies. J. Neuropath. exp. Neurol. **27**: 351-370. 1968.
9. **FARRELL, D. F., H. J. BAKER, R. M. HERNDON, J. R. LINDSEY and G. M. McKHANN.** Feline GM1 gangliosidosis: Biochemical and ultrastructural comparisons with the disease in man. J. Neuropath. exp. Neurol. **32**: 1-17. 1973.
10. **FOLCH, J., M. LEES and G. H. SLOANE-STANLEY.** A simple method for the isolation and purification of total lipids from animal tissues. J. biol. Chem. **226**: 497-509. 1957.
11. **GONATAS, N. K., F. D. TERRY, R. WINKLER, S. R. KOREY, C. J. GOMEZ and A. STEIN.** A case of juvenile lipidosis: The significance of electron microscopic and biochemical observations of a cerebral biopsy. J. Neuropath. exp. Neurol. **22**: 557-580. 1963.
12. **HAGEN, L. D.** Lipid dystrophic changes in the central nervous system in dogs. Acta path. microbiol. scand. **33**: 22-35. 1953.
13. **KARBE, E. and B. SCHIEFER.** Familial amaurotic idiocy in male German Shorthair Pointers. Pathologia vet. **4**: 223-232. 1967.
14. **LANDING, B. H., L. L. UZMAN and A. WHIPPLE.** Phosphomolybdic acid as a staining reagent for lipids. Lab. Invest. **1**: 456-462. 1952.
15. **LUNA, L. G.** Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd Edition. New York: McGraw-Hill. 1968.
16. **McGRATH, J. T., A. M. KELLY and S. A. STEINBERG.** Cerebral lipidosis in the dog. J. Neuropath. exp. Neurol. **27**: 141. 1968.
17. **PERCY, D. H. and B. S. JORTNER.** Feline lipidosis. Archs Path. **92**: 136-144. 1971.
18. **READ, W. K. and C. H. BRIDGES.** Cerebrospinal lipodystrophy in swine. Pathologia vet. **5**: 67-74. 1958.
19. **READ, W. K. and C. H. BRIDGES.** Neuronal lipodystrophy. Pathologia vet. **6**: 235-243. 1969.
20. **RIBELIN, W. E. and L. D. KINTER.** Lipodystrophy of the central nervous system in a dog. Cornell Vet. **46**: 532-537. 1956.
21. **ZEMAN, W. and P. DYKEN.** Neuronal ceroid-lipofuscinosis (Batten's disease): Relationship to amaurotic family idiocy? Pediatrics **44**: 570-583. 1969.