Journal of Neurology, Neurosurgery, and Psychiatry, 1972, **35**, 685–692

A case of G_{M2} gangliosidosis of late onset

P. BUXTON, J. N. CUMINGS, R. B. ELLIS, B. D. LAKE, W. G. P. MAIR, J. R. ROBERTS, AND E. P. YOUNG

From the Neuropathology Laboratory, Walton Hospital, the Department of Neurology, Alder Hey Children's Hospital, Liverpool, the National Hospital, Queen Square, London, and the Institute of Child Health, Great Ormond Street, London

SUMMARY A case of G_{M2}-gangliosidosis commencing by the age of 5 years is described, in which hyperacusis, dementia, and fits were prominent clinical features. In addition to the typical ganglioside pattern on thin layer chromatography and the presence of membranous bodies in electron microscopic studies and characteristic histology and histochemistry, there was biochemical evidence of a gross reduction in heat-labile hexosaminidase activity in white blood cells and brain. A younger unaffected sibling showed the same enzyme defect in white blood cells.

Tay-Sachs disease is a condition usually commencing in very young infants, who survive only a few years. There is now known to be an associated enzyme defect, for there is an absence of hexosaminidase A occurring in the metabolic pathways between the various gangliosides, Müldner, Wherrett, and Cumings (1962) having demonstrated the raised amount of G_{M2} in Tay Sachs disease by thin layer chromatography (TLC).

Recently O'Brien (1969) commented on the presence of at least three types of G_{M2}-gangliosidosis: in type 1, the typical Tay-Sachs, there is an absence of hexosaminidase A, in type 2 hexosaminidase A and B are absent, and in type 3 there is a partial deficiency of hexosaminidase A. The TLC of one such probable case of type 3, in a boy of nearly 6 years, has been illustrated by one of us previously (Cumings, 1968, 1971). Suzuki, Suzuki, Rapin, Suzuki, and Ishii (1970) described one case in a child whose disease commenced at 6 years of age and who died aged 14 years, while other possible cases have been recorded (Bernheimer and Seitelberger, 1968). There appears to be some degree of variation relative to the extent of hexosaminidase A decrease in different cases, suggesting the possibility of more than one type of juvenile G_{M2}-gangliosidosis (Okada, Veath, and O'Brien (1970), Suzuki and Suzuki (1970)).

The present report details a further example of this rare condition, in which biochemical and histological studies demonstrated the lesion to be similar, in many respects, to Tay Sachs disease, yet possessing characteristics of the case of Suzuki *et al.* (1970).

CASE HISTORY

CLINICAL The patient (G.D.) a boy, was the second of three children, the first being a girl 10 years older, of the mother's previous marriage. The third child, a girl, is seven years younger than the patient. The father is half-Jewish, but there is no consanguinity, nor is there evidence at present of any neurological disease in either of the parents or of the other two children. Birth and development of the patient, including all milestones, were normal, but at the age of 4 years he had an ileocolic intussusception which was reduced surgically. He continued normal development and at 5 years old went to school. Soon after he gave evidence of some psychological deterioration and developed an intense dislike of loud noises.

He was first seen at 5 years and 4 months, when his appearance was normal, he was alert and attentive, but movements were clumsy and fine coordination was impaired. There was some increase in muscle tone in all limbs and the tendon reflexes were exaggerated. There was no sensory loss, vision was normal, and the optic discs and fundi showed no abnormality. Progress at school during the first year was negligible.

At the age of 6 years 10 months epileptic attacks

were noted. These occurred up to 10 times a day, each lasting half a minute, with left-sided facial spasm and turning of the head to the left. Abnormal EEG tracings, in which the normal background alpha activity was replaced by slow (2–3 Hz) delta activity, interrupted by frequent paroxysms of high amplitude (greater than 200 μV) spikes, were found. Fits, despite treatment by phenobarbitone (30 mg t.d.s.), were still present at $7\frac{1}{4}$ years, and there was an increasing spasticity. A lumbar encephalogram at this time showed no evidence of cerebral atrophy and the cerebrospinal fluid was normal chemically. Various blood examinations made, such as electrolytes, calcium, blood count, liver function tests, Wassermann and various virological studies as well as urinary analysis and amino acid chromatography, were all normal. Radiological studies were all normal.

The fits now changed in character; from being brief tonic seizures they became generalized tonic-clonic fits, lasting up to five minutes, and despite therapy they occurred 10 to 20 times a day. When aged 8 years, clear evidence of dementia was present, for he was retarded, speechless, doubly incontinent, and could not feed himself. There was a fine spontaneous tremor in both arms and evidence of gross cerebellar involvement. There was no sensory loss; visual attention remained with pupillary responses brisk and the optic discs and fundi normal.

Cerebral biopsy was performed just before the age of 9 years, from which he recovered well. He is still alive one and a half years later with clinical features essentially identical with those that existed before cerebral biopsy.

The clinical diagnosis before biopsy was a progressive neuronal degeneration, probably a lipidosis.

HISTOLOGY Part of the cerebral biopsy, which consisted of a 1 cm cube of frontal tissue containing both white matter and cortex, was sectioned fresh frozen, part after formalin fixation by frozen section and part after paraffin wax embedding.

By all section techniques and conventional staining a general glial increase was noted in both cortex and white matter and there was some general loss of myelin, but the striking feature was distension of nerve and glial cells in the cortex. This distension was often gross, due mainly to finely granular material in the cytoplasm compressing the nucleus to the edge of the cell (Figs 1 and 2). The material was

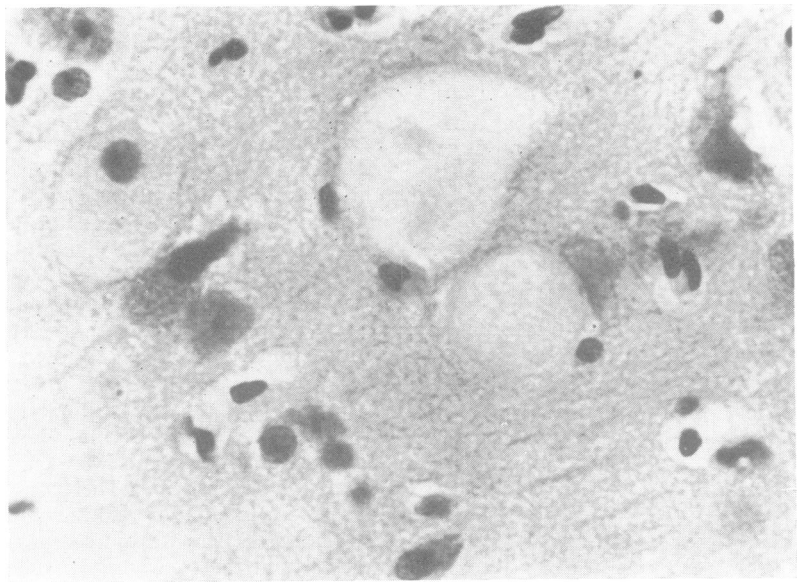

FIG. 1. *Cortex to show distended nerve and glial cells. H and E, ×600.*

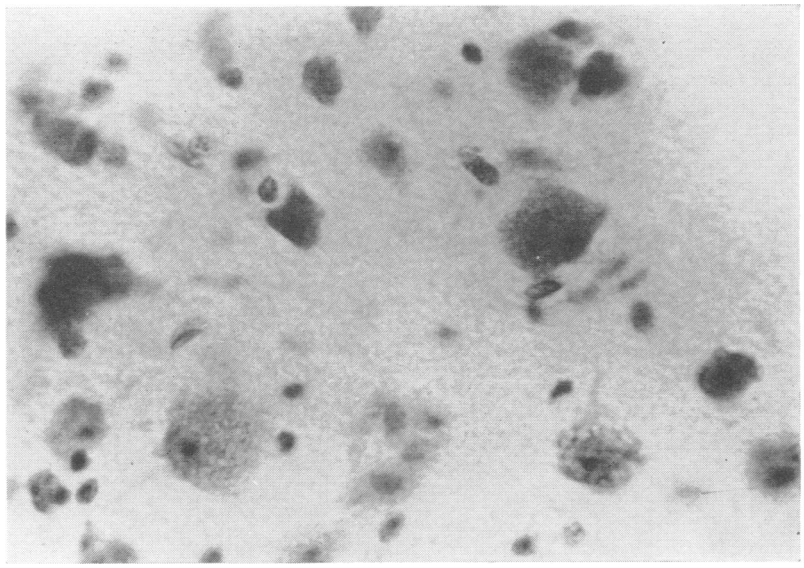

FIG. 2. *Cortex to show distension of nerve cells, some with fine granules and less severe distension of a nerve cell and some with foamy cytoplasm. H and E, × 600.*

faintly eosinophilic in some places, faintly basophilic in others. In some cells where distension was less gross, the cytoplasm appeared foamy, occasionally with small vacuoles. By PAS stain in fresh frozen material the fine granules in distended neurones and glial cells stained brightly, as did the cells with more foamy cytoplasm, although in these the granules appeared coarser. In the white matter a few glial cells showed coarse PAS positive material in the cytoplasm, these cells being mostly in the vicinity of blood vessels. The PAS reaction in frozen sections of formalin fixed tissue was never as strong as that found in fresh frozen sections; many of the distended cells in sections of paraffin wax embedded tissue failed to stain by this technique, which suggests that there has been some loss of substance during processing. By Oil Red O stain the granular material in cortical nerve and glial cells was faint orange-yellow, but scanty bright-red stained perivascular phagocytes were seen in the white matter. Nile blue sulphate stained the granular material blue, but in the greatly distended cells there was a faint suggestion of pink. Strong metachromasia was obtained with Feyrter's thionin in the material staining positively with PAS, but there was no metachromasia in the white matter.

In general the most grossly distended cells were glial with fine granules, occasional neurones were also grossly distended, but many were less so and the cytoplasm tended to be of the more foamy type with coarser granules. Glial satellitosis of less distended neurones was common.

Electron microscopic studies were also made and marked structural changes were seen in the nerve cells: their nuclei were electron-lucent, not even their margins being electron-dense. The nuclear membranes were distinct. Some nerve cells showed distension of the endoplasmic reticulum tubules to which were attached ribosomes: groups of polysomes were scattered throughout the cell cytoplasm. Other nerve cells were distended by varying-sized membranous bodies formed of parallel or concentric arrays of closely applied laminae (Fig. 3). Mitochondria were scarce in such cells. Similar membranous bodies though smaller in dimensions were present in the axons of some of the myelinated nerve fibres in the cortex (Fig. 4). Other myelinated axons were filled with electron dense granular material.

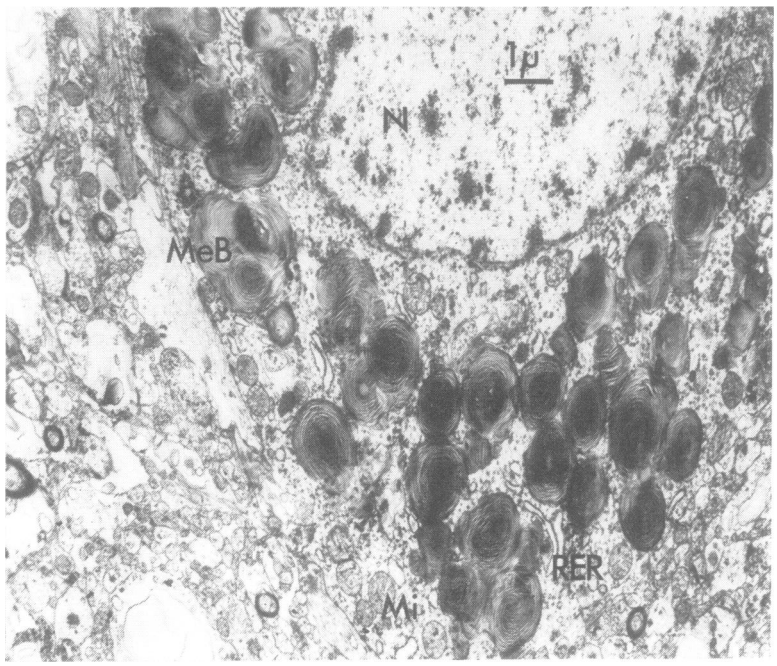

FIG. 3. *A nerve cell presents in its cytoplasm numerous membranous bodies* (MeB), *occasional mitochondria* (Mi), *and some rough endoplasmic reticulum* (RER). *The nucleus* (N) *shows electron density mainly at its margins.* × *6,300.*

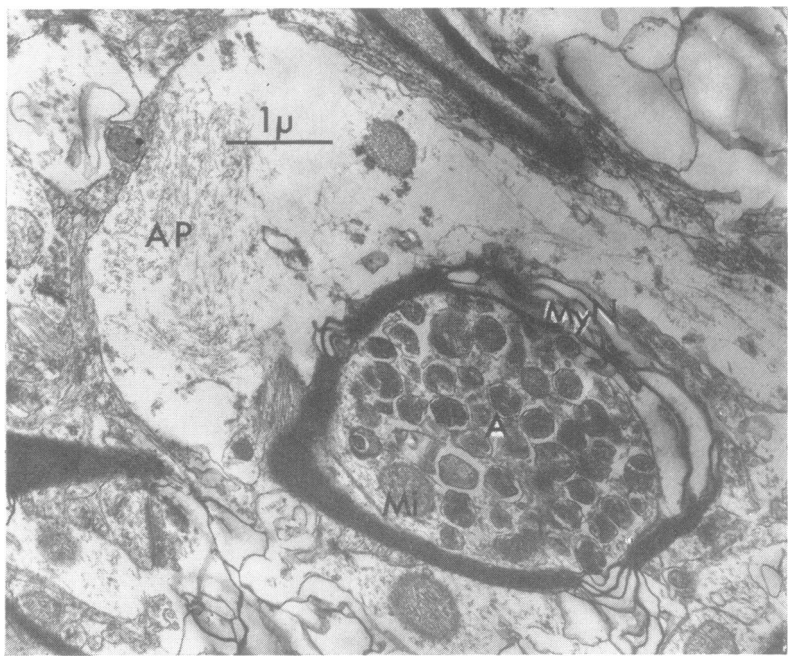

FIG. 4. *A myelinated nerve* (MyN) *surrounded by astrocyte processes* (AP), *exhibits in its axon* (A) *membranous bodies* (MeB) *and some mitochondria.* × *14,000.*

Alongside the nerve cells and myelinated axons were astrocyte processes containing large numbers of glial fibres. The blood capillaries were lined by hypertrophied endothelial cells: many pericytes occurred within the basement membrane of the capillaries. Some endothelial cells and pericytes contained lipid material.

These histological findings are typical of a neuronal lipidosis of the Tay-Sachs type.

BIOCHEMICAL The portion of fresh brain tissue was separated into white matter and cortex. Samples were dried at room temperature to constant weight and the water content obtained. Total phospholipid and N-acetyl-neuraminic acid were estimated as previously described (Dayan and Cumings, 1969), while thin-layer chromatography (TLC) was performed by the methods employed by Müldner *et al.* (1962).

Table 1 shows the results of the analyses of both white matter and cortex, from which it is seen that there is no significant abnormality in amount of either phospholipid or ganglioside. By the TLC examination phospholipids and sphingolipids were in normal relative ratios to each other: cholesterol esters were not present in either white matter or cortex as shown by TLC. The ganglioside pattern was abnormal as can be seen in Fig. 5 in which is a well-marked G_{M2} band in both the cortex and the white matter. The amounts of G_{M2} in both white matter and cortex were estimated and were respectively 54% and 48% of total gangliosides.

TABLE 1

LIPIDS IN BRAIN

	White matter (*g/100 g dry tissue*)	*Cortex*
Total phospholipid	24·5	21·2
N-acetyl-neuraminic acid	0·09	0·27
Water (%)	75·8	80·5

FIG. 5. *Ganglioside patterns by TLC of extracts of cerebral cortex of patient (on left), of a normal control (middle) with an authentic G_{M2} marker (on right).*

ENZYME STUDIES White blood cells were prepared from 5 to 10 ml. heparinized, venous blood by sedimentation in dextran solution (Kampine, Brady, and Kanfer, 1967). After purification, the cells were homogenized in 300 μl. 200 mM KCl, frozen and thawed three times, and the supernatant used for enzyme assays. For brain, the supernatant from a 2% w/v homogenate of grey matter in cold, distilled water was used. The determination of the heat-labile hexosaminidase activity was based on an observation of Robinson and Stirling (1968). Heat-inactivation was carried out at two enzyme concentrations in McIlvaine's buffer, pH 4·5, for 60 min at 50° C. The remaining activity and the total activity of identical non-heated samples were determined as previously described (Young, Ellis, Lake, and Patrick, 1970) and DEAE-cellulose chromatography was carried out by the method of these same authors.

The percentages of the heat-labile hexosaminidase activity in the grey matter and in white blood cell preparations from patient G.D. fell in the same range as those from typical cases of Tay Sachs disease (Table 2). This contrasts with the value found in the brain of a case of late-infantile G_{M2}-gangliosidosis (patient L.A. in Table 2; onset of symptoms at 20 months) which was intermediate between those of controls and cases of Tay Sachs disease.

TABLE 2

PERCENTAGE OF TOTAL HEXOSAMINIDASE ACTIVITY PRESENT AS HEAT-LABILE COMPONENT IN PREPARATIONS OF WHITE BLOOD CELLS AND GREY MATTER

	Age at test (yr)	*Heat-labile activity (%)*
White blood cells		
Juvenile G_{M2}-gangliosidosis		
Patient G.D.	8	9
Father	—	55
Mother	—	49
Half-sister, M.D.	18	65
Sister, S.D.	1½	4
Tay-Sachs disease		
Patients (6*)	½ to 2	5 (0–9*)
Parents (7)	—	43 (36–47)
Controls		
Healthy adults (14)		62 (51–70)
Normal cord blood samples (15)		61 (48–68)
Children with variety of degenerative diseases other than G_{M2}-gangliosidosis (16)		64 (55–77)
Grey matter		
Juvenile G_{M2}-gangliosidosis		
Patient G.D. (biopsy at 8 yr)		10
Late infantile G_{M2}-gangliosidosis		
Patient L.A. (necropsy at 4 yr)		36
Tay-Sachs disease		
Patient T.C. (necropsy at 20 mth)		10
Controls		
1. Biopsy at 4 yr (no abnormality found)		65
2. Post-mortem at 5 mth (congenital heart defect)		65

* Figures in parentheses in the first column refer to the number of cases tested and, in the last column, to the range of values found.

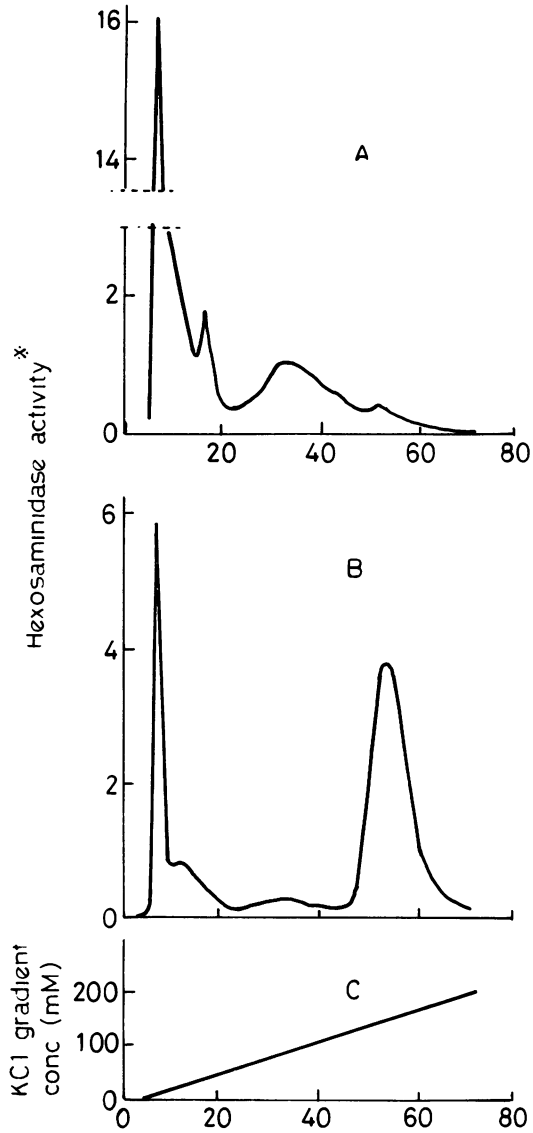

FIG. 6. *Fractionation of brain hexosaminidases of DEAE-cellulose columns.* A = *patient G.D. (biopsy);* B = *control (post-mortem samples from case of leukaemia, 6 years);* C = *KCl gradient used in* A *and* B. * *Hexosaminidase activity is expressed as n-mole substrate hydrolysed/hr/100 µl. of individual fractions in the standard assay system.*

The healthy younger sister, S.D., of patient G.D., was found to have a low level of heat-labile hexosaminidase activity similar to that of her affected brother.

The results from the heat inactivation study were supported by the elution profiles of hexosaminidase activity from brain supernatants chromatographed on DEAE-cellulose (Fig. 6); that for patient G.D. (Fig. 6A) is similar to that found for typical cases of Tay-Sachs disease (Young *et al.*, 1970), although the initial peak was not so greatly elevated above normal and a slight peak was eluted at a position corresponding to the final peak of the control (Fig. 6B). The activity of this peak would hardly be detected by the heat-inactivation method. However, in the case of late-infantile G_{M2}-gangliosidosis, the peak eluted in this position was about one-third of the activity of the control (Young *et al.*, 1970).

DISCUSSION

The description by Tay in 1881 and Sachs in 1898 of the early infantile form of the disease which bears their name led to the recognition of a group of familial diseases with varying ages of

onset and duration, some with progressive blindness as well as dementia. As in the cases previously described (Cumings, 1968, 1971; Suzuki *et al.*, 1970) of type 3 G_{M2}-gangliosidosis, our case G.D. showed no evidence of central nervous system involvement in early childhood until in his sixth year when there was clear evidence of a failure to learn at school accompanied by a psychological disturbance in the form of excessive timidity in the playground.

Hyperacusis, which is a consistent early symptom in Tay-Sachs disease, was also a presenting symptom in our case, although it did not feature in the case described by Suzuki *et al.* (1970). The general clinical pattern of deterioration with signs and symptoms of grey cell damage in this child was similar to that described by Suzuki *et al.* (1970). Epilepsy, myoclonus, and the muscle wasting were followed by a gradually increasing dementia. In neither case did blindness appear. Even six years after the initial symptoms the optic discs and fundi remain normal in this child. The preservation of sight in all these children is in marked contrast to the early amaurosis so characteristic of the Tay-Sachs syndrome and the Batten-Spielmeyer-Vogt group of disorders in which degenerative retinal changes and loss of vision invariably precede dementia. In our patient G.D. the initial symptoms were of hyperacusis, insidious dementia, and mild spasticity beginning in his sixth year and followed within two years by the appearance of seizures and increasing signs of pyramidal, striatal and cerebellar involvement but without the loss of vision or hearing.

The diagnosis of G_{M2}-gangliosidosis is demonstrated by the characteristic histological features, especially the EM findings and the histochemistry as well as by the presence of the ganglioside fraction G_{M2} in the thin-layer chromatography of both cortex and white matter together with the confirmation of the enzyme defect. Though the proportion of total gangliosides present as G_{M2} is greatly raised above the value found in normal brains, it is not so elevated as that found in cases of Tay-Sachs disease (Wherrett and Cumings, 1963) and is comparable with that reported by Suzuki *et al.* (1970) who found a figure of 37·6%. Their patient showed the first signs of the disease when aged 6 years (as compared with ours of 5 years) and died at 14 years, whereas our patient is still alive at 10 years, so that it has not been possible to examine organs apart from the brain.

Deficiencies of heat-labile hexosaminidase (or hexosaminidase A) have been described in three cases of later-onset G_{M2}-gangliosidosis (Okada *et al.*, 1970; Suzuki and Suzuki, 1970). Because of differences in methodology and in tissues or fluids analysed, it is difficult to compare the results from different laboratories, but at least two distinct types of enzyme abnormality appear to occur in these cases. In one type—our case G.D. and case K.L. of Okada *et al.* (1970)—there is a profound deficiency of the enzyme similar to that found in typical cases of Tay-Sachs disease, while in the other—patient L.A., and that of Suzuki and Suzuki (1970) and of case B.H. of Okada *et al.* (1970)—the level of activity is intermediate between those in Tay-Sachs disease and controls. It should be noted that the reported ages of onset of clinical symptoms in these four cases do not appear to correlate with the respective degrees of enzyme deficiency.

The fact that the enzyme deficiency was found in the asymptomatic younger sister of patient G.D. indicates that the defect is present from an early age. However, it is not clear why such children can have a normal life for several years, while those with Tay-Sachs disease are affected in the first year of life.

We wish to thank Dr. A. D. Patrick, Mr. H. Goodwin and the Department of Medical Illustration, Institute of Neurology, for their assistance. Thanks are also due from R.B.E. and E.P.Y. for financial support from The British Tay-Sachs Foundation.

REFERENCES

Bernheimer, H., and Seitelberger, F. (1968). Über das Verhalten der Ganglioside im Gehirn bei 2 Fällen von spätinfantiler amaurotischer Idiotie. *Wienerklinische Wochenschrift*, **80**, 163–164.

Cumings, J. N. (1968). (1968). Genetically determined neurological diseases of children. In *Biochemical Aspects of Neurological Disorders*, 3rd series, p. 268. Edited by J. N. Cumings and M. Kremer. Blackwell: Oxford.

Cumings, J. N. (1971). Inborn errors of metabolism in neurology (Wilson's disease, Refsum's disease, and lipidoses). *Proceedings of the Royal Society of Medicine*, **64**, 313–322.

Dayan, A. D., and Cumings, J. N. (1969). An infantile case of subacute sclerosing panencephalitis with an abnormal ganglioside pattern in the brain. *Archives of Diseases in Childhood*, **44**, 187–196.

Kampine, J. P., Brady, R. O., Kanfer, J. N. (1967). Diagnosis

of Gaucher's disease and Niemann-Pick disease with small samples of venous blood. *Science*, **155**, 86–88.

Müldner, H. G., Wherrett, J. R., and Cumings, J. N. (1962). Some applications of thin-layer chromatography in the study of cerebral lipids. *Journal of Neurochemistry*, **9**, 607–611.

O'Brien, J. S. (1969). Five gangliosidoses. *Lancet*, **2**, 805.

Okada, S., Veath, M. L., and O'Brien, J. S. (1970). Juvenile GM_2 gangliosidosis: partial deficiency of hexosaminidase A. *Journal of Pediatrics*, **77**, 1063–1065.

Robinson, D., and Stirling, J. L. (1968). *N*-Acetyl-β-glucosaminidases in human spleen. *Biochemical Journal*, **107**, 321–327.

Suzuki, K., Suzuki, K., Rapin, I., Suzuki, Y., and Ishii, N. (1970). Juvenile G_{M2}-gangliosidosis. *Neurology (Minneap.)*, 20, 190–204.

Suzuki, Y., and Suzuki, K. (1970). Partial deficiency of hexosaminidase component A in juvenile G_{M2}-gangliosidosis. *Neurology (Minneap.)*, **20**, 848–851.

Wherrett, J. R., and Cumings, J. N. (1963). A contribution to the biochemistry of Tay-Sachs disease. *Transactions of the American Neurological Association*, **88**, 108–111.

Young, E. P., Ellis, R. B., Lake, B. D., and Patrick, A. D. (1970). Tay-Sachs disease and related disorders: fractionation of brain N-acetyl-β-hexosaminidase on DEAE-cellulose. *FEBS Letters*, **9**, 1–4.

ADDENDUM

An important study has recently been published on juvenile G_{M2}-gangliosidosis. See Menkes, J. H., O'Brien, J. S., Okada, S., Grippo, J., Andrews, J. M., and Cancilla, P. A. (1971). *Archives of Neurology*, **25**, 14–22.