An Infantile Case of Subacute Sclerosing Panencephalitis with an Abnormal Ganglioside Pattern in the Brain

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Recent evidence has suggested that subacute sclerosing panencephalitis (SSPE) is due to temperate infection of the brain by a virus, probably measles virus (Symposium, 1968). In the majority of patients with SSPE, chemical analysis of the brain has shown features of inflammation and demyelination (Cumings, 1965), though 2 patients have been described previously whose brains had abnormal patterns of gangliosides (Norton, Poduslo, and Suzuki, 1966; Ledeen, Salsman, and Cabrera, 1968).

An infant is described in this paper, the youngest reported case of SSPE, in whom the histological features of the encephalitis at necropsy were studied by light and electron microscopy, and in whom chemical analysis of the brain showed an unusual pattern of gangliosides.

Case Report

A male infant, the first child of healthy, unrelated English parents, was delivered at full term after an uneventful pregnancy, and appeared to develop normally for the first 2 months of life. His mother, aged 28 years, said she had had measles 'as a small child', and was not known to have been in contact with this or any other infectious disease during pregnancy. Both parents and the baby lived in a small rural community and were not known to have been exposed to any such disease during the child's life. The family's pet dog seemed healthy throughout this period.

At 2 months the child began to dribble excessively, and was seen to have occasional attacks of twitching of the right side of the face. These continued until he was 4 months old, when he began to have grand mal convulsions, with more marked jerking of the right side of the body. The major fits lasted for 2 to 3 hours and occurred 2 or 3 times daily despite symptomatic treatment with anticonvulsants. In between attacks he was a floppy, miserable baby of normal bodily habitus without any localizing signs of neurological damage. His general condition deteriorated rapidly, and he died of bronchopneumonia at the age of 5 months.

Investigations. Hb 9·9 g./100 ml.; WBC 7600/ cu.mm.; 27% polymorphs and 69% lymphocytes; CSF at 4 months—cells 48 lymphocytes/cu.mm.; protein 40 mg./100 ml.; no organisms were grown on routine bacteriological culture.

Electroencephalogram. Periodic sharp wave complexes on both sides, but more marked on the right, suggested a generalized degenerative disorder (Fig. 1). The appearances were compatible with the diagnosis of SSPE.

Necropsy findings. Macroscopically, apart from bronchopneumonia, the viscera and endocrine glands appeared normal. The spleen (20 g.) had prominent Malpighian bodies on its cut surface, the thymus (19 g.) appeared normal, and lymph nodes generally were small.

Microscopical examination did not show any abnormality in viscera and endocrine glands. The thymus was partly involuted. The spleen and lymph nodes appeared normal and did not contain reaction centres. There was a moderate number of plasma cells in the spleen and bone-marrow. Giant multinucleate cells and intracellular inclusion bodies were not found anywhere. Frozen sections of large and small intestine, liver, spleen, and kidney were stained by periodic acid-Schiff, Sudan black, Baker's acid haematein, and Kulchitsky's haematoxylin methods. No abnormal lipid material was found in neurones of the myenteric plexus or in cells of the reticuloendothelial system.

On naked-eye examination, the brain (weight 760 g. after fixation) appeared atrophic, and the cerebellum was shrunken. On coronal slicing of the cerebrum the cortex of the parietal and temporal lobes was thin, and most of the centrum ovale including subcortical U-fibres appeared grey. In the temporal lobes in particular the white matter had a gelatinous grey appearance.

Received September 3, 1968.

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and contained small cavities. There was moderate dilatation of the lateral ventricles. The basal ganglia and brain-stem appeared normal. Folia of the cerebellum were generally shrunken and the white matter was unusually grey coloured. The spinal cord appeared normal.

**Microscopical findings.** Throughout all layers of the cerebral cortex there was an active subacute encephalitis which was more marked in the parietal and particularly in the temporal lobes and opercula of the insula, where there was a devastating loss of neurones (Fig. 2). There were fewer lesions in the occipital and frontal cortex. In affected areas neurones were damaged or had disappeared, and there was gliosis and many active microglia and compound granular corpuscles (Fig. 3). In less affected areas, such as the calcarine and frontal cortex, lesions were restricted to occasional foci of inflammatory cells and degenerating neurones. Small blood vessels in grey and white matter were cuffed by lymphocytes, plasma cells, and other mononuclear cells with pyroninophilic cytoplasm. Similar active lesions of moderate intensity were present in the basal ganglia and thalamus. In the centrum ovale there was widespread gliosis, with many binucleate and gemistocytic astrocytes and thick sleeves of chronic inflammatory cells around blood vessels. The grey cavitated areas seen by naked eye in the temporal lobes were found to be extensive areas of destruction of myelin sheaths and axis cylinders in which only a loose meshwork of scar tissue remained (Fig. 4). Myelination had begun in the centrum ovale and internal capsule, and appeared somewhat deficient in the brain-stem and spinal cord.

There was diffuse gliosis of moderate intensity in both cerebral peduncles, in the pes pontis, and in the anterior part of the medulla. In the brain-stem active inflammatory lesions and perivascular cuffing was found in the corpora quadrigemina and adjacent cranial nerve nuclei. The cerebellum showed two main lesions. There was moderate demyelination and diffuse gliosis of its white matter and patchy radial gliosis of the cortex (Fig. 5). The majority of cerebellar folia appeared normal in Nissl-stained preparations though a few were distorted (Fig. 6). In such preparations, cells of the cortical layers also appeared normal, but in sections stained by Cajal's silver nitrate-pyridine method for the cerebellum, Purkinje cell dendrites were abnormal (Fig. 7). Instead of the usual regular inverted pyramidal pattern of branching, many cells appeared to have a single thick vertical bar-shaped dendrite, at the distal end of which was a fusiform or...
the Unna-Pappenheim methyl-green pyronin method, variably eosinophilic or amphophilic after haematoxylin and eosin, and bluish or unstained in Nissl preparations. Intracytoplasmic inclusions were not found.

**Peripheral nervous system.** Random samples of voluntary muscles and major peripheral nerve trunks appeared normal. Teased, isolated fibres from a sural nerve had normal appearing myelin sheaths.

**Histochemistry.** Small amounts of material staining orange with Sudan III were present in macrophages in frozen sections of formol-fixed cerebral cortex and around a few blood vessels in the centrum ovale. No abnormal coloration was found of neurones or glial cells anywhere in frozen sections stained by the periodic-acid Schiff, Baker's acid haematein, Kulschitsky's haematoxylin for myelin, alcian blue, Hirsch-Peiffer, and Feyrter's enclosure methods. In the white matter, these techniques showed only some myelinated fibres of normal appearances in less damaged areas.

**Electron microscopy.** Formol-fixed necropsy samples, the only material available, of the parietal cortex were post-fixed in osmic acid, embedded in Epon, and uranium and lead-stained sections examined in an AEI EM6 electron microscope.

Over-all preservation of the tissue was poor, and fine details of cellular structure could not be identified.

In many nuclei of parenchymal cells, probably glial in nature, there were intranuclear filaments about 180Å diameter (Fig. 9). At high magnifications, some filaments appeared to have a ribbed structure. Cells containing the intranuclear filaments were those which had intranuclear inclusions on light microscopy of the corresponding thick sections. Filaments or virus-like particles were not seen in the cytoplasm of any cells.

A portion of the parietal cortex was homogenized by sonication and centrifuged. Negatively stained preparations of the suspension were examined by electron microscopy (Fig. 10), and tangled filaments of about 180Å diameter were found which had a regular helical structure (Mrs. J. D. Almeida, 1967, personal communication).

The appearances found on electron microscopy were interpreted as being consistent with the RNA helix of a virus of the measles—canine distemper—rinderpest group.

**Serology.** The patient's serum was not available for examination.

Serum was obtained from his mother 9 months after the onset of the child's illness. It contained no detectable antimeasles antibody by a complement-fixing technique.

**Chemical analysis.** Portions of the frontal lobe of the brain, liver, and spleen frozen at necropsy were examined biochemically. White matter and cerebral cortex were separated and the water content of each determined separately by drying at 37 °C. in vacuo.
Fig. 3(a).—Diffuse inflammatory infiltration and loss of neurones from parietal cortex. (Nissl. × 63.) (b) Perivascular cuffing in white matter of frontal lobe. (Nissl. × 20.)

Fig. 4.—Devastating loss of neurones from temporal cortex, perivascular cuffing by inflammatory cells and severe damage to white matter. (Nissl. × 24.)
Lipid extracts of cerebral white matter and cortex, spleen, and liver were prepared by the technique of Folch, Lees, and Sloane Stanley (1957). The various lipids were estimated by the methods of Cumings and Rozdilsky (1965), except for total cerebroside which was determined by the techniques of Radin, Brown, and Lavin (1956), and Svennerholm (1956). Thin-layer chromatography (TLC) was carried out by the method of Müldner, Wherrett, and Cumings (1962).

The results obtained from the brain are shown in the Table, together with values from a normal child of the same age. Though there is some reduction of
Fig. 8.—Intranuclear inclusion bodies in glial cells and neurones. (*Haemalum and eosin. × 950.*)

Fig. 9.—Electron micrograph of nucleus of unidentified cell in the brain, showing tangled mesh of virus-like particles. (*×12,800.* Insert shows their filamentous structure. (*×80,000.*)
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myelin lipids and a slight increase in esterified cholesterol, none of the findings suggest any specific disease. On TLC there was a normal pattern of phospholipid, cerebrosides, and sulphatides. The ganglioside pattern of both cortex and white matter was markedly abnormal (Fig. 11), as both showed prominent bands of the monosialo-compounds G\textsubscript{M3} and G\textsubscript{M4}, which are rarely present in normal brain and then only in trace amounts.

A small sample of formalin-fixed ulnar nerve was subjected to the same lipid extraction and TLC techniques. A similar pattern of gangliosides was detected, though present in much smaller quantities.

Discussion

The morbid anatomical findings in the brain of the present patient were those of an inclusion body encephalitis. The nature and distribution of the lesions are compatible with the diagnosis of subacute sclerosing panencephalitis (SSPE) though more active than is commonly seen in this disorder (Greenfield, 1963; Symposium, 1968), and having an unusually severe effect on the temporal lobes (Osetowska, 1961). These differences may reflect the particular reactions of an immature brain to the pathological processes of a subacute encephalitis. The age of the child at the onset of the clinical disorder, 2 months, is far younger than

Fig. 10.—Compound helical structure of isolated virus-like filaments is seen in this negatively stained electron micrograph. (\times 341,000.)

Fig. 11.—TLC of lipid extract from cerebral white matter and cortex of patient. The ganglioside bands G\textsubscript{M3} and G\textsubscript{M4} (Svennerholm's nomenclature) are abnormally prominent.
in any previously reported case of SSPE. Even in the Antwerp series of 75 cases reviewed by Canal and Torck (1964), the youngest patient was 3 years old at the onset of the disease.

Further evidence that the child had SSPE comes from the thin section electron micrographs of virus-like helical filaments in parenchymal cells in the brain. The helical structure displayed in the negatively stained preparations (examples of which have not previously been obtained from this disease) shows that the filaments have the morphological characteristics displayed by certain compound helical RNA viruses (Waterson and Almeida, 1966). By analogy with other examples of SSPE recently studied (Dayan et al., 1967; Symposium, 1968), it is probable that they are measles virus which appears to be the cause of classical SSPE. There is no adequate evidence about the source or timing of the infection in the present case, nor is there any simple explanation why SSPE should be a disease largely of those aged 5 to 20 years (Canal and Torck, 1964). The clinical evidence of normal development and behaviour of the child until he was 2 months old suggests infection sometime after birth, and the over-all normal anatomy of the brain (except the cerebellum) is also compatible with the hypothesis of postnatal onset of encephalitis, or, at least late intrauterine infection. The absence from the mother's serum of detectable anti-measles complement-fixing antibody is against an intrauterine infection, which would probably have caused a marked rise in the maternal antibody titre. Full evidence is lacking about the child's immunological reactions to the presumed virus, but, at the morphological level at least, he probably had some normal immune mechanisms, as shown by the many plasma and other pyroninophilic cells seen in the brain, bone-marrow, and spleen.

The bizarre morphology of Purkinje cell dendrites in the cerebellar cortex, which also seems not to have been recorded previously in SSPE, could have resulted from any of four causes. Firstly, they might be due to congenital dysplasia, though this seems unlikely because of the patchy nature of the lesion, its striking differences from other congenital cerebellar atrophies (Norman, 1963), and because it would mean that the child had two independent illnesses. Secondly, it could have been caused by viral infection of Purkinje cells: none of the available morphological evidence can prove or disprove this in terms of the absence of inclusion bodies from Purkinje cells and the lack of inflammatory lesions from the cerebellum, though there was diffuse gliosis of its white matter. If the lesions were due to the virus it would be a good example of permanent morphological change less than cell death, produced by chronic viral infection of susceptible cells. The dendritic abnormalities of the Purkinje cells bear some resemblance to those observed in divers lipidoses (Norman, 1963), and could perhaps be due to the effect of the abnormal pattern of gangliosides found in the brain. Comparable changes were not recorded in the other cases of SSPE with similar chemical lesions (Norton et al., 1966; Ledeen et al., 1968), though the appropriate staining methods for displaying Purkinje cell dendrites were not employed. In the present case, unlike a classical lipidosis, there was no over-all excess of gangliosides in the brain. As we do not know whether Purkinje cell lesions in lipidoses are due directly to an effect of the intracellular excess of these compounds, or to an altered growth pattern of the cells caused by the disordered metabolic pathway, this hypothesis remains untested for the present. Lastly, the Purkinje cell changes could be interpreted as a reaction to damage produced by the encephalitis of the sort called by Bouman (1934) 'hyperdifferntiation', and here due to cell development disturbed by a lack of the normal exogenous controlling influences destroyed by the infection. Such an effect in the present case would suggest antenatal onset of the brain damage.

The biochemical abnormalities in the brain appear to be unique like many of its other features. Various abnormalities in lipid composition of the brain of older patients with SSPE have been recorded (Cumings, 1960; Szliwowski and Cumings 1961), mainly taking the form of increases in esterified cholesterol due to demyelination, and in hexosamine, either in the lipid soluble or insoluble residue fractions. Thin-layer chromatography (TLC) studies of SSPE brains have only recently been reported by Norton et al. (1966) and Ledeen et al. (1968); and those two cases, though resem-
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blending each other, both differ from the present findings. Both previous reports described increased amounts in the brain of the monosialo-gangliosides G_{3A} and G_{4A}. Norton et al. found an over-all twofold increase in total ganglioside concentration, with abnormalities restricted to the white matter. Ledeen et al. illustrate raised levels also of the disialo-gangliosides G_{3A} and G_{4A} (in their Korey nomenclature). Our case showed an increase in G_{3A} (G_{D3} in the Svennerholm terminology used here) but not in G_{4A}. All these compounds may occasionally be present in small amounts in normal brains. Their occurrence in much greater concentration in SSPE (though still not in the marked excess over normal of one type of ganglioside found classically in lipidoses such as Tay-Sachs' disease) might be an effect of the inflammation, or due to the virus perhaps causing a continuing metabolic perversion of infected cells. Both Norton et al. and Ledeen et al. suggested that the changes they found were due to inflammation, but their hypothesis fails to explain why they do not always occur, e.g. the case referred to briefly by Dayan et al. (1967) was analysed by the same methods, and did not show any abnormality of gangliosides on TLC. It is possible that this is an example of a virus-induced alteration in ganglioside metabolism, rather than an expression of the ganglioside composition of the increased numbers of glial cells in the damaged brain, as suggested by Ledeen et al. In our case the absence of abnormal gangliosides from the frozen viscera affords evidence against a possible congenital metabolic defect as the cause of the chemical lesion, because, in proven instances of such inherited enzymatic disorders, e.g. Tay-Sachs' disease, the viscera are also involved (Eeg-Olofsson et al., 1966). In two other cases (not of SSPE) in which we found similar but not identical chemical abnormalities (Dayan and Cumings, in preparation), and where the history suggested an inherited metabolic lesion, visceral storage of the gangliosides had occurred. The finding of abnormal gangliosides in peripheral nerve, albeit in trace amounts only, is possibly due to centrifugal carriage of the compounds from the central nervous system by axoplasmic flow. The presence of an approximately normal total amount of gangliosides in the brain accounts for the inability of histochemical techniques to demonstrate any specific lesion.

At present SSPE is one of the very few disorders the probable cause of which has been identified as a known virus behaving in a temperate fashion (Symposium, 1968). It seems intriguing that from the evidence of the present case at least, it may also be one of the first human disorders in which may be seen persistent and specific structural and metabolic lesions other than death of cells chronically infected by the virus.

Summary

A 5-month-old boy died after a short neurological illness. Histological and electron microscope studies on necropsy samples of brain showed that he had subacute sclerosing panencephalitis, and that there were measles virus-like filaments in parenchymal cells of the brain. There were bizarre abnormalities of Purkinje cell dendrites in the cerebellum.

Biochemical analysis of the brain showed an abnormal pattern of gangliosides in both cortex and white matter, with a relative excess of G_{M3} and G_{M4}.

It is suggested that the temperate viral infection caused both the morphological and the biochemical lesions.

We are grateful to Dr. D. A. J. Williamson for permission to study the patient and to Dr. I. N. L. Johnson for information about the family. We thank Mrs. J. D. Almeida and Dr. M. A. Woodhouse for electron microscope studies, Dr. J. V. T. Gostling for the serological study, Professor H. Urich and the late Dr. R. M. Norman for advice, and Mrs. M. Blackman, Miss M. I. Stokes, and Messrs. H. Goodwin, R. H. Hunt, and J. Mitchell for skilled assistance.

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An infantile case of subacute sclerosing panencephalitis with an abnormal ganglioside pattern in the brain.

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Arch Dis Child 1969 44: 187-196
doi: 10.1136/adc.44.234.187