Sanfilippo Syndrome (Mucopolysaccharidosis-III)

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INTRODUCTION

Amongst the mucopolysaccharidoses Sanfilippo syndrome (MPS III) is a comparatively recent discovery. Harris (1961) described a case of mental retardation associated with urinary excretion of heparin sulphate. In 1963 Sanfilippo and co-workers described similar cases. Since then other authors have described cases of this disorder (Maroteaux and Lamy 1965; McKusick 1966; Wallace, Kaplan, Adachi, Schneck and Volk 1966; Gordon and Thursby-Pelham 1969; Abraham, Shetty, Bhaktaviziam, Chakrapani, Kokrati and Bachhawat 1970; Haust, Gordon, Bryans, Woolin and Binnington 1971). McKusick (1969) suggested that some of the cases described by Jervis in 1950 may belong to this disorder.

In the present communication we describe 2 cases of this disorder which have been studied clinically, radiologically, histopathologically and biochemically.

MATERIAL AND METHODS

Biochemical methods

Urinary glycosaminoglycans (GAG) extraction and their characterisation were carried out according to the method described earlier from our laboratory (Chakrapani and Bachhawat 1968, 1969; Cherian, Chandrasekaran and Bachhawat 1970). Cerebrospinal fluid (CSF) analysis for GAG was carried out according to the method described by Constantopoulos and Dekaban (1970).

Total GAG from the frontal lobe biopsy sample was estimated according to the method of Singh and Bachhawat (1968). The fractions obtained from cetyl-pyrindinium bromide fraction with sodium chloride as well as DEAE–Sephadex chromatography were analysed according to the method described by Singh, Chandrasekaran and Bachhawat (1969).

Thin-layer chromatography (TLC) and characterisation of gangliosides from the brain biopsy samples were carried out according to the method of Borri, Hooghwinkel and Edger (1966). Individual gangliosides were estimated after TLC by the method of Warren (1959), after hydrolysing the material from TLC in 0.1 N H₂SO₄ at 80°C for 1 hr.

Histopathological methods

The brain biopsy tissue from the frontal lobe of both the patients was immediately divided into two portions, one for histopathological and the other for biochemical studies. The histopathological light
microscopic studies were done on frozen tissue and paraffin-embedded tissue sections. The stains used were haemalum and eosin (HE) sudan III, PAS, alcin blue and Luxol fast blue.

Light microscopic studies were also conducted on the sural nerve and gastrocnemius muscle of Case 2. In addition to the light microscopy, electron-microscopic studies were performed on the brain biopsy sample of Case 2. For this purpose fresh tissue was immediately fixed in Dalton’s chromo-osmic fixative. Ultrathin sections were cut with a Cambridge microtome with a glass knife, stained with lead citrate and uranyl acetate and viewed with an electron microscope.

Case 2 later expired and a necropsy study was done on the brain and the spinal cord. From the necropsy tissue sections from cerebral cortex of various regions (frontal, parietal, occipital, hippocampus), corpus callosum, internal capsule, thalamus, caudate and lenticular nuclear masses, hypothalamus, mammillary bodies, cerebellum, midbrain, pons, medulla and cervical spinal cord were studied with the light microscope.

CASE REPORTS

Case 1, M.B. (H. No. 586973)

History: This first-born 7-year-old boy of consanguineous parents (first cousins) was first seen by us in January 1970 with complaints of inability to walk without support, inability to speak more than a few words, poor understanding and distension of the abdomen. Birth was normal but the milestones of development, motor as well as mental, were markedly delayed. There was no history of convulsions at any time. The child was suffering from frequent upper respiratory infections. Two younger siblings had died of unknown causes at the ages of 1.5 and 2 years.

Examination. Coarse “gargoyle” facial features, hirsutism, corneal clouding, hypotonia, short stubby fingers, kyphosis in thoracolumbar region and gross hepatosplenomegaly were noted (Figs. 1 and 2). The child was a dwarf (height 80 cm) and his head circumference was 42 cm. He could hear. He was irritable and markedly mentally retarded. He could utter only a few words like “appa”, “amma”. There was no restriction of movements at any joint. No cardiac abnormality was noted.

Investigations. Haemogram normal, peripheral blood smear for metachromatic granules in leucocytes negative. CSF cell count, sugar and proteins normal. Electrocardiogram (ECG) and electroencephalogram (EEG) normal. Nerve conduction studies showed conduction velocities of median and lateral popliteal nerves 44 and 36 m per sec respectively. Needle electromyography (EMG) from the upper and lower limb muscles showed fibrillation potentials; the interference pattern could not be assessed properly because of poor cooperation. X-ray of the skull showed thick cranial vault. X-rays of the spine and hands showed biconvex vertebral bodies with anterior beaking in the thoracolumbar region and coning of metacarpal

Fig. 1. Facial features and hepatosplenomegaly of Case 1.

bones respectively (Figs. 3 and 4). Pneumoencephalogram showed mild dilatation of ventricular systems and subarachnoid spaces.

Case 2. R.K. (H. No. 618426)

History. This boy born to consanguineous parents (uncle-niece) by Caesarian section, was brought to us at the age of 11 years in August 1970 with complaints of delayed milestones of development (motor as well as mental), aggressive, destructive behaviour and poor comprehension. The child did not suffer from convulsions at any stage. There was a history of recurrent upper respiratory tract infection. There was no family history of similar illness.

Examination. Markedly hyperkinetic, destructive, mentally retarded boy with “gargoyle” facies: no corneal clouding; hirsutism present; moderately dwarfed but without any spinal deformity; liver palpable 5 cm below the right costal margin; spleen not palpable; cardiovascular system normal. Moderate atrophy of biceps brachii, triceps and deltoid muscles bilaterally but other muscles were essentially normal. Patient could hear. Optic fundus was normal.

Investigations. Haemogram normal. Peripheral blood smear for metachromatic granules in leucocytes negative. CSF cell count, protein, and sugar normal. EEG taken during sleep normal. Nerve conduction velocities in median and lateral popliteal nerves were 58 and 56 m per sec respectively. Needle EMG from the biceps brachii muscles showed fibrillations and pseudomyotonia, whereas these were not recorded from distal muscles of the upper limb and muscles of the lower limbs; the interference pattern could not be assessed. X-ray of the skull and spine showed thick skull vault with anterior pouching of the pituitary fossa (Fig. 5) and biconvex vertebral bodies with mild anterior beaking in the lumbar region, respectively. X-rays of the hand did not show any coning of metacarpal bones or “triangulation” of metaphyses of radius and ulna.

RESULTS

Biochemical findings are summarised in Tables 1, 2 and 3.

TABLE 1
URINARY GLYCOAMINOGLYCANs (GAG) IN MPS III

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Initials and Hospital No.</th>
<th>Age and sex</th>
<th>GAG expressed as uronic acid in mg/l</th>
<th>Major GAGs expressed in % of total GAG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Heparan sulphate</td>
</tr>
<tr>
<td>1</td>
<td>M.B. 586973</td>
<td>7 yr male</td>
<td>35.5</td>
<td>73</td>
</tr>
<tr>
<td>2</td>
<td>R.K. 618426</td>
<td>11 yr male</td>
<td>40.0</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td></td>
<td>3.42</td>
<td>1.7</td>
</tr>
</tbody>
</table>
* N.D. = Not detected.

TABLE 2
BRAIN GLYCOAMINOGLYCANs (GAG) IN MPS III (TOTAL GAG WITHIN NORMAL LIMITS)

<table>
<thead>
<tr>
<th>No.</th>
<th>Initials and Hospital No.</th>
<th>Age and sex</th>
<th>Major GAGs expressed in % of total brain GAG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Heparan sulphate</td>
</tr>
<tr>
<td>1</td>
<td>M.B. 586973</td>
<td>7 yr male</td>
<td>57.8</td>
</tr>
<tr>
<td>2</td>
<td>R.K. 618426</td>
<td>11 yr male</td>
<td>43.0</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>10-11 yr</td>
<td>10.6</td>
</tr>
</tbody>
</table>

TABLE 3
GANGLIOSIDES IN BRAIN TISSUE IN MPS III (TOTAL GANGLIOSIDES WITHIN NORMAL LIMITS)

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Initials and Hospital No.</th>
<th>Age and sex</th>
<th>Individual gangliosides expressed in percentage of total gangliosides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GT1 + GD1β</td>
</tr>
<tr>
<td>1</td>
<td>M.B. 586973</td>
<td>7 yr male</td>
<td>31.0</td>
</tr>
<tr>
<td>2</td>
<td>R.K. 618426</td>
<td>11 yr male</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>5 yr</td>
<td>23.0</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>17 yr</td>
<td>28.6</td>
</tr>
</tbody>
</table>
* N.D. = Not detected.

There was marked increase in urinary excretion of GAG in both the cases, the excretion of heparan sulphate predominating. Dermatan sulphate was not detected in either of the patients (Table 1).
Total GAG in the brain tissue was near-normal in both the patients but there was a differential increase in heparan sulphate as compared to normal (Table 2). In Case 1 dermatan sulphate was also mildly increased. CSF analysis in Case 1 showed marked increase in GAG, 60%, of which was heparan sulphate (400 μg/100 ml normal 8 to 10 μg/100 ml).
Total gangliosides were within normal limits but the faster-moving ones (GM2 and GM1), which were not detected in the control brains, were significantly increased in both the patients (Table 3 and Figs. 6 and 7).
Fig. 6. TLC of brain gangliosides in Case 1. N = Normal. M = Patient.

Fig. 7. TLC of brain gangliosides in Case 2. RG = Grey matter of the patient. N = Normal.
Light microscopy of the frontal lobe biopsy samples from both the patients showed neuronal ballooning with PAS-positive material which was stained with fat stains and was coarsely granular (Fig. 8). Sural nerve biopsy from Case 2 showed demyelination, and the muscle biopsy from the gastrocnemius muscle of the same patient showed "group muscle fibre atrophy".

The light microscopy of the central nervous system from various regions of Case 2 showed marked ballooning of neurons at all levels with PAS-positive lipid material. However, no detectable degree of demyelination was noted. Macrophages laden with PAS-positive lipid material were noted in the molecular layer of the cerebellum but in other areas these were scanty (Fig. 9).

Fig. 8. Light microscopy of frontal lobe tissue. PAS stain showing ballooned neurons. × 200.

Fig. 9. Light microscopy of cerebellar tissue in Case 2. PAS stain showing macrophages in molecular layer. × 50.
Electron-microscopic observations

Neurons. The nuclei in most neurons were displaced to the periphery (Fig. 10). The nucleus was composed of fine granular material of moderate electron density. The nuclear membrane was paired and irregularly wavy (Fig. 11). In some cells it was indistinct (Fig. 12). The cytoplasm of all the neurons examined was extensively replaced by abnormal inclusions (Fig. 10). The cell organelles ordinarily seen in the cytoplasm were much less. Several mitochondria could be identified by the presence of their cristae. Neurofibrils were scanty and lysosomes were rare. However, ribosomes were numerous and were present diffusely throughout the cytoplasm.

The inclusion bodies in the cytoplasm showed a variegated appearance. They were single-membrane-bound bodies of varying density and configuration. Some of them were long and oval with numerous transverse striations (Fig. 13). Rarely the lamellar pattern showed a concentric arrangement. Some cytoplasmic inclusions were filled with very fine amorphous homogenous granular material (Fig. 14). In some of these structures fine striations could be made out along with the amorphous granular material. The inclusion bodies in most of the cells studied showed dense osmophilic and this osmophilia may quite possibly have been due to its lipid nature. There were also round and unilamellar-bound clear vacuoles, presumably containing mucopolysaccharides (Fig. 12). The cytoplasmic inclusion bodies occasionally showed empty holes which were artifacts due to the displacement of their contents during section-cutting (Figs. 10 and 11).

Glial cells. Glial cells were few and their cytoplasm showed a few inclusion bodies showing a lamellar pattern (Fig. 15).

Blood vessels. Many small capillaries were seen with swollen endothelial cells. Some of these cells showed unit-membrane-bound vacuoles containing markedly osmophilic granular material in dumps (Fig. 16). The perithelial cells were also swollen and had large, apparently empty vacuoles.

White matter. Numerous non-myelinated and myelinated fibres were seen. There was no definite evidence of demyelination. Occasionally, axonal cytoplasm showed a large number of rounded structures (Fig. 17). Most of them were laminated and osmophilic.

DISCUSSION

Clinical

Facial features in MPS III can vary from normal to those seen in Hurler's disease (MPS I) even though they rarely reach the characteristic "gargoyle" appearance (Abraham et al. 1970; Maroteaux and Lamy 1965; Gordon and Thursby-Pelham 1969). In both of our cases facial features were very coarse and resembled to a great extent those seen in MPS I. Hirsutism, which has been noted by other authors was quite marked in both these cases. Corneal clouding has been reported to be very rare in this disease. Maroteaux and Lamy (1965), Wallace et al. (1966), Gordon and Thursby-Pelham (1969) and Abraham et al. (1970) have not noted any clouding in their cases. In 8 cases described by Sanfilippo, Podosin, Langer and Good (1963) overt corneal clouding was absent in all, but in 1 out of the 5 who had slit-lamp examination, mild haziness was noted. In 1 of our cases (Case 1) overt corneal clouding was present whereas in the other it was absent.

Hepatomegaly is common in this disorder, whereas splenomegaly is less common (Sanfilippo et al. 1963; Maroteaux and Lamy 1965; Wallace et al. 1966). Abraham et al. (1970) in 2 of their cases and Gordon and Thursby-Pelham (1969) in both of their cases did not notice hepatosplenomegaly. Haust et al. (1971) noted mild hepato-splenomegaly. Both the cases in the present study had hepatomegaly whereas splenomegaly was noted only in Case 1. This visceromegaly in mucopolysaccharidosis is due to the deposition of mucopolysaccharides in these organs (Bishon, Norman and Tingey 1956; Brante 1957; Wallace et al. 1966). As with the observations of other

J. neurol. Sci., 1972, 17: 323-345
Fig. 10. Electron-microscopic (EM) picture of a neuronal cell with nucleus (N) and nucleolus (Nₙ). The nucleus is displaced to one side as the cytoplasm is packed with numerous inclusion bodies. The empty holes represent artifacts (A), × 10,000.
Fig. 11. EM: Neuronal cell with distinct nucleolus (Ns). The paired nuclear membrane (NM) is irregular and wavy (indicated by the arrow). × 24,000.
Fig. 12. EM: Neuronal cell showing an indistinct nuclear membrane (NM). A few mucopolysaccharide vacuoles (MV) are seen. Several mitochondria (M) with distinct cristae are present, × 18,000.
Fig. 13. EM: Numerous zebra-bodies (ZB) are present in the cytoplasm of the neuronal cells. Portions of zebra-bodies (ZB) show intense osmophilia. × 32,000.
Fig. 14. EM: Granulomembrane structures (GM) containing amorphous granular material. Fine striations can be made out in some portions of these inclusion bodies, × 88,000.
Fig. 15. EM: A glial cell (GC) with an intracytoplasmic zebra-body (ZB). × 45,000.
Fig. 16. E.M.: Part of the capillary wall with endothelial cell (EC) showing clumps of osmiophilic amorphous material contained in unit-membrane-bound vescicles. Some of this material is found in the cytoplasm without any limiting membrane (VL, vascular lumen; BM, basement membrane), × 32,000.
Fig. 17. EM: Many myelinated (MA) and non-myelinated axons (Ax) are seen. In one axonal cytoplasm numerous osmiophilic laminated inclusions are seen. × 18,000.
authors cardiac involvement was not noted in either of the patients reported here.

Musculoskeletal growth in this disorder has varied from normal to moderate dwarfism (McKusick 1966; Maroteaux and Lamy 1965; Sanfilippo et al. 1963; Abraham et al. 1970). However, severe dwarfism as seen in Hurler and Morquio’s diseases is not seen. Both of our cases were dwarfs, Case 1 being markedly short.

Mental retardation of a severe degree is a striking feature in this disease. McKusick (1966) observes: “Because good bodily strength is combined with severe mental defect, management is often a problem”. Case 2 of the present report was difficult to manage because of his destructive, hyperkinetic behaviour, whereas the other case who resembled MPS I in every respect clinically, though mentally retarded, was docile and easily manageable. These gross mental changes are presumably due to the widespread neuronal involvement in the disease process.

Radiological features

Thickening of the skull vault in this disease has been noted by various authors (Maroteaux and Lamy 1965; McKusick 1966; Gordon and Thursby-Pelham 1969; Haust et al. 1971). Abraham et al. (1970) noted normal thickness of the skull vault in 2 of their cases in which X-rays were taken. Both the cases in the present study had thick vaults and in 1 anterior pouching of the pituitary fossa was also noted. The brain in these cases is invariably atrophic and it is conceivable that thickening of the calvarium, at least in part, is compensatory in nature.

Skiagrams of the spine showed biconvex vertebrae in both cases. Beaking and kyphosis of the vertebral bodies was marked in Case 1, whereas it was mild in the other. McKusick (1966), Gordon and Thursby-Pelham (1969), Abraham et al. (1970) and Haust et al. (1971) did not note any spinal abnormalities. However, other authors (Sanfilippo et al. 1963; Maroteaux and Lamy 1965) have reported vertebral abnormalities similar to those seen in MPS I, but usually of milder degree. Mild “coning” of the metacarpal bones as seen in other mucopolysaccharidoses was noted in Case 1. However, triangulation of the ulnar and radial metaphyses was absent in both patients. X-rays of the pelvis were not taken in our cases. Maroteaux and Lamy (1965) have reported the iliac wings to be often slightly massive in appearance and of slightly reduced height. These authors also observe that the acetabular roofs are sometimes slightly irregular but, more particularly, the development of the head of the femur appears markedly insufficient as compared with the acetabulum.

Nerve conduction and needle EMG studies

Of the 2 cases of Sanfilippo disease on whom EMG findings have been reported earlier by us (Taori, Iyer, Abraham and Mammen 1971), 1 who showed fibrillation potentials is being reported in detail in the present communication (Case 1). He also showed mild reduction in conduction velocity in peripheral nerves. In Case 2 of the present study, fibrillations were noted mainly in the proximal muscles of the upper limbs. The conduction velocities of the motor peripheral nerves were normal. The pathological findings of “group muscle fibre atrophy” (Case 2) and the EMG findings of fibrillation suggest the involvement of lower motor neurons in this disease. Histopathological findings of marked ballooning and loss of some anterior horn cells

J. neurol. Sci., 1972, 17: 323-345
(Case 2) confirms this contention. In our previous communication (Taori et al. 1971) it was observed that the case which resembled MPS I clinically, showed fibrillation potentials, whereas the other case which had the least clinical resemblance to MPS I, did not show this abnormality. It is interesting to note that Case 2 in the present communication also showed many clinical features of MPS I even though these were not as striking as those in Case 1.

Histopathology

Frontal lobe biopsy from both these patients showed identical histopathological changes in the cortical neurons, which were balloononed with PAS-positive lipid material. Similar findings have been reported earlier by other authors (Wallace et al. 1966; Abraham et al. 1970). On necropsy, Case 2 showed similar involvement of the nerve cells at all levels of the central nervous system. However, macrophages and demyelination were not prominent features in these 2 cases. Sural nerve biopsy from Case 2 showed demyelination. The cause of this remains uncertain.

The electron-microscopic appearances of the brain sections noted in this study are very similar to those described in cases of MPS I (Aleu, Terry and Zellweger 1965) and MPS III (Wallace et al. 1966). The intraneuronal zebra-bodies are thought to be due to accumulation of ganglioside, and they bear a striking resemblance to those seen in Tay–Sachs disease, although in the latter, most of the cytosomes are concentrically laminated (Aleu et al. 1965). Membrane systems closely similar to those of Tay–Sachs disease have been created in vitro by incubating three lipid fractions, i.e. cholesterol, ganglioside and phospholipid, in an ionic medium (Samuels, Gonatas and Weiss 1964). This work indicates that the lamellar cytosomes in Tay–Sachs disease and in MPS I and III are the result of a physicochemical phenomenon involving the orientation of the complex lipid molecule in relation to an aqueous milieu.

The intraneuronal fine granular bodies probably represent transitions between these cytosomes and typical zebra-bodies. Aleu et al. (1965) have postulated that these granular forms represent unorganised structures where the membrane formation is retarded for lack of some substrate, rather than representing a product of degradation. Perithelial vacuoles have been thought to correspond to the soluble mucopolysaccharide content (Aleu et al. 1965). Such vacuoles presumably containing mucopolysaccharides are present, but in smaller number, in the neurons also. Small amounts of lamellar material, which may correspond to ganglioside, have been observed in the perithelial cytoplasm (Aleu et al. 1965). However, these authors observe that there is a striking topographic separation of these two major components, the zebra-bodies representing ganglioside accumulating in the neurons mainly, and the vacuoles probably corresponding to mucopolysaccharide content accumulating in the perithelial region. Aleu et al. (1965) have observed that the rarity of the vacuolar material in the neurons makes unlikely the possibility that these cells are concerned with its synthesis. Meyer (1961) had shown that dermatan sulphate and heparan sulphate are the normal constituents of the vessel wall. The latter predominates in MPS III and the former in MPS I. It would seem quite possible then that the perithelial cell is the major site of synthesis of these mucopolysaccharides in health and in mucopoly-

SACCHARIDOSIS. However, the presence of these vacuoles in the neurons raises the question of the origin of mucopolysaccharides in these sites also, even though not as much as in the perithelial region. Similar vacuoles have also been described in parenchymal cells of liver (Wallace et al. 1966; Haust et al. 1971).

The granular osmophilic material noted in the endothelium is considered to be of less specific nature. In the present study some of this material is membrane-bound. Aley et al. (1965) have suggested that it might represent miscellaneous products of cellular degradation coming from neurons, myelin sheaths and perhaps glial and mesenchymal cells.

In some glial cells a few inclusion bodies of lamellar type were noted. The mechanism of this incorporation into the glial cell is not clear. It may result from phagocytosis by the glia or could be due to synthesis in situ. The latter is a possibility in view of the recent evidence of small but definite concentrations of ganglioside in isolated astrocytes (Leden 1970).

The changes in the axons resembled those noted in Tay–Sachs disease (Terry and Weiss 1963) and Hurler's disease (Aley et al. 1965). These changes could be due to axonal degeneration following degenerative changes in the soma of the neuron.

Biochemistry

The increased urinary excretion of GAG, predominantly heparan sulphate and the absence of or only traces of dermatan sulphate are the hallmarks of this disease. The percentage of the heparan sulphate excreted in the urine as reported by various authors has varied from 45 to 100% (Wallace et al. 1966; McKusick 1966; Maroteaux and Lamy 1965; Gordon and Thursby-Pelham 1969; Abraham et al. 1970). Dermatan sulphate has been either absent (McKusick 1966; Maroteaux and Lamy 1965; Gordon and Thursby-Pelham 1969; Abraham et al. 1970) or present only in traces (Muir 1969; Kaplan 1969; Haust et al. 1971). In both the patients in the present communication, no dermatan sulphate was detected.

In the brain tissue, total GAG was within normal limits in both patients. However, heparan sulphate was markedly increased differentially. Similar findings have been reported by Wallace et al. (1966) and more recently from our laboratory (George and Bachhawat 1970). Dermatan sulphate was somewhat increased in Case 1.

Total gangliosides in the brain were within normal limits in both the patients. However, in the TLC pattern it was obvious that the faster-moving gangliosides GM$_2$ and GM$_3$ were differentially increased. This finding is similar to that noted earlier in MPS I (Abraham, Chakrapani, Singh, Kokrady and Bachhawat 1969) and in MPS III (Wallace et al. 1966; Abraham et al. 1970).

CSF analysis for GAG was done only in Case 1 and was found to be markedly increased (400 µg/100 ml) as compared to the reported values of 8–10 µg/100 ml in normal individuals (Constantopoulos and Dekaban 1970). These authors have reported an increase of GAG in the CSF of MPS I and II cases, the GAGs being dermatan and heparan sulphates. The mechanism of increase of GAG in the CSF in these disorders remains uncertain. However, it is interesting to note that in our case the proportion of heparan sulphate in the CSF (60%) was very much similar to that in the brain tissue (57.8%). Therefore, it is tempting to postulate that the

*J. neurol. Sci., 1972, 17: 323–345*
CSF GAG is a reflection of GAG content of the brain tissue. However, this needs to be further studied.

Both the patients described in the present communication differ from those described earlier from our department (Abraham et al. 1970), in that these 2 patients showed a resemblance to MPS I clinically, more so in Case 1, whereas in the previously reported cases the resemblance was slight. Both the patients showed fibrillation potentials on EMG studies whereas, in 1 patient previously studied, fibrillations were not noted (Taori et al. 1971). Biochemically 1 (Case 1) showed a mild disturbance of dermatan sulphate in the brain, in addition to marked derangement of heparan sulphate, whereas the other (Case 2) had biochemical findings similar to those of the patients described earlier (Abraham et al. 1970). These patients came from different families and those described by Abraham et al. (1970) were from 1 family. From the clinical data discussed so far, it seems probable that this syndrome may have at least two subgroups. Haust et al. (1971) have suggested two subgroups on the basis of different patterns of urinary GAG excretion. Kresse, Wiesmann, Cantz, Hall and Neufeld (1971) by cross-correction tests have shown that MPS III fibroblasts can be divided into two groups, each deficient in a different factor, but the mucopolysaccharide metabolism may be corrected by mixing one with the other. A similar difference has been noted in the macromolecular correcting factor in the urine of the patients by these workers (Kresse et al. 1971). However, from the clinical summaries available to these authors, no obvious phenotypic difference between the two biochemical subgroups of MPS III was noted.

The basic defect which explains the abnormal pattern of gangliosides in the brain and the abnormality of mucopolysaccharides in various organs including brain in MPS I, II and III is still not clear. Deficiency of β-galactosidase has been demonstrated in the skin, liver and brain in these disorders (Van Hoof and Hers 1968; Ockerman, Hultberg and Ericksson 1969; MacBrinn, Okada, Woollacott, Patel, Ho, Tappel and O'Brien 1969). Since acid mucopolysaccharides share with gangliosides the presence of hexose units and N-acetyl-hexosamine, it is postulated that β-galactosidase deficiency can explain the disturbance of both these substances. However, the fact that these 3 different genetically determined disorders, 2 of them autosomal recessive and 1 sex-linked, show a common enzyme deficiency, has made MacBrinn et al. (1969) suggest that the deficiency of this enzyme in the tissues may be a secondary phenomenon, probably unrelated to the primary defect. Ockerman (1968) and Ockerman et al. (1969) have found that the fraction of β-galactosidase which is deficient in the tissues of MPS I and II was found in normal or excess quantity in plasma and urine of these patients. Kinetic studies by Fratantoni, Hall and Neufeld (1968) showed that the excessive accumulation of sulphated mucopolysaccharide results from impaired degradation. This excessive accumulation in MPS I, II and III fibroblasts has been shown to be reduced to normal by macromolecular factors, derived from fibroblast secretions, fibroblast homogenates and human urine, provided the donor of the cells or urine is not of the same genotype (Fratantoni, Hall and Neufeld 1969; Cantz, Chrambach and Neufeld 1970; Kresse et al. 1971). The corrective factor is specific for each genotype; it is probably protein and is distinct from common lysosomal hydrolases, including β-galactosidase (Fratantoni et al. 1969;
Cante et al. 1970; Kresse et al. 1971; Barton and Neufeld 1971). Thus, it is possible that the necessary degradative enzymes which may presumably be lacking in the tissue cells in these disorders may either be induced or their loss from the cells is prevented by the corrective factor. The deficiency of β-galactosidase in the various tissues and increased levels in serum and urine of patients (Ockerman 1968; Ockerman et al. 1969), favors the latter possibility. However, this hypothesis, according to which the deficiency of β-galactosidase could occur at the cellular level as a result of a correcting factor deficiency, cannot explain why one rather than the other mucopolysaccharide is involved because the protein-GAG linkage region where this enzyme is supposed to act is the same for all GAGs except hyaluronic acid and keratan sulphate. According to this hypothesis one would expect both dermatan sulphate and heparan sulphate to accumulate in MPS III. However, in this particular disorder, heparan sulphate alone is preferentially accumulated. Therefore, it is more likely that the primary defect of deficiency of corrective factor acts directly on the tissue cells rather than through β-galactosidase or else there could yet be another mechanism by which this GAG is accumulated at the expense of the others. In recent years, in our laboratory complete absence or very low activity of chondroitin-4-sulphate sulphotransferase, normal values for sulphotransferase activity to transfer sulphate to the amino group of heparan sulphate as compared to the hydroxy group of heparan sulphate has been noted (George and Buchhawat 1970). Perhaps the availability of specific GAG sulphotransferase may determine which GAG will preferentially be accumulated.

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SUMMARY

Clinical, radiological, electromyographic, histopathological and biochemical findings of 2 cases of MPS III syndrome are described. Both these patients showed a clinical resemblance to MPS I, the more striking resemblance being noted in Case 1.

On biochemical studies, both the patients showed a marked disturbance of heparan sulphate. In addition, in Case 1, a mild disturbance of dermatan sulphate was noted in the brain. At autopsy, the neuronal involvement in the disease process was noted at all levels of the central nervous system. Peripheral nerve and muscle biopsies done on 1 patient showed evidence of demyelination and "group muscle fibre atrophy". Electron-microscopic findings on the brain tissue are described in 1 of the patients.

These cases are compared with other reported cases of this syndrome and it is suggested that these patients differ phenotypically from some of the cases described

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in the literature. The literature on various aspects (clinical, radiological, histopathological, and biochemical) of this syndrome is reviewed.

REFERENCES


SANFILIPPO SYNDROME