Mannosidosis

Clinical and biochemical study

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SUMMARY The clinical, radiological, and biochemical features of 2 male children with mannosidosis are described. Superficially they appeared to suffer from Hurler's syndrome, but the facies, eye signs, radiological and cytological features were atypical. Excess urinary oligosaccharides were found by thin-layer chromatography. The diagnosis was confirmed by determining the acidic α -mannosidase activity of leucocytes and cultured skin fibroblasts. Prenatal diagnosis is possible from cultured amniotic cells.

Mannosidosis belongs to a group of disorders variously described as oligosaccharidoses or glycoprotein storage diseases and superficially resembles Hurler's syndrome in its clinical manifestations. The disease is genetically determined and is inherited as an autosomal recessive. Characteristically, patients show progressive psychomotor retardation, coarse facies, skeletal deformities, hepatosplenomegaly, lens opacities, and have vacuolated lymphocytes.

The disorder was first defined biochemically by Öckerman (1967) who demonstrated accumulation of mannose-rich oligosaccharides in the tissues of patients which could be accounted for by a severe deficiency of acidic α -mannosidase activity. Mannose-containing oligosaccharides are also excreted in the urine of patients (Nordén *et al.*, 1973). Recently Farriaux and Fontaine (1976) reviewed the few published case reports, gave a general description of the clinical features, and defined the biochemical criteria necessary for establishing the diagnosis.

It is likely that the full spectrum of the symptomatology of this disorder has not yet been defined. For this reason and because no cases have so far been reported for England, we report 2 patients studied at the Hospital for Sick Children and the London Hospital, and St. Albans City Hospital.

Case reports

Case 1. A boy was born normally at term, on 18 August 1957, birthweight 3.6 kg. During the first 3

days of life he had respiratory difficulties, thereafter the neonatal period was normal. From the age of 3 months he had recurrent respiratory infections which led to a right upper lobectomy at 11 months, though development during the early months was considered normal. Crawling was delayed until 1 year of age and walking until 2½ years, at which time he had few clearly recognizable words, and was found to have a severe conductive deafness.

A further respiratory infection at the age of 3 years led to readmission. He was then found to have coarse facies, broad hands, a lumbar kyphosis, and hepatosplenomegaly. There were no eye abnormalities. X-rays of skull and spine were reported to be typical of Hurler's syndrome; urine contained excess glycosaminoglycans, and coarse cytoplasmic vacuoles were present in lymphocytes of peripheral blood.

A developmental assessment on a Ruth Griffith's profile gave an overall development quotient of 49 with particularly marked delay in hearing/speech and performance categories. He was placed in a nursery school for the mentally handicapped at this time and later attended the primary and junior departments of the same school.

Recurrent upper and lower respiratory tract infections continued, adenoids were removed at 4 years, and tonsils at 7 years. The previously noted abnormalities were still present. At the age of 8 he developed a right-sided empyema and osteomyelitis of the left tibia, both due to *Staphylococcus aureus*. Since then he has had no further important infections.

Around the age of 15 years, his gait had become lopsided and over the next 2 years exercise tolerance

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became limited by pain and dyspnoea. On readmission his speech consisted of grunted sounds intelligible only to his parents. Motor function was clumsy. The physical features noted previously were still present; liver and spleen were no longer palpable and now a very obvious kyphoscoliosis is present as shown in Fig. 1.

Fig. 1 Case 1 aged 17 years. The coarse facies and broad hands are still present. The kyphoscoliosis is now very obvious.

Again the lymphocytes had coarse PAS-positive vacuoles, often occurring in clusters around the nucleus. Urinary glycosaminoglycan : creatinine ratio was 25 (normal range for age 18–67). X-rays showed a thickened calvarium and broad ribs. The spine, in addition to the obvious kyphoscoliosis, was abnormal at all levels, with irregular disc spaces and erosions, and beaking of the vertebral bodies (Fig. 2). Respiratory function studies showed that the dyspnoea on exercise was due to moderately severe restrictive lung disease resulting from the structural changes in the spine. Tests of immunological function including leucocyte function tests were normal, and no explanation was found for the recurrent infections. The biochemical studies which led to the diagnosis of mannosidosis are described below.

At the time of writing the patient is 19 years of age, lives at home, and attends a training centre.

Activities within the community are restricted by his difficulty in communication and by pain and dyspnoea on exercise. Communication has improved recently as he has been placed with a group of adolescents with hearing difficulties at his training centre.

Case 2. A boy was born normally at 37 weeks, on 23 January 1973, birthweight 2.5 kg. He progressed normally during the first year, sitting up at 6 months, standing with support at 8 months, and walking at 1 year. Height and weight had followed the 50th centile since birth.

At 18 months he kept falling over and was noted to be overactive, to have a lumbar kyphosis, coarse facies, and broad hands (Fig. 3). Examination of his eyes showed very early cataract formation with punctate opacities arranged like the spokes of a cartwheel. By the age of 21 months he had mild hepatosplenomegaly; and the punctate lenticular opacities had coalesced to form the spoke-like cataracts shown in Fig. 4.

X-rays showed a thickened calvarium, broadened ribs, and kyphosis at the level of the 2nd lumbar vertebrae with beaking of the bodies of the 2nd and 3rd lumbar vertebrae. Lymphocytes of peripheral blood contained PAS-positive vacuoles. The glycosaminoglycan : creatinine ratio was slightly increased at 201 (normal range for age 60–192). The biochemical findings which led to the diagnosis of mannosidosis are described below.

Developmental assessment at 2½ years showed an overall development quotient of 69 on a Ruth Griffith's profile with particular delay in hearing/speech and performance categories. The delay in hearing and speech was also shown on Stycar Hearing Tests and the Reynell Development Language Scale (verbal comprehension 1 year 8 months, expressive language 1 year 6 months).

The patient is now 3½ years of age, and lives at home. His general condition is deteriorating, he suffers from frequent upper respiratory tract infections, and growth velocity has fallen, his height being below the 25th centile. The abnormal physical features are more marked, and developmentally he is falling further behind. A Ruth Griffith's profile gave an overall development quotient of 63; delay in the hearing/speech and performance categories have become more marked, performance being about the 2-year level. Formal hearing testing suggested that he has a conductive deafness.

Biochemical investigations

24-Hour urine specimens were collected from Case 1 and random specimens from Case 2. Purified leuco-

Fig. 2 Case 1. X-rays of the dorsolumbar spine, showing marked kyphoscoliosis. The vertebral bodies show beaking, erosions, and partial collapse.

cyte pellets and plasma specimens were obtained from both patients and their parents. Fibroblast cultures grown in medium 199 plus 15% fetal bovine serum were established from skin biopsies from the 2 patients.

For the chromatography of urinary oligosaccharides, ethanol was added to an equal volume of urine, and the mixture (40 μ l) applied to thin-layer plates of silica gel (Silica Gel 60, No. 5553, Merck). After chromatography for 2 hours in a solvent system, n-butanol-acetic acid-water (2:1:1 by volume) sugars were located with aniline-diphenylamine reagent.

α -Mannosidase was assayed in centrifuged supernatants from homogenates of leucocytes or fibroblasts in 0.2 M KCl. The standard assay mixture contained: 2.5 mM 4-methylumbelliferyl- α -D-mannopyranoside (Koch-Light Ltd.) in 0.05 M citrate-phosphate buffer pH 4.0 (200 μ l), and leucocyte or fibroblast extract (5 μ l, containing 50–70 μ g protein) or plasma (5 μ l). Where appropriate the buffer-substrate solution also contained 0.5 mmol Zn^{++} /l. Blank assays contained no enzyme. After 15 minutes' incubation at 37°C for leucocyte or fibroblast assays, or 60 minutes for plasma assay, 2.3 ml of 0.25 M glycine-NaOH, pH 10.4, was added and the fluor-

Fig. 3 Case 2 aged 18 months. The coarse facies, broad hands, and lumbar kyphosis can be seen.

escence of the liberated 4-methylumbelliferone measured. One unit of activity was equivalent to the hydrolysis of 1 nmol of substrate per hour at 37°C.

Protein was determined by the method of Lowry *et al.* (1951).

Results

Urinary oligosaccharides. Thin-layer chromatography showed a heavy oligosaccharide band as the major abnormality in the urines from the 2 patients. This mannose-containing substance (R_f 0.27) coincided exactly with an authentic specimen of M_2G -trisaccharide isolated from the urine of a patient with proven mannosidosis (Nordén *et al.*, 1973).

α -Mannosidase. Activity in leucocytes, plasma, and cultured fibroblasts of the 2 patients and in leucocytes and plasma of their parents are summarized in the Table. As in other reported cases, the character-

Table α -mannosidase activity (pH 4.0) of leucocytes, plasma, and cultured fibroblasts

	Leucocytes (U/mg protein)	Plasma (U/ml)	Fibroblasts (U/mg protein)
Case 1	6	3	10
Mother	54	16	—
Father	121	38	—
Case 2	4	3	7
Mother	110	35	—
Father	72	30	—
Controls			
range (mean)	101–463 (193) (n=20)	32–178 (70) (n=12)	106–236 (156) (n=10)

Fig. 4 Case 2. Slit lamp photograph of lens, showing spoke-like opacities of the posterior cortex of the lens.

istic enzymic abnormality in the patients was a severe deficiency of α -mannosidase activity at a pH optimum of approximately 4. The residual levels of activity ranged between 2 and 6% of the mean values for corresponding controls. For the patients and controls the enzymic activity at pH 4.0 was stimulated approximately 20% in the presence of 0.5 mM Zn^{++} . Other mannosidase components, with optimum activities in the pH ranges 5.0–5.5 and 6.0–6.5 are present in normal cells and plasma, and these components were shown to be present at normal levels of activity in the patients.

The specific activity of acidic α -mannosidase in plasma and leucocytes of the parents, presumed heterozygotes for the condition, were 23 and 28% respectively of the mean values of the controls. Considerable overlap with the normal range was, however, found so that heterozygosity could not be defined with confidence. Masson *et al.* (1974) showed that the determination of the ratio of α -mannosidase activity to that of an unaffected enzyme, hexosaminidase A, allows differentiation of heterozygotes from homozygous affected and normal subjects. Similarly, in our families determination of the ratio of α -mannosidase to β -galactosidase activity in leucocytes allowed such differentiation to be made.

Discussion

Only few cases of mannosidosis have been reported since the first description by Öckerman (1967) and the full spectrum of the symptomatology cannot yet be defined. The clinical picture is becoming clearer and certain cardinal findings are emerging. In the fully developed picture, symptoms and signs are generally similar to those of Hurler's syndrome, with coarse facies, psychomotor retardation, skeletal abnormalities, hepatosplenomegaly, and opacities of the lens. The 2 patients reported here showed most of these features and in our first patient, slow development and clumsiness were noted towards the end of the first year.

The most marked skeletal changes were a thickened calvarium, eroded and beaked vertebral bodies, and a lumbar kyphosis. With the exception of the case reported by Tsay *et al.* (1974), which resembles Case 2 in his very early stages, vacuolated lymphocytes were present in all the reported cases and were invariably detected in our patients. In both our patients at least 30% of peripheral lymphocytes were vacuolated.

Both patients suffered from a speech defect, due to both defective comprehension and expression; in our first patient, the defect only allowed him to communicate within the close family circle. They both suffer from a conductive deafness with onset in

late infancy which must play an important part in their speech abnormality but it is uncertain whether the deafness is the result of the frequent upper respiratory tract infections or more directly of the mannosidosis. Recurrent infections, which are a feature of Hurler's syndrome, and have been reported in mannosidosis, occurred in both patients, ceasing in the first patient at about the age of 8 years. Bilateral spoke-like cataracts were present in Case 2 whereas no eye abnormality has been found in Case 1. Cataracts have been found in 4 of the previously described cases and in 2 of these were described as wheel-like. Murphee *et al.* (1976) have shown in one patient that the opacities consist of multiple vacuolations in the posterior cortex of the lens. The origin of these vacuolations is uncertain but it is unlikely that they are lysosomal in origin. Ketoacidosis, which led to the death at the age of 4½ years of the patient reported by Kjellman *et al.* (1969), was not noted in our patients nor in the other published cases.

Case 1 is now 19 years old but is seriously handicapped by skeletal and speech defects. The marked kyphoscoliosis has resulted in a lopsided gait and dyspnoea on exercise due to severe restrictive lung disease. Case 2 is now 3½ years old and although perhaps less severely affected than Case 1 at the same age, he seems to be following a similar course in many respects.

The clinical, radiological, and haematological findings in the cases of mannosidosis described here and in previous reports (Farriaux and Fontaine, 1976) point to a diagnosis of a 'storage disorder' but are not specific for mannosidosis. Other lysosomal diseases which result from a failure of degradation of the complex carbohydrate chains of sphingolipids, glycosaminoglycans, and glycoproteins, as well as the more generalized lysosomal defects of the mucopolidoses, manifest similar symptoms and signs. The definitive diagnosis depends on the demonstration of the underlying specific biochemical abnormality; namely, a deficiency of lysosomal α -mannosidase.

α -Mannosidase activity with a pH optimum of approximately 4.0 is reduced to very low levels in all tissues from affected individuals and represents the combined loss of the A and B acidic components of lysosomal α -mannosidase (Carroll *et al.*, 1972). The enzymic deficiency can be detected easily and reliably using plasma or leucocytes (Masson *et al.*, 1974) or cultured skin fibroblasts (Taylor *et al.*, 1975). The low levels of residual α -mannosidase activity determined in these tests at pH 4 is probably due to a small contribution from intermediate and neutral mannosidase components which are unaffected in mannosidosis. This applies particularly to assays using plasma in which the major α -man-

nosidase component has a pH optimum in the intermediate range of 5.0–5.5.

Having established the diagnosis by the finding of low levels of mannosidase activity, it is generally unnecessary to devote much effort to the demonstration of excess mannose-rich oligosaccharides in the urine. Their isolation and characterization (Nordén *et al.*, 1973) is beyond the scope of most clinical laboratories and measurement of the total mannose content of urine may give misleading results (Tsay *et al.*, 1974). The detection of excess oligosaccharides by direct thin-layer chromatography of urine on silica gel (Humbel and Collart, 1975) provides a useful corroborative or screening test in the investigation of patients showing the clinical, radiological, or haematological features of a 'storage disorder'.

Prenatal diagnosis is theoretically possible from cultured amniotic cells. Taylor *et al.* (1975) showed that the specific activity and electrophoretic properties of the acidic α -mannosidase in cultured amniotic cells are similar to those found in cultured skin fibroblasts. To date, however, prenatal diagnosis has not been undertaken though many laboratories are in a position to do so should the occasion arise.

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