10% to between 2% and 4%.

A bifid pelvis may arise by either a splitting of the tip of the developing ureteric bud or the formation of an accessory ureteric outgrowth of the mesonephric duct. Nation found that renal ectopia was the most common anomaly associated with duplication of the renal pelvis. Gray and Skandalakis reported that the incidence of unilateral bifid pelvis is much higher than that of bilateral duplication.

Aside from the developmental and morphological interest of this case, it has practical importance from a clinical viewpoint. An unrecognized persistent renal collar may pose a danger, since a surgeon may identify only the anterior component of this anomaly. Hydronephrosis, lithiasis and infection commonly affect kidneys with double pelves. The presence of a kidney at the level of the aortic and caval bifurcations in a woman with a gravid uterus may pose problems of vascular and ureteric obstruction, especially when the woman is lying in a supine position. Also, awareness of the existence of variations in the vascular supply and drainage of a malascended kidney is of importance to the surgeon who attempts to remove such a kidney or attempts to work on the veins and arteries which it covers.

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Glycogen Storage Disease Type IV
Diagnosed Biochemically

A Case Report

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SUMMARY

A case report of a child with glycogen storage disease type IV is presented. The diagnosis was confirmed by enzyme assay on cultured fibroblasts. Some unusual features of this disorder are discussed and the possibility of antenatal diagnosis is emphasized.


Glycogen storage disease type IV (GSD IV), also known as Andersen's amylopectinosis or branching enzyme deficiency, is a very rare disorder and only 7 definite cases and 4 probable ones have been reported. The demonstration by Howell et al. that the specific enzyme deficiency responsible for the disease can be detected in cultured fibroblasts means that a definitive diagnosis can now be made. The object of this article, in addition to describing the clinical and histopathological features of the condition, is to inform paediatricians and other clinicians of the availability of the enzyme assay in a South African laboratory.
CASE REPORT

A 2½-year-old girl of Portuguese extraction first presented on 29 September 1975 to the Transvaal Memorial Hospital for Children, Johannesburg. She was born in Luanda, Angola, following an uneventful 8-month pregnancy and delivery. There were no complications during the neonatal period. The patient seemed to gain weight steadily for the first few months of life. From 4 months of age, however, periodic vomiting occurred, associated with anorexia and failure to thrive. There was no diarrhoea, and stools were normal in colour. Her motor development was normal until 1 year of age. At that stage she was able to sit and stand but was not able to walk.

At 1 year of age and while she was still in Angola, she experienced a pyrexial illness accompanied by mild jaundice. This was diagnosed and treated as malaria. The jaundice subsequently cleared. By 18 months of age abdominal distension had developed. When the patient was 2 years and 9 months the family moved from Luanda to Maputo in Mozambique. Further investigations were performed and after a liver biopsy the patient was said to be suffering from chronic active liver disease. Shortly thereafter mother and child travelled to South Africa and sought medical advice in Johannesburg.

The patient had never received any blood transfusion or other blood products. There was no history of any other significant illnesses. Immunizations were incomplete, as the child had only received BCG vaccination and one dose of triple vaccine (diphtheria and tetanus toxoids and pertussis vaccine). She was the only child in the family and there was no consanguinity between the parents. The mother had no miscarriages. There was no family history of liver disease.

Examination revealed a thin, underweight and apprehensive child. Her weight (9 kg), length (82 cm) and head circumference (46 cm) were below the 3rd Boston percentile. She was not pale or jaundiced but had palmar erythema and early clubbing of the fingers. No cataracts or Kayser-Fleischer rings were seen. Her pulses were of good volume. There was no cardiomegaly but she had a 2/6 ejection systolic murmur, maximal at the left sternal border. Her chest was clear. Her abdomen was distended and superficial dilated veins were noted. There was an 8-cm firm, non-tender, nodular hepatomegaly, and a 4-cm firm splenomegaly. Ascitic fluid was present. She was generally weak with obvious muscle wasting, needed assistance to sit up, and was unable to walk.

Investigations

The haemoglobin level was 10.8 g/100 ml with reasonably normal indices. The white cell count was 8 800/μl and platelet count 94 000/μl. The prothrombin index was 67%. The SGOT value was 330 units and SGPT 74 units; LDH was 200 units (normal range 24-78 units); and alkaline phosphatase 13 KA units. The total serum protein level was 7.6 g/100 ml, with albumin 2.6 g/100 ml, α-globulin 0.3 g/100 ml, α₂-globulin 0.9 g/100 ml, β-globulin 0.8 g/100 ml and γ-globulin 3.0 g/100 ml. The IgG level was 2 400 mg/100 ml, IgA 392 mg/100 ml and IgM 279 mg/100 ml.

Blood smears for malaria were negative as were the direct Coombs test and Wassermann reaction. Tests for anti-nuclear factor, Australia antigen and liver antibodies were negative. The serum caeruloplasmin level was 28 mg/100 ml (normal range 16 - 33 mg/100 ml) and serological studies for cytomegalovirus were negative. The α-antitrypsin phenotype (Pi), kindly determined by Dr I. Prinsloo of the National Research Institute for Occupational Diseases, was FM.

Material for histological examination was obtained by percutaneous liver biopsy.

Methods

Material was stained for glycogen by Best’s carmine and iodine methods. The iodine method was that described by Culling except that oil of cloves was used in place of oreganum.

Fibroblast cultures were established from skin biopsy specimens of the patient and her mother. When these had reached confluence the cells were scraped from the flasks, suspended in 1 ml of de-ionized water and subjected to three cycles of freezing and thawing. The lysate was then centrifuged for 10 minutes at 10 000 g and the clear supernatant used for enzyme assays.

The branching enzyme (amylo-1,4→6-transglucosidase) was assayed according to the method of Brown and Brown.9 The reaction rate was monitored by following the release of inorganic phosphate as measured by the method of Ernster et al. after the reaction had been stopped with 10% trichloro-acetic acid. Protein was measured by the method of Lowry et al.6

RESULTS

Histological examination of the liver showed disruption of the normal architecture with formation of nodules of liver cells surrounded by dense connective tissue (Figs 1 and 2). Many of the hepatocytes showed a plant-like appearance with central nuclei and a fine reticular cytoplasm. There was also vacuolation, but no fat was demonstrated. The sections were positive for glycogen. The glycogen stained red with Best’s stain but deep blue with iodine. A diagnosis

![Fig. 1. Liver biopsy specimen showing islands of hepatocytes surrounded by connective tissue.](image-url)
Fig. 2. Reticulin stain showing micronodular cirrhosis.

was made of micronodular cirrhosis with features suggestive of amylopectinosis.

The results of enzyme assay showed very low levels of enzyme activity in the patient's fibroblasts (0.19 μmol/min/mg protein) compared with the control values (1.61 ± 0.18 mean (±SD) of 4 individuals tested). The fibroblasts of the patient's mother (0.91 μmol/min/mg protein) showed enzyme activity in the intermediate range in keeping with what would have been expected in a heterozygote.

DISCUSSION

The unusual feature of amylopectinosis is cirrhosis. This is absent in the other varieties of glycogen storage disease although it has been described as occasionally present in GSD type III.

The cause of cirrhosis in GSD IV is obscure. Andersen has postulated that the hepatic fibrosis and eventual advanced cirrhosis are due to the presence of an abnormal glycogen which leads to a foreign body reaction, yet Sidbury et al. could find no histological evidence for this. Analysis of the glycogen led to the suggestion of a branching enzyme (amylo-1,4→1,6-transglucosidase) deficiency.

During the normal biosynthesis of glycogen, peripheral chains of glycosyl units in 1,4 linkages are formed. When these chains contain 7-21 glycosyl units the branching enzyme creates a side-chain. It does this by transferring some of the glycosyl units to a branch position either on the same chain from which they were derived or onto another chain. Glycosyl units may then be added to this new side chain, thereby increasing its length. In the absence of the branching enzyme an abnormal glycogen is produced. This has been analyzed and found to resemble the amylopectin of plant starch — hence the name amylopectinosis.

The glycogen that accumulates in GSD IV is characterized by outer branches of 14 or 15 glycosyl units and inner branches of 5-6 units in length. Normal glycogen has outer and inner chains of 7-8 units and 3-4 units, respectively. The greater chain length and the fewer branches of amylopectin are believed to account for its lower solubility when compared with normal glycogen. This abnormal and less soluble glycogen may be the cause of the cirrhosis of the liver in patients with GSD IV.

Nevertheless, a substantial amount of branching of the hepatic glycogen still occurs and a number of hypotheses have been suggested to explain this. Recent electron microscopical studies demonstrated the glycogen to be in the form of inclusions with low electron density. The inclusions consist of a rim of normal-appearing glycogen and a large central core of abnormal glycogen. The abnormal glycogen had a fine granular and fibrillar appearance with the fibrils being 60-65 Å units in diameter.

The abnormal structure of this glycogen results in an altered iodine-poly saccharide complex absorption spectrum, a property which has been used to help differentiate amylopectinosis from other forms of glycogenesis. Whereas iodine stains normal glycogen a brownish-red colour, it stains the amylopectin-like material deep blue. This is also in contrast to the red colour obtained with Best's carmine. Fresh frozen material has been recommended for staining since prolonged fixation abolishes or decreases the blue colour.

Although GSD IV appears to be the rarest of the glycogen storage diseases, it is possible that some cases are being misdiagnosed. The weakness which often accompanies this condition can lead to a diagnosis of Werdnig-Hoffmann's disease. Furthermore, the vacuolation of the hepatocytes may be mistaken for fatty infiltration.

A definitive diagnosis can be made by assaying enzyme activity in hepatic tissue, leucocytes and cultured skin fibroblasts. Leucocyte enzyme activity in parents of patients with GSD IV has occasionally been normal rather than heterozygous.

This autosomal recessively inherited condition is invariably fatal and the risk of its occurrence in future offspring is 1 in 4. This, however, need not deter carrier parents from further pregnancies. Howell et al. demonstrated branching enzyme activity in cultured fibroblasts and cultured amniotic fluid cells obtained between the 15th and 17th weeks of pregnancy. Antenatal diagnosis is therefore feasible, provided that there is no embryonic type of branching enzyme which later disappears. The ability to determine whether a fetus is affected would greatly assist couples who know, usually through having had a child with this distressing disease, that they are at risk.

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