Two Cases in Glycogenosis Type IV.

I. Light Microscopic Observation.

Tokuhiro ISHIHARA, Fumiya UCHINO,
Hironobu ADACHI, Mutsuo TAKAHASHI,
Seishiro WATANABE, Hiromi ONISHI
Yoshihiko IKEE* and Sachiyo MISAWA*
First Department of Pathology, Yamaguchi University School of Medicine, Ube, Japan.
**Shimonoseki National Hospital.*
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INTRODUCTION

Glycogenosis type IV, which was first reported by Andersen in 1952[1], is one of the less common forms of glycogen storage diseases and is characterized by hepatosplenomegaly, poor prognosis, progressive cirrhosis and deposits of the polysaccharide similar to amylopectin in the liver, heart and reticuloendothelial system.

As far as we know, the disease has been reported in only twelve cases in foreign countries[1-16] and two cases in Japan[17].

In this report we will describe two cases in siblings with hepatosplenomegaly, liver cirrhosis and deposits of polysaccharide in the hepatic parenchymal cells. One of the cases, the elder sister of the other case, showed only clinical signs and underwent a liver biopsy while the other case, the brother, had clinical signs, a liver biopsy and a partial autopsy. Both cases are probably considered to glycogenosis type IV from histochemical findings of deposited polysaccharide in the biopsy and autopsy specimens.

CASE REPORTS

Case 1: A 3 month old girl, the third of unrelated parents, complained of fever, decreased appetite and vomiting. The pregnancy and delivery were uncomplicated and the birth weight was 3,000 g. After birth she experienced intermittent pulmonary infections. At 2 months of age, hepatosplenomegaly was noticed.

Physical examination; She was slightly undernourished and anemic.

The liver, which was firm and smooth, was palpable 4 cm below the right costal margin and the spleen 3 cm below the left costal margin. Ascites was not detected. There was no lymphadenopathy.

Laboratory findings; A hemogram showed slight anemic and neutrophilia. The glutamic oxalacetic transaminase (sGOT) was 148 u, the glutamic pyruvic transaminase (sGPT) 81 u and lactic dehydrogenase (LDH) 400 u. Fasting blood glucose was within normal limits. A glucose tolerance test was not done.

On the ninth day, a liver biopsy was performed and the histopathological findings were as follows; The specimen was immediately cut in two parts which were fixed in 10% buffered formalin and 4.15% glutaraldehyde, respectively. Paraffin sections were stained with hematoxylin eosin and periodic acid Schiff (PAS) with or without diastase digestion.

Light microscopical findings; Moderate cirrhosis was noted in the specimen (Fig. 1) Hepatic parenchymal cells were enlarged and contained large, slightly basophilic material which was strongly stained with PAS. The central PAS positive granules of the hepatic parenchymal cells showed diastase resistant staining quality, however the peripheral PAS positive granules were not diastase resistant. Kupffer cells had slightly basophilic material in the cytoplasm. The material was strongly stained with PAS and showed diastase resistance. The proliferated connective tissues contained numerous macrophages with the same PAS positive material. The pathological diagnosis was deposition of polysaccharide with liver cirrhosis resembling postnecrotic type.

On the 34th day, she was discharged because of decreased complaints. At 6 months of age, she collapsed suddenly and died in another hospital. An autopsy was not performed.

Case 2; A 6 month old boy, the younger brother of case 1, complained of fever, anorexia and vomiting. The pregnancy and delivery were normal and the birth weight was 3,440 g. After birth he frequently had had common colds, acute tonsillitis and bronchitis.

Physical findings; He was 6 kg in weight, slightly undernourished and anemic. Cardiomegaly was not detected and the lungs were clear. Lymphadenopathy was not revealed. The liver, which was smooth and firm, was enlarged 5 cm below the right costal margin. The spleen was palpable 4 cm below the left costal margin. Ascites was minimal.

Laboratory findings; A hemogram showed slight anemia and neutrophilia. Platelet count was normal. sGOT was 510 u, sGPT 152 u, cholinesterase 0.56 u, LDH 580 u and CPK 50 u. Fasting glucose was 59 mg/100 ml. Blood glucose in a glucose tolerance test was 90 mg/100 ml at 30 minutes,

120 mg/100 ml at 60 minutes and 124 mg/100 ml at 120 minutes. Blood glucose with glucagon test was 85 mg/100 ml at 30 minutes, 99 mg/100 ml at 60 minutes, 150 mg/100 ml at 90 minutes and 150 mg/100 ml at 120 minutes.

On the 9th day, a liver biopsy was performed. The findings were similar to those of case 1 and the diagnosis was deposits of the polysaccharide with liver cirrhosis (Fig. 7). Subsequently, the right testicular hydrocele was moderately detectable and he received an operation for hernia.

On the 30th day, the liver became smaller and firmer than before and the spleen became progressively larger. The superficial abdominal vessels became prominent and ascites developed. Subsequently, he had respiratory infections.

At 10 months of age, he suddenly died of respiratory and cardiac failure. A partial autopsy was performed 3.5 hours after death.

Autopsy findings; The child was undernourished. The abdomen markedly distended with ascites. Marked testicular hydrocele was noted. The scar from the hernia operation was detected at the right inguinal region. The muscles of the abdominal wall, thorax and extremities were slightly atrophic. Fluid was not detected in the pleural and pericardial cavities. The abdominal cavity was completely filled with white-yellow colored fluid. The heart was normal in weight (160 g) and pale brown in color. The free wall of the left ventricle measured 7 mm in thickness and that of the right ventricle 2 mm. The left lung weighed 90 g and the right 100 g. Both showed slight congestion, partial atelectasis and edema. The liver was nearly two times normal weight (600 g) and was transparent, yellow and firm. The outer surface was nodular. The size of the nodules ranged from 2 mm to 10 mm in diameter. The spleen was nearly three times normal weight (180 g). It was reddish violet and slightly firm. On the cut surface the follicles were indistinct. Esophageal varices were noted. The lymph nodes were enlarged in paratracheal, pulmonary hilar, paragastric, paraortic and mesenteric areas. No abnormalities were noted in the remainders of the abdominal organs. Cervical organs, brain and spinal cord were not examined.

For light microscopy, specimens from all organs were fixed in 10% buffered formalin. In addition, the liver, the heart, skeletal muscles from the rectus abdominalis, iliolumbalis and diaphragm, the spleen and lymph nodes from multiple sites were fixed in Carnoy's solution and 4.15% buffered glutaraldehyde. Lymph nodes from multiple sites and bone marrow from the vertebrae were fixed in Zenker's solution. Pieces of formalin fixed liver and spleen were boiled for 2 hours in distilled water

before paraffin embedding. The paraffin sections were stained with hematoxylin eosin, toluidine blue, PAS, Best's carmine, colloidal iron, Mayer's mucicarmine, alcian blue and sulfate alcian blue. PAS stains were used to evaluate digestion with diastase.

Light microscopic examination; In the liver, the findings were similar to those of case 1 (Fig. 2, 8, 9). Furthermore PAS positive material reacted strongly with Best's carmine (Fig. 10). It was stained purple brown with iodine (Fig. 11), blue with colloidal iron (Fig. 12), blue with sulfate alcian blue (Fig. 13) and blue with toluidine blue (pH 2.0). It was not stained with Mayer's mucicarmine. A piece of formalin-fixed liver was boiled for 2 hours in distilled water and was slightly digested with diastase and α. amylase.

The macrophages containing the same PAS positive material were detected in the spleen (Fig. 3, 14), lymph nodes of multiple sites (Fig. 5), the gastro-intestinal tract, lungs, kidneys and heart.

The cardiac muscle showed deposits of the same PAS positive material (Fig. 6). In addition, marked infiltration of neutrophils were detected in the anterior wall of the left ventricle.

Sarcolemma of skeletal muscles contained a large amount of the same PAS positive material.

In smooth muscles of gastro-intestinal tract, a few deposits of the same PAS positive material were revealed.

The spleen showed numerous clusters of the same macrophages, slight congestion, fibrosis and extramedullary hematopoiesis (Fig. 11).

In the lymph nodes, marked fibrosis and a large number of macrophages were noted (Fig. 4, 5).

The lungs, partially in bilateral lower lobes, revealed bronchopneumonia. Macrophages with identical PAS positive materials were scattered in the septum and alveolar spaces.

No significant abnormalities, except for the above mentioned macrophages, were found in the other organs.

PATHOLOGICAL DIAGNOSIS

1. glycogenosis type IV
   a. the deposition of the polysaccharide in the hepatic parenchymal cells, cardiac muscle, skeletal muscles, smooth muscles and reticuloendotherial cells.
   b. liver cirrhosis
2. acute myocarditis.

3. bronchopneumonia.
4. varix of the esophagus.
5. bilateral hydrocele testises.
6. pulmonary congestion and edema.
7. extramedullary hematopoiesis of the spleen.

DISSCUSSION

The two present cases were characterized by hepatosplenomegaly, poor prognosis, liver cirrhosis resembling postnecrotic type and deposits of polysaccharide in the hepatic parenchymal cells. In addition, in case 2, two kinds of the polysaccharide were contained in the cardiac muscle, skeletal muscle, smooth muscle and macrophages. The one was normal glycogen as indicated from various histochemical stainings. It was strongly stained with PAS, Best's carmine and colloidal iron which is characteristic of staining for normal glycogen. The other was stained slightly basophilic with hematoxylin eosin and was diastase resistant PAS positive. It was stained purple brown with iodine and slightly positive with alcian blue.

Table. 1.

Histochemical Features of the Polysaccharide

	Present Case	Motoi	Normal Glycogen
PAS	+	+	+
Diastase Digestion	Resistance	Resistance	Not Resistance
Best's Carmine	+	+	+
Mayer's Mucicarmine	−	−	−
Iodine	Purple Brown	Purple Brown	Brown
Colloidal Iron	Pale Blue	0	Blue
Alcian Blue	±	−～±	−
Sulfate Alcian Blue	+	0	−
Toluidine Blue	Blue	0	Blue

+ Positive − Negative 0 Not Detect

From these findings, the latter was identified as abnormal glycogen (Table 1). Some investigators have described such a material as similar to amylopectin by analysis[6),8),10),16)].

From the above mentioned findings, the present cases were coincident

with glycogenosis type IV which had been first reported by Andersen in 1952[1]. Furthermore, the chemical studies, enzymatic studies and fine structures in case 2 will be reported elsewhere.

Two[1),11)] of the fourteen cases which have been reported in the world literature were only described in the family history and one[4)] only in clinical studies. Although three of fourteen cases were reported by Craig and Uzman[4)] as familial metabolic disorders with storage of unusual polysaccharide complex, it seems likely, as Levin et al.[9),10)], Motoi et al.[17)] and Schochet et al.[14)] have suggested, that they were also glycogenosis type IV. Although a case which was reported by Ferrans et al.[18)] was described by Schochet et al.[14)] as the same disease, we suggest that the case was not identifiable as glycogenosis type IV. There are three reasons; that the material in hepatic parenchymal cells and cardiac muscle was not stained with Best's carmine, that the material was not deposited in the spleen and that the patient died at 17 years of age.

The family history was significant in ten[1),4)-6),11),13)-17)] out of fourteen cases (six[1),4)-6),11),13),17)] out of twelve families[1),4)-6),11),13)-17)]). Three cases[13),17)] had related parents and other seven[1),4)-6),11)] cases had one[1),4)] or two[5),6),11)] siblings with an identical disease (Mercier et al.[11)] described that a 6.5 month old male reported by Holleman et al.[6)] and a 11 month old male reported by Fernandes et al.[5)] were brothers and had another brother with the identical disease.). In our studies, three of four siblings which had unrelated parents had the identical disease. Levin et al.[10)] reported that an autosomal inheritance was more likely but that an x-linked one could not be excluded. Löhr[19)] described that an x-chromosomal recessive inheritance would be more likely. On the other hand, Hers[20)] had a dominant mode of transmission in type IV. From above mentions, we suggest that the glycogenosis type IV was inherited.

The sexual dominent was male in eleven out of fourteen cases (contain our cases).

The prognosis in every case was extremely poor, the age at death ranging from 4 months to 48 months.

In every case, the hepatic parenchymal cells, the cardiac muscle and the reticuloendothelial cells were affected. In seven cases[1),4),5),11),12)], skeletal muscle was not described, in three cases[1),13),17)], skeletal muscle was reported as normal and in four cases[6),14)-17)] deposits of abnormal glycogen were detected. In the case 2 of this study, skeletal muscle contained large deposits of normal and abnormal glycogen. In the smooth muscle of the gastro-intestinal tract, a few deposits of the same material were revealed. Parenchymal cells in the kidney, pancreas and adrenal

gland were not affected.

In every case which examined light microscopically, the livers showed cirrhosis. A cause of liver cirrhosis in glycogenosis type IV described by Andersen[1),2)] was that the hepatic parenchymal cells were destroyed by deposits of abnormal glycogen and fibrosis followed. On the contrary, Sidbury et al.[16)] and Motoi et al.[17)] suggested that cirrhosis was caused by nutritional disturbance as well as alcoholic cirrhosis. They described the reason that fibrosis is not always noted in every tissue with abnormal glycogen. In the present case, fibrosis was revealed in the liver, lymph nodes and spleen which contained a large deposits of polysaccharide. Fibrosis was especially prominent in the liver and the lymph nodes. If cirrhosis is caused by nutritional disturbances, the same type of cirrhosis must be observed in the other glycogenosis types. But, in the other type a few cases[21)-23)] had cirrhosis in spite of a large amount of the normal glycogen. Therefore, we support Andersen's theory[2)] that abnormal glycogen which was not digestive and remained for long time reacting as a foreign body might give rise to a different cirrhotic type.

SUMMARY

Glycogenosis type IV, which is characterized by deposits of polysaccharide in the liver and liver cirrhosis resembling postnecrotic type, were described in two children. They were siblings, had a sister with an identical disease and had unrelated parents. Furthermore, in one case in which a partial autopsy was performed, two kinds of the polysaccharide were noted in the cardiac muscle, skeletal muscle, smooth muscle and reticuloendothelial cells. The one was normal glycogen and the other was not identical with normal glycogen because of its diastase resistance. The genesis of the cirrhosis may have been a reaction to the stimulation by the polysaccharide as a foreign body.

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EXPLANATION OF FIGURES

Fig. 1. The proliferation of connective tissue, formation of pseudolobules and the enlarged hepatic parenchymal cells are revealed (Case 1). H. E.

Fig. 2. Formation of pseudolobules, the enlarged hepatic parenchymal cells and macrophages with slightly basophilic material are noted (Case 2). H. E.

Fig. 3. Numerous macrophages with basophilic material are detected in the spleen (Case 2). H. E.

Fig. 4. Marked fibrosis is noted in the mesenteric lymph node (Case 2). H. E.

Fig. 5. A large number of macrophages with the same material are shown in the paratracheal lymph node (Case 2). H. E.

Fig. 6. Deposits of the same PAS positive material are noticed in the sarcolemma of cardiac muscle (Case 2). H.E.

Fig. 7. PAS positive material is detected in the hepatic parenchymal cells, Kupffer cells and macrophages (Case 2, biopsy specimen). PAS

Fig. 8. The hepatic parenchymal cells, Kupffer cells and macrophages among connective tissue contain PAS positive material (Case 2). PAS

Fig. 9. The central PAS positive granules of the hepatic parenchymal cells give negative reaction with diastase digestion, but the peripheral granules give positive reaction (Case 2). PAS with diastase digestion

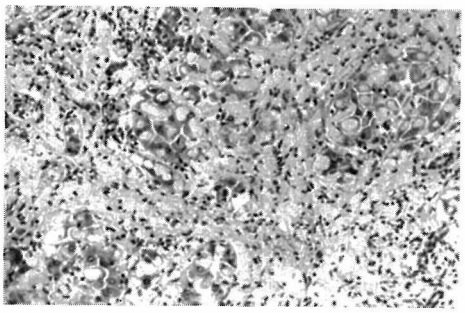
Fig.10. PAS positive material is reacted strongly with Best's carmine (Case 2). Best's carmine

Fig.11. The material is stained purple brown with iodine (Case 2). Iodine

Fig.12. The material is reacted with colloidal iron (Case 2). Colloidal iron

Fig.13. The material is stained blue with sulfate alcian blue (Case 2). S.A.B.

Fig.14. Numerous macrophages with PAS positive material are noted in the spleen (Case 2). PAS



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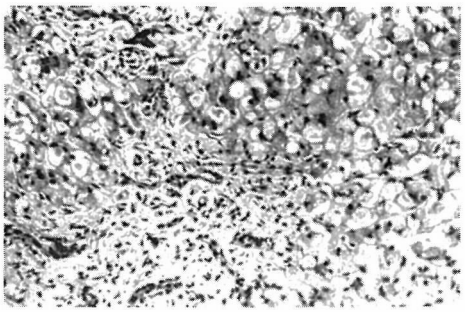

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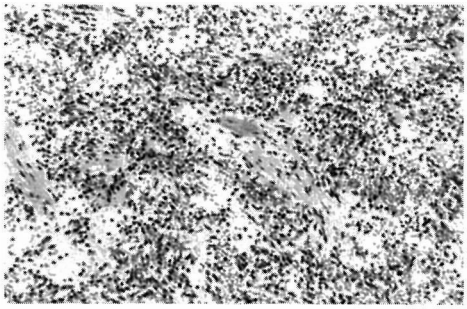

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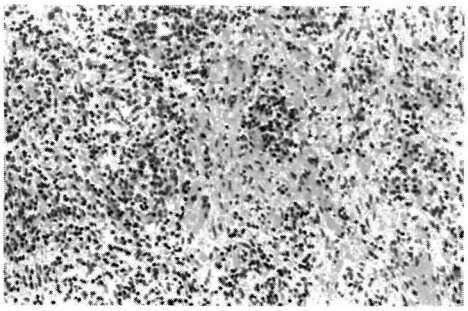

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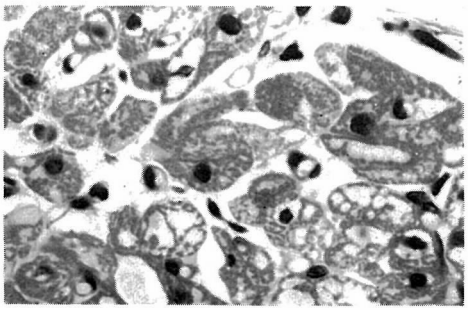

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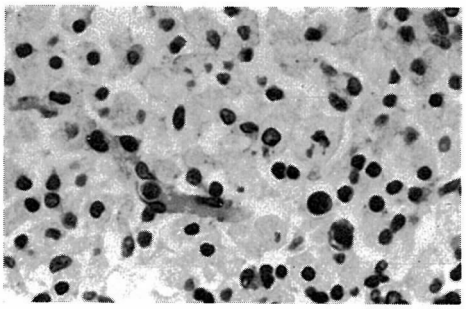

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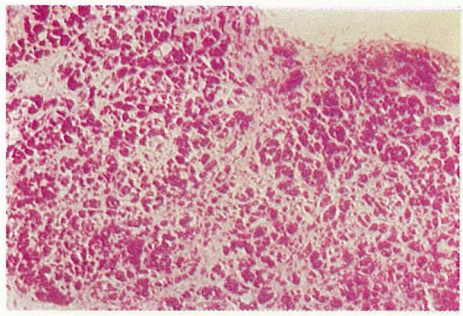

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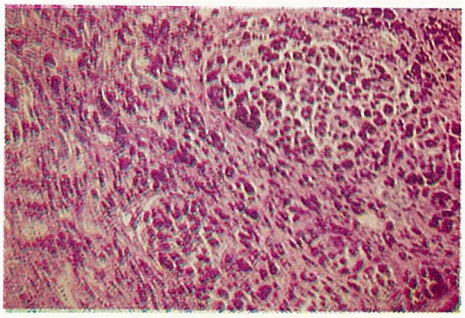

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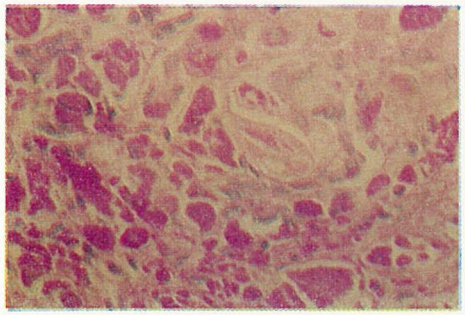

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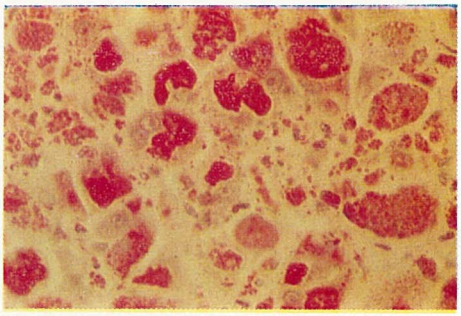

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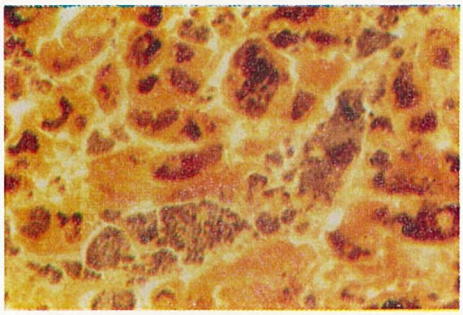

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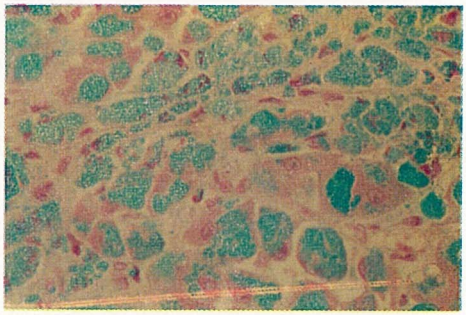

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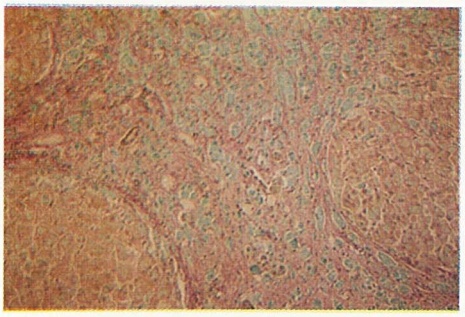

Fig. 13

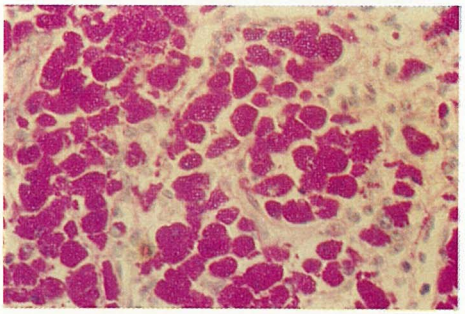

Fig. 14.