Congenital Steatosis of the Liver: Biochemical Approach to Its Pathogenesis

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Congenital steatosis of the liver¹⁾—“Fettspeicherleberkrankheit²⁾”—is said to be a congenital metabolic disorder characterized by hepatomegaly, retardation of physical development, muscular hypotonia, etc., and further by fasting hypoglycemia and a poor response of blood sugar to the adrenaline test. From viewpoint of clinical features congenital steatosis of the liver is unable to be differentiated from glycogen storage disease of the liver.^{1,2)} The differential diagnosis between them is achieved only by liver biopsy—conspicuous fatty liver in congenital steatosis, which is not a common finding of the liver in glycogen storage disease.^{1,2)} It is, however, not seldom to find the descriptions of accumulation of both glycogen and fat in hepatic cells among the reported cases of glycogen storage disease with liver biopsy.³⁾

In 1934, Debré *et al.*⁴⁾ advocated a new conception: “hépatomégalie polycorique”, in which congenital steatosis of the liver and glycogen storage disease of the liver shall be included, emphasizing strongly the similarity of clinical features between both disorders. In the present paper a case of congenital steatosis of the liver—the first case reported in Japan—is investigated clinically and biochemically in order to try to elucidate the pathogenesis of this peculiar disorder. So far as we know, the work reported here is the very first designed to try to elucidate the metabolic derangement in the liver of this particular disease.

CASE REPORT

N. C., a girl, was born as the first child on January 9, 1961 and of an uneventful, full-term pregnancy and spontaneous delivery. The birth weight was 3,008 g. The parents were healthy but consanguineous. There seemed to be no history of metabolic disease in the relatives of the patient, although no

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detailed information was obtained.

Soon after birth, she suffered from frequent vomiting and occasional episodes of fever (37–38°C). At the age of 4 months, the patient was admitted to our University Hospital because of retardation of physical development.

Physical examinations showed a poorly nourished infant whose face was pale and expressionless. The palpebral conjunctiva was not icteric but anemic. No jaundice was noticed on the skin. The chest was clear to percussion and auscultation. The abdomen was distended with presence of slight varicosis. No ascites was seen. The non-tender liver with smooth surface was palpable 7 cm below the costal margin on the right mid-clavicular line. The spleen was not palpable. The extremities were slender and hypotonic. There were no patellar and ankle reflexes. No pathological reflexes were present. Dentition was observed at the age of 5 months. The head-fixing was delayed and became possible at the age of 8 months.

The results of laboratory examinations are shown in Table I. Abnormal findings are summarized as follows; fasting hypoglycemia, a poor response of blood sugar to adrenaline (cf. Fig. 1), liver damage of moderate degree, hyperlipemia and hypercholesterolemia. Although hypoproteinemia and hypochromic anemia were observed on admission, they were improved soon by forced feeding. Hypoproteinemia and hypochromic anemia on admission were probably due to the insufficient feeding owing to frequent vomiting.

Under the diagnosis of glycogen storage disease basing on the above described findings (cf. Table I), the first liver biopsy was performed at the age of 5 months. The findings of liver biopsy (cf. Fig. 2) showed a remarkable accumulation of fat in the liver cells, namely, a picture of so-called fatty liver — a finding against our expectation that the accumulation of glycogen would be remarkable. The second biopsy was carried out at the age of 8 months when the nutritional condition of the patient was improved by forced feeding with well-balanced diet (the body weight had increased from 5.41 kg to 7.56 kg during these three months). The findings (cf. Fig. 2) showed a fatty liver picture similar to that in the first biopsy. In addition, a proliferation of connective tissue in the portal area was recognized. In the face of the fact that the infant had shown a remarkable improvement of nutritional condition by the well-balanced diet during the hospitalization, the fatty infiltration of the liver was probably of a chronic, progressive course as would be presumed from the biopsied specimens. It is not to be considered at all that our own case had had a lipotropic factor deficiency which would have played an important part for the development of that fatty liver.

Basing upon the clinical features, the laboratory findings (cf. Table I) and the biopsy findings of the liver (cf. Fig. 2), the diagnosis of congenital steatosis of the liver was established.

At the age of nine months, a liver specimen was taken from the patient by

TABLE I. Laboratory Findings of the Infant with Congenital Steatosis of the Liver

	Age	4 months	7 months	9 months
Serum protein	Total protein (mg/dl)	4.6	6.2	6.6
	{ Al.	2.83		3.42
	{ α -Gl.	0.40		0.94
	{ β -Gl.	0.48		1.06
	{ γ -Gl.	0.49		1.18
Serum electrolytes	K (mEq/L)	5.90		6.00
	Na "	139.4		134.0
	Cl "	97.0		98.5
	Ca "	3.84		4.60
	P (mg/dl)	6.36		5.50
Serum	Total lipids (mg/dl)			860
	Cholesterol (mg/dl)			
	{ Total		380	375
	{ Free		226	208
	{ Esterified		154	167
Blood	Sugar (mg/dl)	71.0	68.0	40.0
	Glucose tolerance test	Normal		
Enzyme in erythrocyte	Aldolase (units/ml)			816 (within normal range)
	G6PD (units/ml)			2400 (within normal range)
Tuberculin R.		Negative		
Wassermann's R.		Negative		
I ¹³¹ -uptake			30 m. 28.1% 24h. 18.1%	
E.C.G.		No abnormality		No abnormality
X-ray examination				
	{ Chest	No abnormality		
	{ Stomach	No abnormality		
	{ Bone	No abnormality		
Urine	Protein	—	—	—
	Sugar	—	—	—
	Urobilinogen	±	+	+
	Bilirubin	—	+	+
	Aceton	—	+	—
	Sediment	Normal	Normal	Normal
	17 KS (mg/day)		3.08	2.16
	17 OHCS (mg/day)		0.06	0.10
	Amino-N (mg/day)			22.6
	Diastase			2 ⁵

Feces	Occult blood Trypsin	—	—	— 400 × (+) (within normal range)
Hematological examination	R.B.C.	324 × 10 ⁴	269 × 10 ⁴	400 × 10 ⁴
	Hb. (g/dl)	3.2	7.2	9.7
	Ht. (%)		26	36
	W.B.C.	17800	11750	24600
	B (%)	0.5	0	0
	E	0	1.0	0.5
	L	30.5	27.0	32.5
	M	3.0	3.0	2.0
	N	66.0	66.0	65.0
	Nuclear shift to the left	—	—	±
Liver function	GPT (units)	45	72	82
	GOT (units)	63	144	131
	TTT (units)	1.2	9.1	1.7
	ZST (units)		1.32	5.6
	CCF		##	+
	Alkaline phos- phatase (B.L. units)	2.45		6.65
	Bilirubin (mg/dl)			
	Total		4.6	5.4
	Direct		2.7	3.1

Fig. 1. Adrenaline test.

exploratory laparotomy. At the laparotomy, the liver was found to be of smooth surface and soft in consistency with absence of tumor. The examination of the abdominal muscle specimens obtained at the laparotomy showed no evidence of accumulation of fat or glycogen.

(a) The first biopsy: A remarkable accumulation of fat in the liver cells was noticed by hematoxylin-eosin stain. (Glycogen was demonstrated by PAS stain in a relatively small amount on the whole.)

(b) The second biopsy: A remarkable accumulation of fat in the liver cells and a proliferation of connective tissue were found by hematoxylin-eosin stain. (Glycogen was demonstrated by PAS stain in a relatively small amount on the whole.)

Fig. 2. Biopsy findings of the liver in our own case.

Using the liver specimen obtained at this laparotomy, the following analyses were performed in order to try to elucidate the pathogenesis of this particular disorder.

METHODS AND MATERIALS

A liver specimen, weighing about 3 g, was removed surgically from our own patient with congenital steatosis of the liver. As controls, surgical patients without liver involvement were subjected to liver biopsy (Controls I and III, adults; Control II, infant). Each of the removed liver specimens was divided into two portions. One of the portions, after weighing, was homogenized with a chilled isotonic KCl solution and the other portion was subjected to make liver slices. These procedures were quickly carried out at 0°C.

The homogenates were analysed according to the following procedures: — Micro-Kjeldahl's method for total nitrogen; Kahn's method⁵⁾ for glycogen; Smith's method⁶⁾ for total fat (fatty acid + neutral fat); Boor's method⁷⁾ for

phospholipid, expressed as P; Zak's method⁸⁾ for cholesterol; Goto's method⁹⁾ for ATP, expressed as acid labile P.

Glucose-6-phosphatase (G6Pase) was determined by Swanson's method¹⁰⁾: The reaction mixture (2.0 ml) consisting of 4×10^{-3} M glucose-6-phosphate, 1.0 ml of citrate buffer (pH 6.5, 0.1 M) and 0.5 ml of enzyme solution to be tested, was incubated at 37°C for 30 minutes. The activity was expressed as mg of phosphorus liberated from glucose-6-phosphate under the condition described above.

Phosphoglucomutase (PG) was determined by Sutherland's method¹¹⁾: The reaction mixture (3.0 ml) consisting of 6×10^{-3} M glucose-1-phosphate, 1.5×10^{-3} M MgSO_4 , 1.2×10^{-2} M cysteine, 2.5×10^{-5} M glucose-1, 6-diphosphate, 1.3 ml of tris buffer (pH 7.6, 0.1 M) and 0.5 ml of enzyme solution to be tested, was incubated at 37°C for 30 minutes. The activity was expressed as μ M of glucose-1-phosphate decreased under the condition described above.

Phosphorylase (PR) was determined by Cori and Sutherland's method¹²⁾: The reaction mixture (3.0 ml) consisting of 0.016 M glucose-1-phosphate, 0.1 M NaF, 0.0013 M AMP, 10 mg of glycogen (as a primer), 1.0 ml of cysteine-glycerophosphate buffer (pH 6.4, 0.02 M) and 0.5 ml of enzyme solution to be tested, was incubated at 37°C for 30 minutes. The activity was expressed as mg of phosphorus liberated from glucose-1-phosphate under the condition described above.

Aldolase (AD) was determined by Sibley's method.¹³⁾ The activity was expressed as cmm of fructose-1, 6-diphosphate split per hour at 38°C.

Lactic dehydrogenase (LD) was determined by Hill's method^{14, 15)}: The activity was expressed as one unit for a decrease of optical density of 0.001 at 340 $\text{m}\mu$ per minute at 37°C.

Glucose-6-phosphate dehydrogenase (G6PD) was determined by Zinkham's method.^{15, 16)} The activity was expressed as one unit for an increase of optical density of 0.001 at 340 $\text{m}\mu$ per minute at 37°C.

Liver slices, weighing about 100 mg, were prepared from the ice-cold liver tissue. Each slice, after weighing exactly, was put into incubation flasks of the Warburg manometer under the condition described in Table II.

In Experiment I, the content of lactic acid produced for one hour at 37°C under anaerobic condition was manometrically estimated using glucose as substrate, in order to investigate the activity of glycolysis in the liver.

In Experiment II, the production of lactic acid was determined in the same way as Experiment I using glycogen as substrate, in order to investigate the activity of breakdown of glycogen to lactic acid.

In Experiment III, the *in vitro* conversion of C^{14} -labelled glucose to glycogen was measured in order to investigate the activity of glycogenesis. At the end of one-hour incubation at 37°C, glycogen fraction was isolated from the reaction mixture by Abder-Akher's method.¹⁷⁾ Then the radioactivity both in the

TABLE II. Experimental Condition

	Experiment I	Experiment II	Experiment III	Experiment IV
In cup	K.R.B.* 2.0 ml Slice	K.R.B. 2.0 ml Slice	K.R.B. 2.0 ml Slice	K.B.R. 2.0 ml Slice
Side	Glucose 0.2 ml (10 mg)	Glycogen 0.2 ml (10 mg)	Glucose 0.2 ml (10 mg) C ¹⁴ -Glucose 0.1 ml (2 μ c)	Glucose 0.2 ml (10 mg) C ¹⁴ -Glucose 0.1 ml (2 μ c)
Atmosphere	95%N ₂ , 5%CO ₂	95%N ₂ , 5%CO ₂	95%N ₂ , 5%CO ₂	Air
Time (h)	1	1	1	3
Determination	Lactic acid	Lactic acid	Radioactivity in glycogen fraction	Radioactivity in fat fraction
Pathway to be investigated	Glucose→ Lactic acid	Glycogen→ Lactic acid	Glucose→ Glycogen	Glucose→ fat

Note: Temperature 37°C * Krebs-Ringer-bicarbonate solution.

glycogen fraction and the remaining whole fraction was counted by gas flow counter with the scaler of Radiation Counter Aloka DC-5. The percentage of counts in the glycogen fraction for the whole counts was calculated.

In Experiment IV, the in vitro conversion of C¹⁴-labelled glucose to fat was determined in order to investigate the activity of lipogenesis from glucose. The incubation was carried out for three hours at 37°C under the aerobic condition. At the end of the incubation, the fat fraction was isolated from the reaction mixture by hot alcohol extraction¹⁸⁾ as follows: The reaction mixture was triturated and dehydrated with anhydrous sodium sulfate and then extracted with hot ethanol. The extracted fraction was completely evaporated to remove ethanol and the fat fraction was isolated from the residue by reextraction with ether. The radioactivity in both the fat fraction and the remaining whole fraction was counted in the same way as in Experiment III. The percentage of counts in the fat fraction for the whole counts was calculated.

RESULTS

The contents of total nitrogen, glycogen, total fat, phospholipid, total cholesterol (free and esterified) and ATP in the liver are shown in Table III and Fig. 3. In the liver from the patient with congenital steatosis, the content of total nitrogen was remarkably decreased to about half of those in the control livers. Such a decrease of nitrogen in the affected liver is to be expected since the pathological findings of the biopsied liver revealed a picture of so-called fatty liver (Fig. 2). The content of liver glycogen of the control subjects showed a considerable fluctuation under the influence of feeding conditions and its normal

TABLE III. Chemical Analyses of the Liver.

		Nitrogen	Glycogen	Total fat	Phospho- lipid	Cholesterol			ATP
						Total	Free	Esteri- fied	
		mg	mg	mg	γ	mg	%	%	γ
Patient		8.9	94.3* (10.6)**	109.0 (12.25)	425 (47.8)	10.88 (1.22)	47.0	53.0	168 (18.9)
Controls	I	20.9	113.5 (5.48)	27.5 (1.33)	225 (10.9)	6.38 (0.308)	37.5	62.5	379 (18.3)
	II	15.7	17.5 (1.12)	18.0 (1.15)	295 (25.2)	3.30 (0.200)	49.5	51.3	232 (14.8)
	III	21.3	52.4 (2.45)	19.0 (0.90)	460 (21.6)	5.03 (0.238)	52.3	47.7	274 (12.9)

* Figures are given as values per 1 g of the liver.
** Figures in parentheses indicate values per 1 mg of nitrogen in the liver.

Fig. 3. Chemical analyses of the liver (1).

range has been reported to lie between 0.82 to 7.09%¹⁹⁾ or 2.42 to 6.1%²⁰⁾. Accordingly, the glycogen content in the affected liver of our own patient — 9.43% — is not high in value per unit weight of the liver. The content of total fat in the affected liver was highly elevated about five times as compared with that in the control livers. Cholesterol also showed about two times higher content in the affected liver as compared with that in the controls. There was, however, no significant difference in the ratio of free to esterified cholesterol between the affected

liver and the controls. The content of phospholipid in the affected liver did not much differ from that in the control livers. The content of ATP was lower in the affected liver.

Now the affected liver contained less nitrogen component than the control, the contents of glycogen or other ingredients in the livers were not to be compared with each other between the affected and control livers, but these values were to be expressed per mg of nitrogen in the liver, namely on protein basis (cf. Fig. 4). In the affected liver the glycogen content was definitely higher; the cholesterol content, approximately 10 times higher; the phospholipid content, about two times higher; and ATP content, of almost the same level.

Fig. 4. Chemical analyses of the liver (2).

Molecular structure of the glycogen isolated from the liver was examined by iodine reaction.^{21,22)} No significant difference in coloring by the reaction was found between glycogen from the affected liver and that from the controls. Therefore glycogen in the affected liver seems to have normal structure. Activities of liver enzymes are shown in Table IV and Fig. 5. The affected liver showed lower activities of G6Pase, PG, PR, AD and LD, in values per 1 g of the liver, as compared with the control livers. Especially the activity of G6Pase in the affected liver was remarkably decreased below one fifth of that in the control liver. On the contrary, the activity of G6PD was higher in the affected liver than in the controls. When these activities were calculated in terms per 1.0 mg of nitrogen in the liver, namely in specific activity (cf. Fig. 6), there were no remarkable differences in activities of PG, PR and LD between the affected liver and the controls.

TABLE IV. Enzyme Activities in the Liver

	G6Pase	PG	PR	AD	LD	G6PD
Patient	1.04* (0.117)**	218 (24.5)	1.50 (0.169)	4100 (460)	41×10^3 (4.6×10^3)	23×10^3 (2.58×10^3)
Controls	I	5.86 (0.283)	480 (23.2)	2.70 (0.131)	6400 (309)	76×10^3 (3.7×10^3)
	II	4.29 (0.273)	470 (29.9)	2.63 (0.168)	6000 (382)	42×10^3 (2.7×10^3)
	III	5.14 (0.241)	460 (21.6)	3.35 (0.157)	8400 (395)	46×10^3 (3.0×10^3)

* Figures are given as values per 1 g of the liver.

** Figures in parentheses indicate values per 1 mg of nitrogen in the liver.

Note: G6Pase=glucose-6-phosphatase, PG=phosphoglucumutase, PR=phosphorylase, AD=aldolase, LD=lactic dehydrogenase, G6PD=glucose-6-phosphate dehydrogenase.

Fig. 5. Enzyme activities in the liver (1).

On the other hand, both LD and G6PD represented higher specific activities in the affected liver than in the control livers, especially the latter enzyme was of the activity amounting about 4 times of that in the controls. The specific activity of G6Pase in the affected liver, however, was definitely reduced below half of that in the controls.

Table V, Figs. 7 and 8 represent the results of the Experiments (I-IV) using liver slices. Fig. 7 illustrates comparative values of the experimental results in terms per 100 mg of the liver and Fig. 8 does those in terms per 1 mg of

Fig. 6. Enzyme activities in the liver (2).

nitrogen in the liver. In Experiment I, in which the activity of glycolysis from glucose to lactic acid was investigated, the production of lactic acid was greater in the affected liver than in the controls, especially when calculated on protein basis. In Experiment II, in which the activity of glycogen breakdown to lactic acid was investigated, the production of lactic acid was greater in the affected liver than in the controls, especially when calculated on protein basis. In Experiment III, in which the conversion of glucose to glycogen was investigated

TABLE V. Manometric and Isotope Studies.

	Experiment I	Experiment II	Experiment III	Experiment IV
	Glucose→ Lactic acid Q N_2 μ CO ₂ /h.	Glycogen→ Lactic acid Q N_2 μ CO ₂ /h.	C ¹⁴ -Glucose→ Glycogen Rate of conversion %	C ¹⁴ -Glucose→ Fat Rate of conversion %
Patient	99.0 * (111.0) **	60.5 (67.5)	0.135 (0.151)	44.5 (50.0)
Controls {	I	73.0 (35.2)	53.6 (25.9)	/
	II	26.2 (16.9)	18.1 (11.5)	0.307 (0.196)
	III	39.6 (18.6)	36.5 (17.2)	0.421 (0.198)

* Figures are given as values per 100 mg of the liver.

** Figures in parentheses indicate values per 1 mg of nitrogen in the liver.

Fig. 7. Manometric and isotope studies (1).

Fig. 8. Manometric and isotope studies (2).

by using labelled glucose, the conversion rate was lower in the affected liver than in the controls in comparison per 100 mg of the liver, however, there was no definite difference of the rate between both livers when calculated on protein basis (cf. Figs. 7 & 8). In Experiment IV, in which the conversion of glucose to fat was investigated by using labelled glucose, the conversion rate was far greater in the affected liver than in the controls, especially when calculated on protein basis.

DISCUSSION

In our own patient, a definite decrease of the G6Pase activity in the affected liver was demonstrated. This supports the two findings of fasting hypoglycemia

and of a poor response of blood sugar level after the injection of adrenaline observed in the patient. On the other hand, the specific activities of PG and PR were within normal limits and glycogen structure also was normal in the affected liver. These results are in agreement with the findings which were reported by Cori^{21,23}) in Type 1 of glycogen storage disease. Since congenital steatosis of the liver and glycogen storage disease of the liver (Type I) showed, as has been quoted above, quite similar clinical features, Debré⁴⁾ included both disorders in a single category under the new term "hépatomégalie polycorrique". Later, cases of glycogen storage disease of the liver with coexistence of fat in addition of abnormal accumulation of glycogen in hepatic cells were discussed by Koch,³⁾ who was of the opinion that block of G6Pase might bring about acceleration in fat synthesis. Koch's suggestion is very interesting to us, but experimental data concerning this point were lacking in Koch's report.

Among the experimental results in our investigation, it is worthy of notice that the affected liver revealed a marked elevation of G6PD activity. G6PD, which is responsible for the first step of pentose phosphate pathway, has glucose-6-phosphate as a substrate in common with G6Pase (cf. Fig. 9). It is, therefore,

Fig. 9. Schema of carbohydrate metabolism.

possible to presume that a compensative elevation of the G6PD activity is induced by a defect of G6Pase. For instance, in oligophrenia phenylpyruvica the conversion of phenylalanine to tyrosine is blocked by a defect of phenylalanine hydroxylase, resulting in an enhancement of the conversion of phenylalanine to phenylpyruvic acid.^{24,25)} Now an enhancement of the G6PD activity, namely, a stimulation of pentose phosphate pathway, results in an increase of TPNH which is essential factor for fat synthesis. Siperstein *et al.*^{26,27,28)} examined the incorporation of C¹⁴-acetate to fatty acid or cholesterol with liver homogenates of rat using glucose-6-phosphate as a substrate. They found that the rate of incorporation was remarkably enhanced when pentose phosphate pathway was stimulated by an addition of TPN into the medium. Furthermore, basing upon the results of similar experiments for diabetic rats, they concluded that the impaired synthesis of fatty acid in diabetes was ascribed primarily to the deficiency of glycolysis via pentose phosphate pathway in the disease. Our own results in this investigation showed that lipogenesis from glucose in the affected liver was noticeably enhanced as compared with that in the control livers (cf. Experiment IV). This enhancement of lipogenesis might be ascribed to an increased activity of G6PD mentioned above. On the other hand, the affected liver represented an almost normal level of specific activity of AD, a slightly higher level of specific activity of LD and an increased activity of glycolysis under anaerobic condition (cf. Experiment I). Therefore there may be no disturbance in the Embden-Meyerhof pathway of glucose to pyruvate in this particular disease. Although it is insufficient to say from our results which pathway, the Embden-Meyerhof or the pentose phosphate pathway, is much more utilized in the liver of the disease in question; it seems that glucose is *in vivo* probably utilized relatively much by pentose phosphate pathway because of remarkable increase of the G6PD activity as compared with that of AD or LD in the affected liver. According to recent studies, the conversion of carbohydrate to fat is *in vivo* considerably active. Stetten *et al.*,²⁹⁾ by means of isotope, demonstrated that 40% of ingested glucose was synthesized to fatty acid while only 5% of that to glycogen. On the contrary the conversion of fat to carbohydrate is thought to be quantitatively small. According to Strisower's experiment,³⁰⁾ only 5% of ingested C¹⁴-palmitic acid was converted to carbohydrate.

Basing on our own findings and the above-mentioned discussion, the following illustration for the mechanism of the development of fatty liver in this particular disease may be given: The conversion of carbohydrate to fat in the liver of this particular disorder might be accelerated probably by an enhanced activity of pentose phosphate pathway, resulting in excessive accumulation of fat in the liver depending upon an imbalance between synthesis and breakdown of fat. On the other hand, glucose-6-phosphate which is converted from glucose by hexokinase may not return to glucose because of a defect of glucose-6-

phosphatase, resulting in much production of pyruvate by way of Embden-Meyerhof or pentose phosphate pathway and glycogen also may take the same route in process of its breakdown. This view is supported by the results of Experiments I and II. The excessive pyruvate, thus produced, may be directed to lipogenesis beyond the capacity of TCA cycle by which pyruvate is burnt.

Although a considerable number of publications concerning glycogen storage disease have appeared in recent years, little is investigated into liver enzymes other than G6Pase. Weber³¹⁾ reported that the liver from the patient with von Gierke's disease had a decreased activities of G6Pase and of G6PD and an increased activities of PG, of Ad, of LD and of phosphohexoisomerase. Therefore, it is of our opinion that the difference between the typical von Gierke disease and the congenital steatosis of the liver might be due to the difference in activation of pentose phosphate pathway. Although both disorders have a defect of G6Pase of the liver in common with each other, lipogenesis is activated in case of congenital steatosis, resulting in accumulation of fat because of increase of the G6PD activity, while in case of von Gierke's disease lipogenesis is not activated, the result being an accumulation of glycogen because of decrease of the G6PD activity.

Now a question is arisen from our experimental results why the breakdown of glycogen to lactic acid in the affected liver under in vitro anerobic condition should increase rather than that in the controls in spite of a deficiency of G6Pase. This may be due to the driven conversion to lactic acid via Embden-Meyerhof pathway as a result of block of the route; glucose-6-phosphate $\rightarrow$ glucose. The fact that the affected liver revealed a higher specific activity of LD may favor the alleged increase of lactic acid production. Either way, our own results do not suggest any block in glycogen breakdown due to the deficiency of G6Pase. Whether the accumulation of liver glycogen is caused by a only defect of G6Pase is questionable, although it is easily understandable that glycogen accumulates in the liver by defect of either debranching enzyme²¹⁾ or phosphorylase³²⁾ as is suggested in some types of glycogen storage disease. The defect of G6Pase should firstly result in an accumulation of glucose-6-phosphate, however, there is no report of its accumulation in the liver of glycogen storage disease. In addition, glucose-6-phosphate could be converted by G6PD or phosphohexoisomerase to either pathway. In this respect, Weber³³⁾ also had some doubts and found that there was no correlation between the activity of G6Pase and the content of glycogen in the liver. It has been known in hepatoma that the activity of G6Pase in the liver is remarkably reduced ^{34,35)} and the liver glycogen also is scanty.³⁶⁾ By the way, the activity of G6PD in the liver is known to show a high level in hepatoma.³⁵⁾ Weber^{37,38)} demonstrated in his animal experiment that the injection of cortisone caused an enhancement of the G6Pase activity as well as an increase of the glycogen content in the liver. Therefore, it seems to be insufficient to ascribe an abnormal

accumulation of liver glycogen to a defect of G6Pase alone.

A possibility may exist that the elevation of the G6PD activity in the affected liver is due to an effect of some hormone or other. According to Pearson,³⁹⁾ the G6PD activity in erythrocyte elevates in case of hyperthyroidism. Since the G6PD activity in erythrocyte from our own patient was within normal limits (cf. Table I), it does not seem likely that the elevated level of G6PD in the affected liver from the patient is secondarily due to hormonal effect. If some hormone acts on activity of enzyme, its effect should exert itself not only on the liver but also on erythrocyte. By the way, the endocrinological examinations concerning thyroid or adrenal gland revealed no evidence of abnormality in our own case (cf. Table I).

SUMMARY

The first case reported in Japan of congenital steatosis of the liver was described. In order to try to elucidate the pathogenesis of this particular disorder, the analysis of the chemical composition, the determination of the enzyme activities and the investigation of the activity of glycolysis, glycogenesis and lipogenesis by means of isotope were carried out on the liver specimens obtained from the patient.

The results obtained were as follows: —

1. The affected liver contained remarkably larger amount of fat and cholesterol. The glycogen content was not higher in weight % of the liver but was higher on protein basis of the liver. There was no structural abnormality of glycogen isolated from the affected liver.

2. In the affected liver, the specific activities of aldolase, phosphoglucose mutase and phosphorylase were within normal limits, the specific activity of lactic dehydrogenase was higher, and that of glucose-6-phosphate dehydrogenase was remarkably higher. The activity of glucose-6-phosphatase, however, was definitely decreased in the affected liver.

3. Experimental results with manometric and isotopic procedures demonstrated that glycolysis seemed to be activated, glycogenesis to be normal and lipogenesis from glucose to be highly activated.

CONCLUSIONS

Congenital steatosis of the liver has in common a defect of glucose-6-phosphatase with von Gierke's disease (Type 1 of glycogen storage disease). For the development of fatty liver in congenital steatosis, an enhanced lipogenesis from carbohydrate, probably due to an increase in the activity of pentose phosphate pathway, will, we believe, play an important role.

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