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KEYWORDS: CC14 liver injury, galactosamine liver injury, partial, hepatectomy, phosphorylase activity, glycogen metabolism

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STUDIES OF LIVER PHOSPHORYLASE IN HEPATIC INJURIES
I. ALTERATION IN ENZYME ACTIVITY

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Abstract. Phosphorylase activities (total and *a* form) were determined in the livers of experimental hepatic injuries with carbon tetrachloride or galactosamine and the livers of patients with liver diseases. Experimental liver injuries caused a slight decrease in total activity in later stages and a marked increase in *a* form activity in earlier stages. In human livers, low values of total activity were found in acute hepatitis and cirrhosis of the liver with no consistent alteration in *a* activity. Phosphorylase activities in hepatocellular carcinomas were also low. The importance of the altered phosphorylase activities in hepatic injuries is discussed in relation to the disorder in glycogen metabolism in the injured liver.

Key words: CCl_4 liver injury, galactosamine liver injury, partial hepatectomy, phosphorylase activity, glycogen metabolism

The level of glycogen in the liver is an important factor in determining the susceptibility of parenchymal liver cells to hepatotoxins and other agents which cause hepatic injuries. Decreased glycogen content in injured livers is also well recognized. Accordingly, the elucidation of altered glycogen metabolism in the injured liver is essential for both the prevention and treatment of liver diseases. Although the glycogen phosphorylase (PLase; 1, 4-α-D-glucan : orthophosphate α-glucosyltransferase, EC 2. 4. 1. 1) has been well studied since the discovery of interconversion of active (*a*) and inactive (*b*) forms of the enzyme through phosphorylation and dephosphorylation reactions (1), reports on phosphorylase activities in injured livers are limited in number. Oeckerman (2) reported unaltered PLase activity in cholelithiasis without referring to the *a* and *b* activities. The discovery made by Appleman and others (3) that the *b* form is active in the presence of both sulfate ion and AMP made it possible to estimate an approximate ratio of the active and inactive forms.

The present study was undertaken to determine the PLase activities in injured livers of experimental animals and of hepatitis and cirrhosis patients. PLase activities in partially hepatectomized rats were also studied to see the

enzyme alteration in hepatic regeneration without injury and to compare the results with those of hepatotoxin induced hepatic injuries, which also entail hepatic regeneration following liver cell necrosis. Although no attempt was made in this study to estimate the *in vivo* levels of active and inactive forms because of some fundamental difficulties involved in the differential enzyme analysis (4), several lines of evidence indicating an altered *a* and *b* interconversion in hepatic injury were obtained in addition to the decrease in total PLase activity.

MATERIALS AND METHODS

Male Sprague-Dawley rats, maintained on Oriental Laboratory chow MF and water *ad libitum* and weighing 135-180 g, were used throughout the experiments. Acute carbon tetrachloride (CCl_4) intoxication was produced by a single administration of 0.5 ml of 20% CCl_4 dissolved in olive oil per 100 g body weight to 12 h fasted rats through a stomach tube intubated under light ether anesthesia. Acute galactosamine intoxication was induced by a single intraperitoneal injection of 150 mg of D-galactosamine hydrochloride (Gal-NH_2), as a neutralized solution, per 100 g body weight to similarly starved rats. The animals without those hepatotoxin treatments served as controls. Those three groups of rats were further fasted for 24 h and given 5 g of the laboratory chow per 100 g body weight for the second 24 h, 10 g per 100 g body weight for the third 24 h and *ad libitum* for the next four days. These dietary manipulations were made in order to minimize the differences in dietary intake among the three groups of differently treated rats. Partial hepatectomy was performed on well-fed rats according to the method of Higgins and Anderson (5). Sham-operated rats served as controls to the hepatectomized rats. These two groups of rats received the standard chow *ad libitum* throughout the experimental period until sacrificed.

The animals were killed at indicated time intervals by decapitation and exsanguination after an ether overdose. Livers were excised and homogenized in four volumes of 63 mM Tris-HCl, pH 7.4, containing 25 mM 2-mercaptoethanol and 6.3 mM EDTA, in a glass homogenizer with a Teflon pestle. Aliquots of the homogenates were used for determination of glucose 6-phosphatase (G6Pase; EC 3.1.3.9) activity by the method of Koide and Oda (6, 7), and the remainders centrifuged for 10 min at $5,000 \times g$. Aliquots of the supernatants were immediately analyzed for total and *a* form PLase activities according to the procedure of Sato and others (4). The remaining supernatants were centrifuged for 45 min at $105,000 \times g$ for estimation of glucose 6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) activity with the resulting supernatants as described by Taketa *et al.* (8). The protein concentrations of those tissue extracts were determined by the method of Lowry and others (9). Alanine aminotransferase (GPT; EC 2.6.1.2) activities in sera were determined colorimetrically (10). Glycogen contents in liver tissues were assayed by a modified anthrone method (8). The procedures of tissue extraction and centrifugation were carried out at 0-4°C. All the chemicals used and described above were obtained from Sigma Chem. Co., St. Louis.

Biopsy specimens of human liver, amounting to 10-20 mg, obtained at perito-

neoscopy or operation were processed similarly with a small scale homogenizer and centrifuge tubes for analysis of PLase activities with the same system* as in the assay of rat PLase (4). The enzyme activities were expressed as units per g homogenate or supernatant protein depending on the tissue extracts analyzed for respective enzymes; one unit being defined as one μmol of substrate reacted per min at 37°C. All the results are given as means and standard errors of the means for each group of animals.

RESULTS

Experimental liver injuries. Activities of liver PLase (total, *a* form and *a*/total) in hepatotoxin treatments and partial hepatectomy are listed in Tables 1 and 2 together with several parameters of liver injury (serum GPT, liver G6PD and G6Pase activities and liver glycogen content). Time course of PLase activities and glycogen content in livers of those experimental animals is illustrated in Figs. 1, 2 and 3.

In CCl_4-treated rats, total PLase activities decreased gradually, reaching a minimum in 3 days and returned toward the initial value in 7 days. The control activity also decreased for the first one day of starvation and recovered after resuming dietary intake. Thus, a statistically significant decrease in activity was found only in 3 days, when the activity of G6PD reached a peak and the activity of G6Pase and the level of the glycogen were still low in the injured livers. In contrast with the decrease in total PLase activity, the activity of *a* form and the ratio of *a* to the total activity both increased markedly in the first 2 days of CCl_4 injury. Thus, the change in PLase *a* activity roughly paralleled that of serum GPT elevation.

The results obtained with Gal-NH_2-injured rats were quite similar to those with CCl_4-intoxicated animals in respect to the alteration in PLase activity, although a rise in *a* form activity was noted in 7 days in association with a marked increase in liver glycogen content. A retarded peak in the rise of serum GPT activity and less marked decreases in liver G6Pase activity were also found as compared with CCl_4-treated rats.

In partially hepatectomized rats, the decreases in total PLase activity and glycogen content in liver were slightly less than in CCl_4-treated rats, but the differences from the sham-operated controls were larger. The increase in PLase *a* activity was noted only when the activity was expressed relative to the total, as similar increases in *a* form activity were also observed in the control livers. No significant changes suggestive of hepatic injury were observed in other parameters following partial hepatectomy except for a decrease in liver G6Pase activity in 72 h.

* The incubation system was similar to that adopted for human PLase assay by Oeckerman (2) except for the presence of sodium sulfate.

TABLE 1. ACTIVITIES OF GPT IN SERA AND OF G6PD, G6PASE AND PLASE IN LIVERS AND CONTENT OF GLYCOGEN IN LIVERS OF CCL_4 AND GALACTOSAMINE-TREATED, AND CONTROL RATS

Conditions		GPT (Karmen units/ml)	G6PD[a]	G6Pase[a]	Total PLase[ab]	Active form of PLase[ab]	Active PLase/ Total	Glycogen (mg/g wet liver)
CCl_4	24h(5)[c]	1740± 51	54.6± 7.0	20.4±4.2	107.7± 6.6	77.6±16.6	0.69±0.13	0.19±0.02
	48h(6)	820±178	91.1±10.7	31.4±3.5	76.2±11.4	70.0± 2.4	0.91±0.03	0.26±0.02
	72h(6)	440±213	149.3±23.5	29.2±4.0	60.6± 4.6	9.7± 3.2	0.17±0.06	1.1 ±0.2
	1 w(3)	24± 4	60.3± 1.8	41.8±0.5	145.9±18.5	17.1± 1.9	0.12±0.01	9.5 ±3.5
Galactosamine	24h(5)	82± 26	46.9± 5.9	62.2±3.0	99.6±18.4	124.5±24.8	1.23±0.04	1.7 ±0.4
	48h(4)	1250±543	76.7± 2.3	50.8±2.3	108.6± 2.1	127.7± 2.1	1.18±0.03	1.0 ±0.2
	72h(5)	488±233	105.4±19.4	42.1±5.4	72.7±11.1	13.8± 4.2	0.24±0.09	9.2 ±2.2
	1 w(6)	27± 2	83.5± 5.8	45.1±3.3	117.0± 0.2	76.5± 6.8	0.68±0.09	80.0±7.0
Control	24h(4)	20± 2	32.4± 1.6	79.6±5.6	90.3± 4.8	10.4± 1.2	0.12±0.02	5.9 ±0.19
	48h(4)	16± 1	32.0± 2.4	60.9±2.5	99.2± 5.0	28.8±14.1	0.29±0.14	3.5 ±1.9
	72h(5)	22± 2	47.6± 4.5	53.6±3.0	98.0± 3.7	14.4± 6.7	0.14±0.06	6.5 ±3.0
	1 w(2)	30± 7	74.4± 6.0	55.8±3.6	138.8± 8.4	16.6± 2.9	0.12±0.01	23.0 ±1.0
Well-fed control	(4)	26± 1	42.2± 4.3	56.2±2.7	141.1± 5.0	6.3± 0.5	0.05±0.002	46.0 ±4.0

a Units/g protein; *b* Total PLase activity was assayed in the presence of 0.5 M Na_2SO_4 together with 1 mM AMP. Activity of active form PLase was assayed in the absence of Na_2SO_4 and AMP. *c* Number in parenthesis, number of animals.

TABLE 2. ACTIVITIES OF GPT IN SERA AND OF G6PD, G6PASE AND PLASE IN LIVERS AND CONTENT OF GLYCOGEN IN LIVERS OF HEPATECTOMIZED AND SHAM-OPERATED RATS

Condition		GPT (Karmen units/ml)	G6PD[a]	G6Pase[a]	Total PLase[ab]	Active form of PLase[ab]	Active PLase/ Total	Glycogen (mg/g wet liver)
Hepatectomized	24h(5)[c]	126±26	39.2± 4.4	52.2± 6.9	116.5±10.2	111.3±11.9	1.02±0.17	12.6± 2.2
	48h(5)	48± 6	53.8± 3.0	52.3± 8.6	106.9± 7.0	71.1±11.6	0.66±0.12	12.6± 3.4
	72h(4)	50± 9	69.3± 4.2	28.8± 2.6	94.7± 2.9	63.0± 5.8	0.67±0.07	12.7± 2.7
	1 w(3)	28± 3	71.1± 3.5	40.6± 4.5	96.6± 1.7	24.0± 6.3	0.25±0.07	15.4± 4.7
Sham-operated	24h(3)	27± 1	37.9± 1.2	59.3± 2.3	120.9± 2.2	101.0±12.8	0.34±0.10	43.0±10.0
	48h(4)	36± 9	38.7± 2.6	54.6± 1.9	181.3± 5.3	43.4±11.0	0.24±0.05	34.8± 6.9
	72h(2)	27± 3	60.8±13.2	69.1±10.5	134.5±14.9	75.9± 0.3	0.58±0.06	29.5± 1.8
	1 w(2)	30± 1	46.0± 5.5	59.6±14.5	114.8± 6.7	29.8± 1.2	0.26±0.01	14.2± 5.5
Well-fed control	(4)	26± 1	42.2± 4.3	56.2± 2.7	141.1± 5.0	6.3± 0.5	0.05±0.002	46.0± 4.0

a Units/g protein; *b* Total PLase activity was assayed in the presence of 0.5 M Na_2SO_4 together with 1 mM AMP. Activity of active form PLase was assayed in the absence of Na_2SO_4 and AMP. *c* number in parenthesis, number of animals.

TABLE 3. PLASE ACTIVITY IN HUMAN LIVER DISEASES

Case	Diagnosis	Liver			Serum				
		PLase activity[a]		Active form/Total	Total bilirubin (mg/dl)	GOT[b]	GPT[b]	Choline esterase (ΔpH)	KICG
		Total	Active form						
1	Fatty liver	288.6	146.5	0.51	0.68	82	161	1.23	0.15
2	Fatty liver	130.7	91.3	0.70	1.30	44	42	0.98	0.033
3	Acute hepatitis	90.3	47.5	0.53	1.11	28	43	0.77	0.15
4	Acute hepatitis	64.4	57.9	0.90	0.71	41	141	0.92	—
5	Acute hepatitis	45.8	38.8	0.85	1.19	31	40	0.82	—
6	Chronic persistent hepatitis	80.7	25.0	0.31	0.94	22	66	0.92	0.16
7	Chronic persistent hepatitis	106.5	34.4	0.32	0.70	37	49	0.98	0.19
8	Chronic persistent hepatitis	243.7	157.5	0.65	0.42	31	38	0.98	0.18
9	Chronic persistent hepatitis	77.8	0	0	0.49	119	168	0.98	0.14
10	Chronic aggressive hepatitis	173.4	123.7	0.71	1.16	157	202	0.78	0.16
11	Chronic aggressive hepatitis	221.0	114.7	0.52	0.70	41	26	0.75	0.12
12	Chronic aggressive hepatitis	144.8	52.8	0.36	0.37	229	299	0.73	0.19
13	Chronic aggressive hepatitis	85.8	80.5	0.94	1.41	94	76	0.68	0.13
14	Liver cirrhosis	83.2	70.1	0.84	0.92	88	129	0.59	0.11
15	Liver cirrhosis	57.4	28.0	0.49	0.47	46	35	—	0.13
16	Liver cirrhosis	62.5	32.8	0.52	1.33	86	52	0.62	—
17	Liver cirrhosis	107.7	7.8	0.07	1.02	259	239	0.84	0.099
18	Liver cirrhosis	92.4	36.6	0.40	1.52	70	66	0.63	0.11
19	Liver cirrhosis[c]	81.1	4.7	0.06	0.71	90	91	0.59	0.11
20	Alcoholic liver injury	183.4	6.3	0.03	1.42	163	150	0.63	0.076
21	Alcoholic liver injury	188.5	65.2	0.35	0.29	44	56	0.95	0.12
22	Alcoholic liver cirrhosis	104.1	20.7	0.20	0.72	72	87	0.75	—
23	Alcoholic liver cirrhosis	114.6	0	0	1.05	272	250	0.46	0.079
24	Primary biliary cirrhosis[c]	214.4	0	0	3.97	187	120	0.70	—
25	Hepatoma[c], Tumor	82.2	71.8	0.87	1.97	136	76	—	—
	Tumor-bearing liver	65.6	0.8	0.01					
26	Hepatoma[c], Tumor	36.0	6.0	0.17	0.93	38	28	0.54	—
	Tumor-bearing liver	140.0	5.0	0.04					

a Units/g protein; *b* Karmen units/ml; *c* Livers obtained at operations, others at peitoneoscopy.

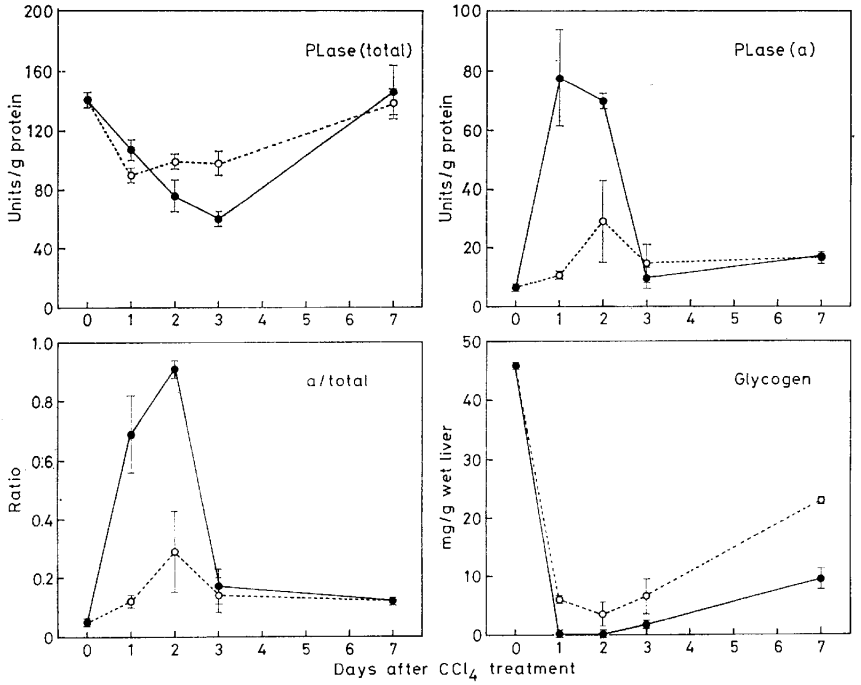


Fig. 1. Activities of PLase (total and *a*) and glycogen content in livers of CCl_4-treated and control rats. •——•, CCl_4-treated; ○- - -○, control.

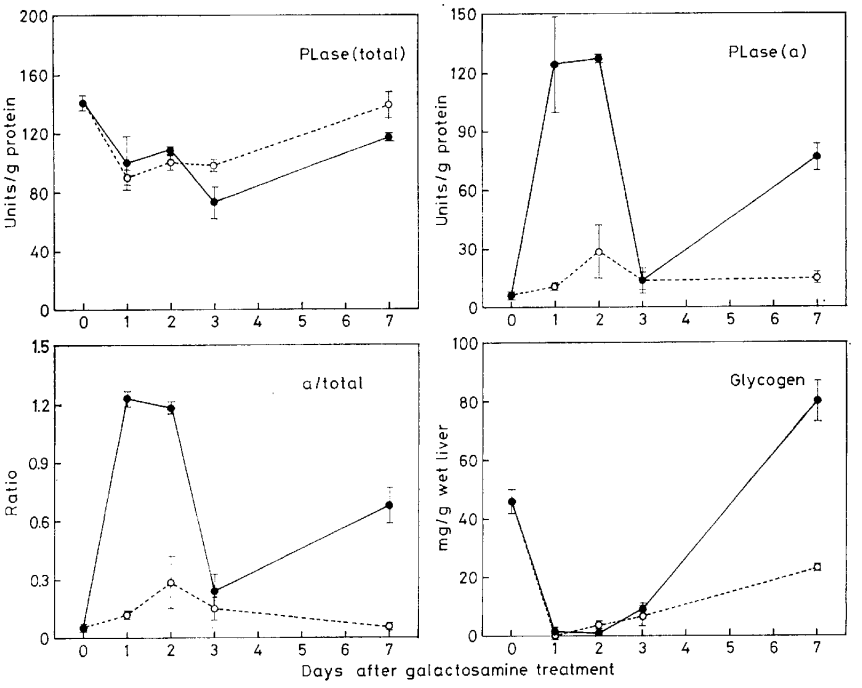
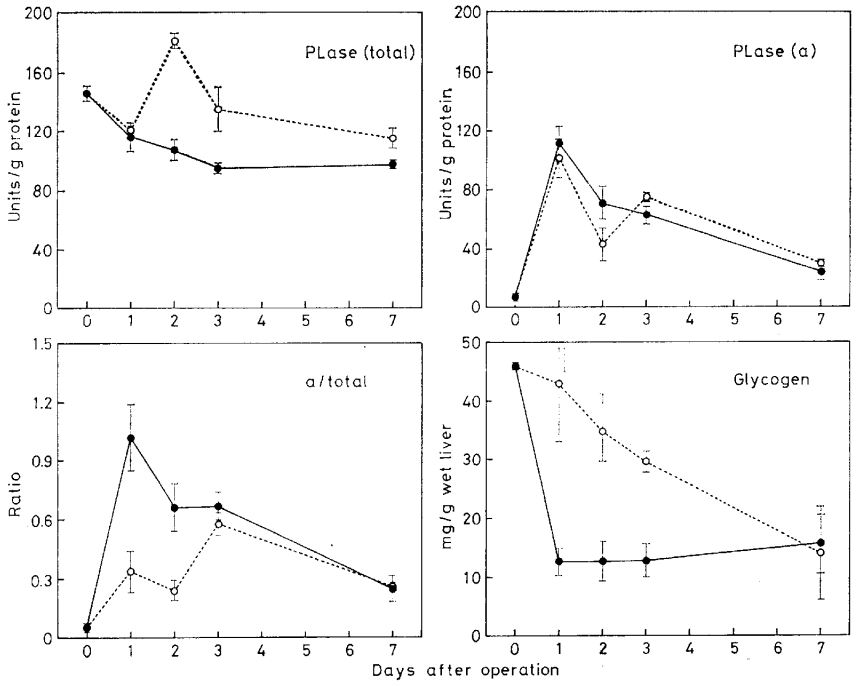


Fig. 2. Activities of PLase (total and *a*) and glycogen content in livers of Gal-NH_2-treated and control rats. •——•, Gal-NH_2 treated; ○- - -○, control.



Pig. 3. Activities of PLase (total and *a*) and glycogen content in livers of partially hepatectomized and sham-operated rats. •——•, hepatectomized; ○- - -○, sham-operated.

Human liver diseases. Liver PLase activities in various liver diseases are shown in Table 3 together with results of some liver function tests. Although the total PLase activity ranged widely, it tended to be lower in acute hepatitis (convalescent), cirrhosis of the liver (postnecrotic) and hepatocellular carcinoma. On the other hand, lower activities of *a* relative to the total were found in alcoholic liver injuries. No consistent tendency was found between the changes in PLase activities and the impairments in liver function tests.

DISCUSSION

In acute hepatic injuries produced by CCl_4 and Gal-NH_2 treatments, relatively small decreases in total PLase activity were found in later stages of the injuries as compared with the marked rises in PLase *a* activity with unaltered total activity in earlier stages of the injuries. In the present system of preparing tissue extracts, no sodium fluoride, an inhibitor of PLase phosphatase, was included in the medium because of the reasons mentioned in the introduction. Since the content of ATP in the liver supernatant is markedly reduced, the increase in *a* form PLase may indicate the change *in vivo*, even though sodium

fluoride was not added to the medium. The relative increase in the active form in early phases of liver damage is most likely to be caused by an insufficiency of PLase phosphatase activity. Another possibility of increased PLase kinase activity is also plausible, provided that a sufficient amount of ATP remains in the enzyme extracts. Under conditions *in vivo*, such a mechanism may be operating in view of the fact that plasma glucagon and liver cyclic 3′, 5′-adenosine monophosphate concentrations are reported to be elevated in various hepatic injuries (12, 13).

The increase in active form of PLase by liver injury would explain the marked drop in glycogen content of injured liver as was observed in CCl_4- or Gal-NH_2-intoxication, assuming that PLase *a* activity *in vivo* is elevated in injured liver. No suitable explanation is available at present for the parallel rises of PLase *a* and glycogen levels in 7 day rats after Gal- NH_2 injection. The same also applies to the discrepancy found in sham-operated controls; *i. e.* the increased PLase *a* activity with minimum diminution in glycogen level. This implies that the importance of the glycogen synthetase level in the injured liver, although not fully understood, should be considered together with PLase.

A moderate decrease in total PLase activity and a relative increase in *a* form activity found in regenerating livers after partial hepatectomy are somewhat similar to those demonstrated with liver injuries. An enhanced regeneration of hepatocytes usually follows the liver cell necrosis. Accordingly, the changes in PLase activities caused by hepatic injuries could be ascribed to the mechanism related to the regenerative process rather than the degenerative one, although the contribution of the latter mechanism, namely hepatic damage *per se*, is also evident, as it was revealed by further analysis of PLase isozyme patterns under identical conditions (14). In most of the carbohydrate-metabolizing enzymes examined, the changes induced by liver damage are generally much greater than those produced by partial hepatectomy (15). This holds true for the enhanced α-fetoprotein production by hepatic injury; this being much greater than that caused by partial hepatectomy (16, 17).

The PLase activities in injured livers from patients varied widely even within the same group of diseases. However, such a wide individual distribution of PLase activity is reported in normal controls (2). The variation of PLase activity in human liver is much greater than that of other enzyme activities of carbohydrate metabolism (18). Therefore, it may not be due to simple technical problems in analyzing the enzyme activity with small amounts of liver tissues. Although the exact cause of the variation in PLase activity is not known, some difference in dietary conditions of the individuals might be related. Reduced dietary intake appears to result in decreased actitivity of total PLase as was found in normal rats starved for one and a half days. In diseased human livers,

the total PLase activity tended to decrease not only in primary hepatoma but also in acute hepatitis or cirrhosis of the liver. This is in accord with the observation made by analyses of other enzymes of carbohydrate metabolism; namely, parenchymal liver damage causes undifferentiated gene expression (15, 18, 19). No consistent increase in *a* form activity was found even in cases with elevated serum GPT activity, which was associated with increased *a* form activity in experimental animals. The significance of lower PLase activities in alcoholic liver injuries is not clear at present. The cases included in the present study had relatively less severe hepatic damage because of the clinical indication for peritoneoscopy and biopsy. This may partly account for the minor change in PLase activity of injured livers and also for the failure of obtaining significant correlations between the change in PLase activity and the impairment in routine liver function tests. The lower values of PLase activity obtained for hepatitis and cirrhosis of the liver are close to the levels reported for glycogen storage disease (2), suggesting the physiological importance of the observed alteration in PLase activity in acquired liver diseases in addition to the generally accepted disorders in hormonal or dietary factors in terms of the dysregulation of carbohydrate metabolism found in liver diseases.

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REFERENCES

1. Wosilait, W. D. and Sutherland, E. W.: The relationship of epinephrine and glucagon to liver phosphorylase. II. Enzymatic inactivation of liver phosphorylase. *J. Biol. Chem.* **218**, 469-481, 1956.
2. Öeckerman, P. A.: A technique for the enzymatic diagnosis of glycogen storage disease on very small tissue specimens. *Acta Paediatr. Scand.* **57**, 105-109, 1968.
3. Appleman, M. M., Krebs, E. G. and Fischer, E. H.: Purification and properties of inactive liver phosphorylase. *Biochemistry* **5**, 2101-2107, 1966.
4. Sato, K., Satoh, K., Sato, T., Imai, F. and Morris, H. P.: Isozyme patterns of glycogen phosphorylase in rat tissues and transplantable hepatomas. *Cancer Res.* **36**, 487-495, 1976.
5. Higgins, G. M. and Anderson, R. M.: Experimental pathology of the liver: restration of the liver of the white rat following partial surgical removal. *Arch. Pathol.* **12**, 186-202, 1931.
6. Chen, P. S. Jr., Toribara, T. Y. and Warner, H.: Microdetermination of phosphorus. *Anal. Chem.* **28**, 1756-1758, 1956.
7. Koide, H. and Oda, T.: Pathological occurrence of glucose 6-phosphatase in serum in liver diseases. *Clin. Chem. Acta* **4**, 554-561, 1959.
8. Taketa, K., Kaneshige, Y., Tanaka, A. and Kosaka, K.: Differential responses of glucose 6-phosphate dehydrogenase and lipogenesis to fat free diets. *Biochem. Med.* **4**, 531-545, 1970.
9. Lowry, O. H., Rosebrough, N. T., Farr, A. L. and Randall, R. J.: Protein measurement

with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265-275, 1951.
10. Reitman, S. and Frankel, S.: A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* **28**, 56-63, 1957.
11. Rall, T. W. and Sutherland, E. W.: *Methods in Enzymology*, ed. S. P. Colowick, and N. O. Kaplan, Academic Press, New York, Vol. 5, pp. 384-391, 1962.
12. DeRubertis, F. R. and Craven, P.: Effects of reduced ATP content on hepatic responses to glucagon. *Metabolism* **25**, 57-67, 1976.
13. Jaspan, J. B., Huen, A. H-J., Morley, C. G., Moossa, A. R. and Rubenstein, A. H.: The role of the liver in glucagen metabolism. *J. Clin. Invest.* **60**, 421-428, 1977.
14. Kobayashi, M.: Studies of liver phosphorylase in hepatic injury. II. Alteration in isozyme pattern. *Acta Med. Okayama* (in press).
15. Taketa, K., Tanaka, A., Watanabe, A., Takesue, A., Aoe, H. and Kosaka, K.: Undifferentiated patterns of key carbohydrate-metabolizing enzymes in injured livers. I. Acute carbon-tetrachloride intoxication of rat. *Enzyme* **21**, 158-173, 1976.
16. Watanabe, A., Miyazaki, M. and Taketa, K.: Differential mechanisms of increased α-fetoprotein production in rats following carbon tetrachloride injury and partial hepatectomy. *Cancer Res.* **36**, 2171-2175, 1976.
17. Taketa, K., Watanabe, A. and Kosaka, K.: Different mechanisms of increased rat α-fetoprotein production in rats following CCl_4 intoxication and partial hepatectomy. *Ann. N. Y. Acad. Sci.* **259**, 80-84, 1975.
18. Taketa, K., Shimamura, J., Takesue, A., Tanaka, A. and Kosaka, K.: Undifferentiated patterns of key carbohydrate-metabolizing enzymes in injured livers. II. Human viral hepatitis and cirrhosis of the liver. *Enzyme* **21**, 200-210, 1976.
19. Taketa, K., Watanabe, A. and Kosaka, K.: Undifferentiated gene expression in liver injuries. In *Onco-Developmental Gene Expression*, ed. W. H. Fishmen and S. Sell, Acad. Press, New York, pp. 219-226, 1976.