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Suppressed Dietary Inducibility of Glucose 6-Phosphate Dehydrogenase and Elevated Cyclic AMP in Acute Hepatic Injury[1] (40302)

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Glucose 6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) is a key enzyme of the pentose phosphate pathway and induced by dietary glucose and amino acids, but not by either alone (1–3). Thus, the dehydrogenase level in rat liver is under a dual dietary control, *i.e.* transcriptional and posttranscriptional regulations; a glucose-dependent step of the induction being sensitive to actinomycin D (3) and blocked by increasing cyclic 3′,5′-adenosine monophosphate (cyclic AMP) level (4). An entirely different type of G6PD induction could be brought about by intoxication of rat with carbon tetrachloride and other hepatotoxins (5–7). Although the synthesis *de novo* of the enzyme protein is involved in the hepatotoxin-induced increase in G6PD activity, it does not require newly synthesized RNA (5) and is insensitive to manipulations to raise hepatic cyclic AMP level (8). We found in our preliminary experiments with acute thioacetamide intoxication of rat that the dietary induction of G6PD was markedly depressed in the injured liver (9).

A further study of this observation, reported in the present communication, revealed that the reduced dietary inducibility of G6PD in the acute hepatic injury could be explained at least by a dietary unresponsive increase in cyclic AMP level in the injured liver. Thioacetamide was chosen in this study to produce an acute liver damage with elevated G6PD activity, because the intoxication with thioacetamide, unlike carbon tetrachloride, caused no reduction in dietary intake.

Materials and methods. Male Sprague-Dawley rats, weighing 130–150 g, were deprived of food for 24 hr before intraperitoneal injection of 20 mg thioacetamide (Merck Co., Darmstadt, Germany; dissolved in saline) per 100 g body weight. The animals were further fasted for 24 hr and divided into the following three groups: GC, refed on a glucose–casein (7:3 in weight) mixture; G, placed on a sole glucose diet; and S, starved for additional 24 hr. Control rats received equivalent amounts of saline in place of the thioacetamide solution and treated identically with respect to the dietary change.

The animals, each group consisting of four rats, were killed at indicated time intervals by ether overdose (10), which gives hepatic cyclic AMP and cyclic 3′,5′-guanosine monophosphate (cyclic GMP) values both closest to those obtained by a freezing method (10, 11). A small portion of the liver was quickly removed, weighed (10) and extracted twice by fixing with trichloroacetic acid (TCA) (12). After removal of TCA with ether (12), the aqueous extract was evaporated at 65° under nitrogen and reconstituted in water to give an original volume. The cyclic nucleotide concentrations were determined, after appropriate dilution and succinylation, by a radioimmunoassay method using Yamasa cyclic AMP and cyclic GMP kits (Yamasa Shoyu Co., Chiba, Japan) (13).

The activities of G6PD, low-Km hexokinase (EC 2.7.1.1) and glucokinase (EC 2.7.1.2) in liver supernatants and of alanine aminotransferase (GPT, EC 2.6.1.2) in sera and the contents of glycogen in liver tissues were determined as described previously (2, 7, 14). The enzyme activities and cyclic nucleotide concentrations in liver were expressed on the basis of unit supernatant protein, because the liver weight increased markedly in refed groups of rat due to an excessive glycogen deposition (Table I). All the results are given as means and standard errors of the means for each group. Histological studies were made on liver specimens by a hematoxylin–eosin staining.

Results. Alterations of G6PD activity and

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cyclic nucleotide contents in liver following dietary and thioacetamide treatments are illustrated in Fig. 1 and those of other parameters of hepatic injury and dietary effect are summarized in Table I. The specific activity of G6PD in control animals increased markedly upon refeeding with glucose and casein, but not with glucose alone. In thioacetamide-treated rats, G6PD activity increased significantly in 24 and 48 hr of the hepatotoxin treatment even if the animals were starved and degeneration and necrosis of hepatocytes were evident histologically. These data are consistent with our previous findings (1–3, 5). When the starved and thioacetamide-treated rats were placed on a glucose-casein diet, only a minute further increase in G6PD activity was produced by the refeeding (GC *vs.* G or S) in contrast with the steep rise found in the control rats. There was no difference in the amount of diet consumed between the injured and control groups. The results apparently indicated that the dietary induction of G6PD was impaired in the injured liver despite the fact that the enzyme activity was increased by hepatic injury itself. That the extent of hepatic injury *per se* was not affected by the different dietary treatments was evident from the similar increases in low-Km hexokinase activity in the three different dietary groups of injured rats (Table I). The hepatic level of this enzyme increases by liver injury (6, 9, 14) and is unresponsive to dietary change as the data for control groups given in the same table reveal. There were no significant differences either in serum GPT activity, a sensitive marker of liver injury, among the three injured groups (Table I). The activity of glucokinase, another dietary inducible enzyme (1, 15), was reduced by thioacetamide intoxication and the induction of this enzyme by glucose or glucose–casein refeeding was also diminished in the injured livers as may be seen in the table.

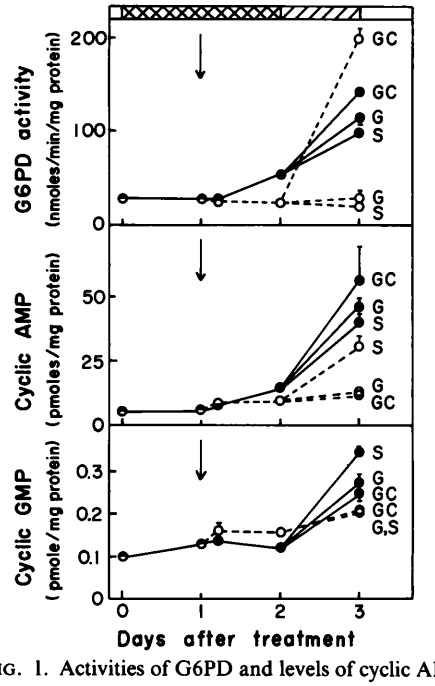

FIG. 1. Activities of G6PD and levels of cyclic AMP and cyclic GMP in livers of control and thioacetamide-treated rats placed on different dietary regimens. The arrows indicate the time of administration of thioacetamide (●——●) or saline (○-----○). ⊠, period of fasting for all the groups; and ▨, period of refeeding for Groups G and GC and of continued fasting for Group S.

The values of cyclic AMP obtained with livers of well-fed rats (0-day value in Fig. 1) fell in the range of reported values (10–12, 16). The hepatic cyclic AMP level increased significantly in 3 days of starvation in both thioacetamide-injured and control groups, although the extent of the increase was slightly larger in the injured group than in the control. An important result of this experiment is that the rise in hepatic cyclic AMP content on prolonged fasting of injured rats could not be suppressed by refeeding glucose-containing diets in contrast with the rise in the control animals. The hepatic levels of cyclic GMP in control groups agreed well with the reported values (11, 12) and changed little by dietary alteration. In thioacetamide-treated rats, however, the cyclic GMP content increased significantly upon prolonged starvation. The increase was much less, although above the control levels, in the refed groups of intoxicated rats. A possibility of overestimating cyclic GMP level in the presence of high concentrations of cyclic AMP was neglected by obtaining constant values with different dilutions of liver extract in radioimmunoassay.

In thioacetamide-treated rats, the amount of glycogen deposited in the liver after refeeding was significantly less than in untreated rats, even though dietary intakes were

TABLE I. ACTIVITIES OF GPT IN SERA AND OF GLUCOKINASE AND LOW-Km HEXOKINASE IN LIVERS AND CONTENTS OF GLYCOGEN AND PROTEIN IN LIVERS OF CONTROL AND THIOACETAMIDE-TREATED RATS PLACED ON DIFFERENT DIETS.

Experimental conditions		Enzyme activities			Tissue constituents	
		GPT	Gluco-kinase	Hexo-kinase	Glycogen	Supernatant protein
Dietary manipulation	Thioacetamide treatment	(Karmen u./ml)	(nmoles/min/mg protein)		(mg/g liver)	
Well-fed	–	32 ± 2	20.3 ± 4.2	3.2 ± 1.3	42.0 ± 7.7	96 ± 2
1-day starved	–	24 ± 1	8.9 ± 0.4	4.4 ± 0.3	3.6 ± 3.6	113 ± 2
1-day and 5 hr starved	–	27 ± 2	10.7 ± 1.8	3.4 ± 0.4	1.9 ± 0.8	115 ± 4
	+ (5 hr)	24 ± 4	9.1 ± 0.6	5.0 ± 0.5	1.1 ± 0.5	108 ± 2
2-day starved	–	21 ± 3	10.6 ± 1.8	3.2 ± 0.2	0.2 ± 0.1	110 ± 4
	+ (1 day)	224 ± 53	6.2 ± 1.7	10.2 ± 0.4	0.2 ± 0.1	103 ± 3
3-day starved	–	32 ± 6	2.5 ± 0.6	3.0 ± 0.2	0.2 ± 0.1	123 ± 3
	+ (2 days)	261 ± 66	0.4 ± 0.3	20.1 ± 1.4	0.2 ± 0.1	102 ± 2
2-day starved and 1-day refed on G	–	21 ± 3	27.5 ± 2.2	3.1 ± 0.2	69.4 ± 7.1	80 ± 4
	+ (2 days)	323 ± 100	9.0 ± 2.2	21.8 ± 1.3	35.8 ± 8.4	88 ± 2
2-day starved and 1-day refed on GC	–	29 ± 4	41.9 ± 2.7	3.9 ± 0.2	80.0 ± 9.8	84 ± 4
	+ (2 days)	229 ± 50	7.6 ± 2.7	20.9 ± 0.6	31.5 ± 16.1	86 ± 1

similar in both groups of rat and almost no ingested dietary mass remained in the gastrointestinal tracts at the time of sacrifice.

Discussion. G6PD is a unique enzyme in a sense that a single molecular species is involved in a wide variety of inductive responses; such as those to dietary, hepatotoxic and neoplastic changes (17). Thus, the induction mechanism of this enzyme appears to be different depending on the type of inductive stimuli. The dietary induction of G6PD requires *de novo* RNA synthesis at a low cyclic AMP level (3, 5), whereas carbon tetrachloride-induced increase of G6PD synthesis obligates neither of them (7). The latter mechanism would also apply to the increased hepatic G6PD level in thioacetamide-injured rat (17). Accordingly, the impairment of dietary induction of G6PD in injured liver is possibly at the level of transcription. The block at this step could be accounted for at least by the high hepatic level of cyclic AMP observed in the thioacetamide-injured rats refed on glucose and casein. The increased level of cyclic AMP appears to be also responsible for the reduced accumulation of hepatic glycogen in the injured refed rats. Incidentally, the low hepatic cyclic AMP level alone is not sufficient to induce this enzyme, since in control rats a sole glucose diet lowered the cyclic AMP level without inductive effect.

Whether the dietary unresponsive increase in cyclic AMP level by thioacetamide treatment is due to a sustained hyperglucagonemia or an altered adenylate cyclase-phosphodiesterase system is to be solved in future studies. Although an increased portal level of glucagon is reported in acute ethionine intoxication of rat, glucose infusion has been shown to decrease the hepatic cyclic AMP content (16). Prostaglandin may well be an attractive candidate for such a stimulant as to the dietary insensitive elevation of cyclic AMP in injured liver.

A reduced dietary response of G6PD in regenerating liver following partial hepatectomy (18) could be similarly explained by elevated cyclic AMP levels in the remnant liver (10). Since, however, the thioacetamide-induced hepatic degeneration and necrosis is also followed by a rise in DNA synthesis (7), some conditions associated with cell division may serve as another common underlying mechanism for the suppression of dietary induction of G6PD. The small increases in hepatic cyclic GMP content found in the late stage of thioacetamide injury might be more or less related to the regenerative process of the injured liver (7, 19), although a direct effect of the carcinogen can not be excluded (20).

It is of some interest to note that another dietary inducible and cyclic AMP-sensitive

enzyme, glucokinase (1, 15), was also shown to be less responsive to glucose-containing diets in the injured liver. Since G6PD and low-Km hexokinase could be induced by hepatic injury itself (5, 6, 9, 14), the decrease in glucokinase activity may also represent a specific metabolic response of hepatocyte to the injury rather than a mere destructive process of general protein synthesis. Thus, in hepatic injury, the induction of more differentiated liver enzymes is suppressed and that of primitive or fundamental enzymes is enhanced, resulting in an undifferentiated enzyme pattern (6, 14). A similar loss of dietary response of G6PD and other carbohydrate-metabolizing enzymes in preneoplastic livers has been demonstrated by Poirier and others (21). An altered inducibility of some enzymes of amino acid metabolism in chronic administration of carbon tetrachloride and a noncarcinogenic azo dye is also reported from their laboratory (22). Although thioacetamide is a hepatocarcinogen, its acute intoxication, as employed in the present experiment, could be interpreted better as a hepatic injury, which bears little significance as precancerous lesion. Elucidation of the mechanisms of altered enzyme induction in acute hepatic injury would provide a clue for the understanding of undifferentiated gene expression in preneoplastic livers and in turn hepatomas.

Summary. The dietary induction of liver G6PD was found to be markedly impaired in the acute hepatic injury of rat caused by thioacetamide intoxication. The level of cyclic AMP in the injured liver was increased and could not be reduced by glucose-containing diets. The results indicated that the suppressed dietary inducibility of G6PD in hepatic injury is accounted for at least by the dietary unresponsive increase in cyclic AMP level in the injured liver.

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