Effect of Liver Damage on Plasma FFA Response to Epinephrine.* (30277)

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The importance of the liver for the synthesis of plasma glycerides is established(1). The liver takes up albumin-bound fatty acids and releases them as glycerides and phospholipids incorporated in plasma protein(2). Since the rate of uptake of FFA by the liver is normally a function of the plasma concentration of FFA, the liver acts as an important regulator of blood lipids.

Both *in vivo* and *in vitro* studies support the hypothesis that epinephrine causes FFA mobilization from depot adipose tissue(3). The plasma FFA level at any time is thus a reflection of both the release into (mobilization) and the removal from the circulation of FFA (utilization and storage). If mobilization of fatty acids from the depots is normal, then a change in plasma FFA concentration may reflect a change in liver function. The purpose of the present study, therefore, was to investigate the acute epinephrine effect on plasma FFA and blood glucose in animals with 2 kinds of liver damage: 1. partial hepatectomy and 2. chronic CCl_4 poisoning, in order to assess the role of the liver in this aspect of lipid regulation.

Methods. Male, albino rats of the Sprague-Dawley strain with an average initial body weight of 220 ± 20 g were used. The animals had access to food (Purina rat chow) and water *ad libitum*. They were divided into 3 groups (36 animals in each): I. normal, intact; II. partially hepatectomized; III. chronic CCl_4 poisoned. All experimental animals were treated with a single injection of epinephrine, 1 mg/kg s.c. in oil (Adrenalin—Parke, Davis & Co.). The effect of epinephrine on plasma FFA was measured using Dole's method(4) and on blood glucose using the Somogyi method(5). Blood samples were obtained from 6 animals in each group by decapitation after 30 minutes and every hour thereafter from similarly treated animals for a period of 4 hours. After the epinephrine injection food was removed but drinking water was available.

Partial hepatectomy was performed by Drabkin's method(6) under ether anesthesia. After laparotomy, the left and the median lobes of the liver were tied with surgical thread and excised. Little or no bleeding occurred. The abdominal layers were joined by 2 sutures and the skin incision closed with metal clips. The animals were allowed to recover from the operation for 48 hours. The percentage of the liver removed was calculated as follows: The excised portion of the liver was weighed at time of operation. At the end of the experiment the remaining liver tissue was removed and weighed. The combined weights were taken as approximate initial weight of the intact liver and the percentage of liver excised was thus:

$$\frac{\text{excised liver weight}}{\text{total liver weight}} \times 100.$$

From this type of calculation the degree of hepatectomy was found to be 58.0 ± 3.8%. This is somewhat less than the actual value because of the regeneration of some hepatic tissue during the 48-hour experimental period. On the basis of our own and other observations the amount of regeneration may be assumed to be of the order of 10-15% of the weight of the remaining liver fragment. CCl_4 was given for 7 days in a single daily dose of 1 ml/kg s.c. in corn oil 50/50 volume %. The last injection was given one day before the experiment was performed.

Results. Normal fed rats are known to respond to epinephrine in oil with a prompt but transient elevation of plasma FFA levels (4). Our results demonstrate that normally, changes in FFA and glucose levels coincide in time (Tables I, II). Both the increased level of plasma FFA and the hyperglycemic effect lasted approximately 4 hours. After partial hepatectomy we observed a relatively

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TABLE I. Plasma FFA Response to Epinephrine Injection.

Time in hr after epinephrine inj	Plasma FFA, mEq/l ± S.D.				
	Normal intact	Partial hepatectomy	p value	CCl_4 poisoning	p value
0	.416 ± .064	.582 ± .036	<.01	.632 ± .057	<.01
½	.893 ± .087	.943 ± .069	N.S.	.756 ± .130	N.S.
1	1.029 ± .091	.706 ± .052	<.01	.641 ± .074	<.01
2	.912 ± .083	.814 ± .042	N.S.	.688 ± .069	<.01
3	.759 ± .042	.909 ± .043	<.01	.600 ± .065	<.01
4	.581 ± .116	1.018 ± .062	<.01	.557 ± .106	N.S.

TABLE II. Blood Glucose Response to Epinephrine Injection.

Time in hr after epinephrine inj	Blood glucose, mg % ± S.D.				
	Normal intact	Partial hepatectomy	p value	CCl_4 poisoning	p value
0	106.9 ± 12.6	103.8 ± 7.2	N.S.	88.1 ± 7.6	N.S.
½	214.1 ± 23.4	181.3 ± 17.0	<.01	157.1 ± 12.5	<.01
1	263.7 ± 29.2	217.5 ± 22.6	<.01	182.0 ± 10.3	<.01
2	225.0 ± 16.5	171.0 ± 7.2	<.01	184.8 ± 32.9	N.S.
3	172.8 ± 14.6	133.2 ± 13.8	<.01	187.5 ± 17.1	N.S.
4	121.5 ± 22.0	105.0 ± 12.2	N.S.	196.8 ± 13.9	<.01

normal response of the blood glucose to exogenous epinephrine (Table II). The plasma FFA response, however, was abnormal (Table I). The initial FFA concentration was higher than in normal animals and a further peak increase was reached 30 minutes after the epinephrine injection. This was followed by a transient decrease but the FFA then rose gradually and remained high after 4 hours.

In animals chronically poisoned with CCl_4, both blood glucose and plasma FFA response to epinephrine were altered (Tables I, II). Blood glucose gradually increased over pre-injection levels and hyperglycemia was still manifest after 4 hours (Table II). The plasma FFA, on the other hand, failed to show significant change during the entire 4-hour post-injection period (Table I).

Discussion. Catecholamines are known to activate lipolysis of triglycerides in adipose tissue both *in vivo* and *in vitro*. Some of the free fatty acids so produced are utilized by the liver for triclyceride and phospholipid synthesis and then are redistributed throughout the body. Dury and Treadwell showed that large increases in hepatic triglyceride content occur within an hour after administration of epinephrine to intact rats(7). Subsequent studies with labelled FFA established that the liver triglycerides originated from plasma FFA(8). The increase in plasma triglycerides and phospholipids is due apparently to increased hepatic synthesis and release of these lipids since hepatectomy abolishes(9) these plasma changes.

Normally, epinephrine induces an elevation in blood glucose as well as increased lipolysis. The hyperglycemia elicits insulin release followed by increased glucose utilization by adipose tissue and increased triglyceride synthesis in this tissue. At the same time hepatic uptake of FFA also increases. Thus, the initial elevation of plasma FFA due to epinephrine injection is counteracted and blood levels are restored to pre-injection levels.

It had been shown that hepatectomy in rats produced an altered plasma FFA response(10,11). Our data confirm this observation. There is no evidence to suggest that this phenomenon is due to a primary defect at the adipose tissue level. The persistence of elevated plasma FFA in these euglycemic animals probably reflects, therefore, the inability of the remaining mass of liver cells to remove FFA from blood at an adequate rate. When an injection of epinephrine imposes a further increase in plasma FFA on this already saturated liver system, the plasma FFA rise to still higher levels and counterregulatory responses are ineffective even after 4 hours. It is of interest that this same

degree of partial hepatectomy does not impair markedly the regulation of blood sugar. In our animals the time course of blood sugar response to epinephrine was relatively normal. It is possible that the changes observed in animals with a regenerating liver may be a reflection of altered metabolism during this period of accelerated protein synthesis. Our data do not rule out this possibility but work by others using a wide variety of functional tests after partial hepatectomy has been largely negative with the exception of urinary urobilinogen levels and serum flocculation tests(12).

A deficit in liver function which is induced by chronic CCl_4 poisoning seems to present quite another kind of alteration in the dynamics of adipose-plasma-liver lipid relations. A characteristic effect of CCl_4 is to produce a block in the release of triglycerides from the liver into the circulation. The development of a "fatty liver" is accompanied by a fall in the plasma concentration of triglycerides and phosphatides in such rats(13). There is, however, no significant effect of the CCl_4 on rate of release of FFA from adipose tissue as measured *in vitro*(14). Our data demonstrate that these animals do not respond to exogenous epinephrine with an elevation in plasma FFA. It is suggested therefore, that the synthesis and accumulation of triglycerides in the liver compensates entirely for the epinephrine-induced mobilization of depot FFA and no significant change in blood levels of FFA is observed. On the other hand, CCl_4-damaged liver cells are known to synthesize glycogen poorly. This probably accounts for the persistent hyperglycemia we observed in the poisoned animals treated with exogenous epinephrine.

These data suggest that it may be possible to distinguish different kinds of liver damage by virtue of the plasma FFA response to injection of epinephrine.

Summary. Three groups of male Sprague-Dawley rats, control, partially hepatectomized and chronically poisoned with CCl_4, were injected with 1 mg/kg epinephrine in oil. Plasma FFA and blood glucose were measured over a 4-hour post-injection period. Control animals showed the usual hyperglycemic and hyperlipemic response to epinephrine within the first hour after injection. Blood glucose and FFA were back to pre-injection levels at 4 hours. After partial hepatectomy, pre-injection blood levels of glucose were normal but FFA concentration was elevated. The hyperglycemic response to epinephrine was normal but the FFA response was markedly altered. A further elevation in plasma FFA was observed after ½ hour and these high levels persisted during the entire 4-hour post-injection period. Chronic CCl_4 induced liver damage elicited a different kind of epinephrine response. There was an abnormally sustained hyperglycemia during the 4-hour post-injection period but the plasma FFA never showed any elevation above pre-injection levels. The data suggest that CCl_4 damages most liver cells in such a way as to lead to abnormal storage of lipids with a consequent increase in utilization of plasma FFA. Partial hepatectomy, however, reflects a diminution below a critical mass of relatively normal cells and total triglyceride synthesis is not sufficient to utilize the plethora of plasma FFA elicited by epinephrine treatment. It is possible that the two types of liver damage may be distinguished in this way.

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