Ketone-body metabolism after partial hepatectomy in the rat

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Fed or 24 h-starved rats were subjected to two-thirds partial hepatectomy or sham-operation and subsequently starved for 4, 14 or 24 h. Despite high plasma fatty acid concentrations, the partially hepatectomized rats failed to respond to post-operative starvation with increased blood and liver ketone-body concentrations or to maintain the high ketone-body concentrations associated with pre-operative starvation. Hypoglycaemia and hyperlactaemia were observed within 30 min of functional hepatectomy, but not partial hepatectomy, of 24 h-starved rats, and, even after a further 24 h starvation of partially hepatectomized rats, blood glucose concentrations were only slightly decreased. The results are discussed with reference to fat oxidation and gluconeogenesis in the liver remaining after partial hepatectomy.

INTRODUCTION

In adult rats, hepatocytes rarely divide, but after two-thirds partial hepatectomy the remaining liver promptly starts to grow. For the first 24 h the cells increase in size but not in number, but subsequently, at 24–30 h, there is a relatively synchronized burst of mitosis (see Fex, 1970). The cells continue to divide until the liver mass is restored (approx. 4–8 days).

We have previously observed (Schofield *et al.*, 1985) that, if fed rats are subjected to partial hepatectomy and then starved post-operatively, steady-state concentrations of the ketone bodies in the liver remnant sampled at 24 h are less than those in the livers of sham-operated controls. Others have demonstrated that the infusion of (+)-octanoylcarnitine, an inhibitor of long-chain fatty acid oxidation, inhibits DNA synthesis in the liver remnant during the first and second post-operative days (Nakatani *et al.*, 1982). This indicates that fatty acid oxidation is required to provide the ATP needed for liver cell DNA synthesis and mitosis, and raises the possibility that the low hepatic ketone-body concentrations result from increased complete oxidation of fatty acids to CO₂ at the expense of ketogenesis, secondary to the increased energy demands associated with cell division. To test this, we determined the time course of changes in hepatic and blood concentrations of the ketone bodies at intervals during the first 24 h after partial hepatectomy (i.e. before DNA synthesis and mitosis). Hepatic lipid and plasma non-esterified fatty acid concentrations were also measured.

EXPERIMENTAL

Two-thirds partial hepatectomies or sham-operations were performed on albino Wistar rats (180–210 g) under light ether anaesthesia as described previously (French *et al.*, 1985). The rats were either fed *ad libitum* or starved for the 24 h before surgery, and were starved post-operatively until sampling at 4, 14 or 24 h later. Liver weights at these respective times of sampling, expressed as g/100 g rat, were as follows: partially hepatectomized rats fed before surgery, 1.40 ± 0.06 (8), 1.41 ± 0.06 (9),

1.78 ± 0.08 (9); partially hepatectomized rats starved before surgery, 1.17 ± 0.07 (8), 1.41 ± 0.04 (4), 1.59 ± 0.04 (4); sham-operated rats fed before surgery, 3.38 ± 0.19 (8), 3.70 ± 0.09 (9), 3.41 ± 0.09 (9); sham-operated rats starved before surgery, 2.96 ± 0.08 (8), 2.92 ± 0.08 (4), 2.96 ± 0.17 (4). Functional hepatectomies were performed in 24 h-starved rats as described by Blackshear *et al.* (1974): in these experiments rats were sampled at 30 min after surgery.

Immediately before sampling, rats were anaesthetized with sodium pentobarbital (60 mg/kg body wt.) and dissected after 5 min. Arterial blood samples were withdrawn and the livers were freeze-clamped. Concentrations of NEFA (plasma) and lactate, glucose, the ketone bodies (3-hydroxybutyrate and acetoacetate) and glycogen (KOH-neutralized HClO₄ extracts) were determined spectrophotometrically (Hohorst *et al.*, 1959; Williamson *et al.*, 1962; Slein, 1963; Keppler & Decker, 1974; Schofield *et al.*, 1985). Liver samples were also extracted for determination of neutral-lipid concentrations (Gove & Hems, 1978).

RESULTS AND DISCUSSION

Plasma NEFA and fatty liver after partial hepatectomy

Partial hepatectomy resulted in marked and sustained increases in plasma NEFA concentrations, which were observed within 4 h of surgery irrespective of whether the rats were fed or starved before the operation (Fig. 1a). Starvation before surgery exaggerated the response to partial hepatectomy. Although sham-operation was also associated with increased NEFA, concentrations were never as high as those observed in partially hepatectomized rats. The NEFA concentrations in the sham-operated rats at 24 h were similar to those found in unoperated 24 h-starved rats (see Fig. 1a).

The accumulation of neutral lipid in the rat liver remnant for up to 2 days after partial hepatectomy is well established (see Mangiavane *et al.*, 1973; Gove & Hems, 1978). Increased hepatic lipid accumulation after partial hepatectomy has been variously attributed to increased lipogenesis (see Gove & Hems, 1978), increased glycerol-

Fig. 1. Plasma non-esterified fatty acid (a) and hepatic triacylglycerol (b) concentrations after partial hepatectomy or sham-operation of fed or 24 h-starved rats

For experimental details see the text. Rats were either fed *ad libitum* (●, ▲) or starved for 24 h (○, △) before surgery. All rats were starved post-operatively before sampling. Each point represents the mean $\pm$ S.E.M. for four to nine observations. Plasma NEFA concentrations (a) in partially hepatectomized (●, ○) or sham-operated (▲, △) rats are presented as mmol/l. Hepatic triacylglycerol concentrations (b) are presented as mg/g wet wt. of liver. Statistically significant differences between partially hepatectomized and sham-operated rats are indicated by: *** P < 0.001; ** P < 0.01; * P < 0.05. Values in fed or 24 h-starved unoperated rats are also shown (◆, ◇).

Fig. 2. Hepatic and blood ketone-body concentrations after partial hepatectomy or sham-operation of fed (a) or 24 h-starved (b) rats

For experimental details see the text. Rats were either fed *ad libitum* or starved for 24 h before surgery. All rats were starved post-operatively before sampling. Each point represents the mean $\pm$ S.E.M. for four to nine observations. Liver ketone-body concentrations in partially hepatectomized (●) or sham-operated (▲) rats are presented as μ mol/g wet wt. of liver. Blood ketone-body concentrations in partially hepatectomized (○) or sham-operated (△) rats are presented as μ mol/ml of whole blood. Liver (◆) and blood (◇) ketone-body concentrations in fed or 24 h-starved unoperated rats are also shown. Statistically significant differences between the partially hepatectomized or sham-operated rats are indicated by: *** P < 0.001; ** P < 0.01; * P < 0.05.

lipid synthesis (Infante *et al.*, 1969; Delahunty & Rubenstein, 1970; Mangiapane *et al.*, 1973), or increased mobilization of NEFA from adipose tissue. In the present series of experiments, increased lipid accumulation occurred within 14 h of partial hepatectomy if the rats were fed pre-operatively, but within 4 h if the rats were starved pre-operatively. This suggests that increased NEFA mobilization and availability is a major factor contributing to the development of the fatty liver.

Hepatic and blood ketone-body concentrations after partial hepatectomy

Liver ketone-body concentrations in rats that were fed pre-operatively (Fig. 2a) remained low at 4 h after surgery. Subsequently hepatic ketone-body concentra-

tions increased in the sham-operated rats, but not in the partially hepatectomized rats. A similar pattern of changes in ketone-body concentrations was observed in the blood.

The increase in ketone-body concentrations in the liver and blood of the sham-operated rats was proportional to the duration of the post-operative starvation period, and roughly correlated with the progressive rise in NEFA concentrations (Fig. 1a). This would be expected, since NEFA are major precursors used for hepatic ketogenesis. However, although NEFA concentrations were similar at 4 and 14 h post-operatively, ketone-body concentrations were clearly increased at the latter time. This result emphasizes the importance of consideration of both rates of production and rates of utilization of substrates in the

Fig. 2(b) shows the changes in blood and liver ketone-body concentrations occurring in rats that were starved pre-operatively. Although ketone-body concentrations were maintained or increased after sham-operation, concentrations declined after partial hepatectomy. This demonstrates that, despite the increased availability of NEFA, there is substantial utilization of ketone bodies by extrahepatic tissues in the partially hepatectomized rats. The decline in ketone-body concentrations was most marked during the first 4 h after liver resection. Thus, during this period, the rate of ketone utilization by extrahepatic tissues exceeds the rate of ketone-body production by the liver remnant. In contrast, between 4 and 24 h, ketogenesis in the liver remnant is adequate to maintain blood and liver ketone-body concentrations. Steady-state concentrations in partially hepatectomized rats that had been starved pre-operatively were higher than those in partially hepatectomized rats that had been fed pre-operatively (cf. Figs. 2a and 2b). Studies *in vivo* and *in vitro* (reviewed by McGarry & Foster, 1980) have indicated that starvation causes a stable increase in the ability of the liver to oxidize fatty acids, and the present experiments therefore imply that the resected liver retains the characteristics induced by pre-operative starvation.

A comparison of the acute effects of sham operation, partial hepatectomy and functional hepatectomy on blood metabolite concentrations in 24 h-starved rats is shown in Table 1. The rats were under pentobarbital anaesthesia throughout these experiments. Decreased blood ketone bodies were observed at 30 min after partial or functional hepatectomy (see also Blackshear *et al.*, 1974), but the concentration decrease in the partially hepatectomized rats was approximately two-thirds of that observed in the functionally hepatectomized rats. Initial ketone-body concentrations were similar in both groups (Table 1). Since ketone-body utilization is proportional to the prevailing ketone-body concentration (Bates, 1971; Keller *et al.*, 1978; Reed *et al.*, 1984), the results confirm that the liver remnant is capable of ketone-body formation. The correlation between the amount of liver removed and the decline in blood ketone-body concentrations after resection strongly suggests that the inability of the partially hepatectomized rats to maintain the high ketone-body concentrations produced by pre-operative starvation is related to the decreased liver mass, but it should be stressed that, in the absence of measurements of ketone-body turnover, this explanation is, of necessity, speculative, and decreased

Table 1. Comparison of effects of partial and functional hepatectomy on blood metabolite concentrations in 24 h-starved rats

Rats (starved 24 h) were subjected to partial hepatectomy (PH) or functional hepatectomy (FH). Venous blood was sampled before and at 30 min after surgery. The rats were under pentobarbital anaesthesia throughout the procedure. Values are means $\pm$ S.E.M. for the numbers of rats in parentheses. Statistically significant effects of PH or FH compared with the sham-operation (SO) are indicated by: $\dagger P < 0.05$; $\ddagger P < 0.01$; $\text{***} P < 0.001$. Statistically significant differences between the PH and FH rats are indicated by: $* P < 0.05$; $** P < 0.01$.

Rats...	Blood metabolite								
	D-3-Hydroxybutyrate + acetoacetate				Glucose			Lactate	
	SO	PH	FH	SO	PH	FH	SO	PH	FH
Initial concn. (mm)	1.57±0.10 (8)	1.6±0.15 (6)	1.68±0.13 (7)	5.13±0.95 (6)	5.27±0.34 (6)	5.67±0.18 (5)	1.77±0.15 (5)	1.31±0.13 (5)	1.84±0.32 (5)
Change in concn. (mm)	+0.43±0.08 (8)	-0.80±0.14 (6)†††	-1.14±0.11 (7)†††	-0.14±0.48 (6)	-0.08±0.23 (7)	-2.37±0.64 (3)†††	-1.02±0.25 (5)	-0.42±0.19 (3)	+2.64±0.73 (3)††
Change in concn.(%)	+27±6 (8)	-48±7 (7)†††	-68±5 (7)†††	-1.68±7.38 (6)	-1.36±4.62 (7)	-58.2±11.3 (3)†††	-54.7±10.7 (5)	-15.80±4.65 (3)†	+143.3±39.4 (3)†††

ketone-body production by the remnant liver may also be a contributory factor.

An important aspect of the provision of metabolic fuels in the partially hepatectomized rat is the efficiency of gluconeogenesis in the remaining liver, since gluconeogenesis is usually coupled to fat oxidation (see, e.g., Tutwiler & Dellevigne, 1979). Whereas, as observed by others (Blackshear *et al.*, 1974), functional hepatectomy was associated with decreased blood glucose concentrations (Table 1), significant decreases were not observed in the partially hepatectomized rats (Table 1). Hepatic glycogen concentrations in the liver remnant removed at partial hepatectomy were 0.19 ± 0.05 (7)% wet wt., indicating that the maintenance of blood glucose was due to glucose synthesis. Even when the pre-operatively starved partially hepatectomized rats were subjected to a further 24 h of starvation, blood glucose concentrations were not markedly decreased compared with those in sham-operated rats [4.99 ± 0.19 (4) and 5.11 ± 0.14 (4) mM respectively; $P > 0.5$]. Others have observed increased activities of a number of gluconeogenic enzymes after partial hepatectomy (Katz, 1979; Curtin & Snell, 1983). Taken together, the results suggest that gluconeogenesis in the liver remnant is unimpaired or may even be increased, and in this context it is noteworthy that increases in blood lactate concentrations observed within 30 min of partial hepatectomy were relatively minor compared with the marked increases observed in the functionally hepatectomized group (Table 1).

Conclusions

Liver resection is associated with an inability either to respond to post-operative starvation with increased blood and liver ketone-body concentrations or to maintain high ketone-body concentrations produced by pre-operative starvation. This metabolic disturbance is likely to be a direct consequence of the decreased liver mass. It cannot be attributed to decreased availability of ketogenic precursors (NEFA), is not linked to DNA synthesis and/or liver cell division (being observed as early as 4 h after surgery), and is unlikely (as gluconeogenesis is unimpaired) to be secondary to decreased β -oxidation. In view of the importance of the ketone bodies as both substrates and regulators, the

findings have clear implications for fuel homeostasis in conditions where liver mass is decreased or liver function impaired.

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