Sequential changes in alanine metabolism following partial hepatectomy in the rat

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After partial hepatectomy, the liver undergoes an array of metabolic changes until regeneration is complete. Since carbons derived from alanine can be incorporated into most metabolic pools, we studied the metabolism of ^{14}C-labeled alanine during the early phase of regeneration. Sham operated (controls) and partially hepatectomized rats weighing about 200 g each were injected intraperitoneally with 1-[U-^{14}C]alanine at 9, 18, and 36 hours after surgery. The animals were killed 2 hours after injection. Compared to the controls, alanine oxidation was markedly depressed ($P < 0.05$) in the 9- and 18-hour groups, but was restored in the 36-hour group. The specific activity of plasma glucose and hepatic glycogen was elevated 9 and 18 hours after partial hepatectomy. There was a corresponding increase in the activities of fructose-1,6-diphosphatase and phosphoenolpyruvate carboxykinase. Hepatic protein specific activity increased by 30, 74, and 120%, respectively 9, 18, and 36 hours after partial hepatectomy. Hepatic fatty acids followed a similar pattern. In a separate set of experiments, the distribution of radioactivity in glutamic acid was measured. The results showed that alanine carbons enter the citric acid cycle primarily via the acetyl CoA pathway in the controls, but via the oxaloacetate pathway in partially hepatectomized rats. The results demonstrate significant changes in the activities of metabolic pathways of alanine in the early phase of hepatic regeneration.

Keywords: Alanine, metabolism, rats, partial hepatectomy

Introduction

Reading of the literature reveals that liver regeneration after partial hepatectomy is associated with profound changes in the activity of numerous metabolic pathways.[1-3] Briefly, following a short lag period of about 2 hours, the polyamine synthetic pathway is stimulated, as indicated by the ornithine decarboxylase activity which reaches a peak at 16 hours postoperatively.[4,5] The rate of RNA and DNA synthesis begins to increase about 14 hours after surgery, and reaches a peak at 24 hours.[6] At 18 hours, the activity of RNA polymerase I doubles, and the activity of RNA polymerase II increases by 50%.[7,8] Alterations in protein metabolism encompass both synthetic and degradative pathways.[9] A net increase in protein content is measurable by 12 hours following surgery, and the rate of protein synthesis is maximally stimulated during the ensuing 24 hours.[10] During the first 36 hours of liver regeneration, the average rate of protein degradation decreases to one-half the normal values.[11] The decrease is accompanied by a significant decrease in the activity of the urea cycle enzymes. The activity of all five enzymes decreases shortly after surgery and reaches minimal values about 4 hours later. At 24 hours the activity of the urea cycle enzymes returns to normal levels.[12] The synthesis rate of metallothionines increases after partial hepatectomy.[13] At 12 hours after partial hepatectomy, the level of ATP decreases, the levels of ADP and AMP markedly increase, the hepatic oxidation of lactate to pyruvate and the ratio of 3-hydroxybutyrate to acetoacetate increases.[14] These observations suggest that there is a decreased level of available chemical energy in the hepatocytes after partial hepatectomy. The production of ketone bodies

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is markedly decreased for up to 48 hours after partial hepatectomy.[15,16] Within the first 24 hours following surgery there is a twofold increase in liver fat content, an increase in the synthesis of phospholipids and cholesterol, and increased incorporation of fatty acids into triglycerides.[17] Liver glycogen is rapidly depleted 24 hours postoperatively, but returns to normal levels within 96 hours.[18] The normal level of blood glucose is maintained by glucose formed from several glucogenic substrates, including alanine,[19,20] lactate, and fructose[21] in the liver remnant and kidney cortex. Corresponding changes in the activity of several gluconeogenic enzymes in the remaining liver also have been found.[21-23]

Here we examined hepatic metabolism of ^{14}C-labeled alanine during the early phase of regeneration. The use of alanine allowed us to study the activity of multiple metabolic pathways since alanine carbons access most metabolic pools.[24] In addition, the activity of fructose-1,6-diphosphatase, phosphoenolpyruvate carboxykinase, acetyl CoA carboxylase, and fatty acid synthetase was determined.

Methods and materials

Male Sprague-Dawley rats ranging in weight from 180–200 g were used in all experiments. They were individually housed at 25° C in stainless steel wire cages and subjected to a 12 hour light/dark cycle. Rats were fed a complete semipurified diet for 10 days before and after experimentation.[25] Food was removed at midnight and the operation was consistently performed between 8:00 and 9:00 a.m. to minimize effects of diurnal rhythm. Partial hepatectomy (HX, comprising laparotomy, and removal of two-thirds of the liver) or sham operation (SO, laparotomy) was performed under medium depth anesthesia, using air-diethyl ether, according to a previously described procedure.[26] After the operation, the rats were returned to their cages with food and water available at all times. The rats were fully conscious within 10 minutes after the operation. During the recovery period and up to 3 hours post surgery, the rats were exposed to an infrared heating lamp to maintain a comfortable body temperature. Our preliminary observations had indicated that this procedure led to a faster recovery and, in particular, to a rapid resumption of food intake. Four experiments were conducted as follows:

Experiment 1—Thirty rats were divided into three treatment groups, each group consisting of five SO (controls) and five HX rats. Nine hours after surgery, each rat in group 1 was injected intraperitoneally with a saline solution of 1-[U-^{14}C]alanine (0.2 ml containing 2 uCi/100 g body weight, specific activity 10 mCi/mmole). The rats in group 2 and 3 were administered a similar dose of the solution 18 and 36 hours, respectively, after surgery. Immediately after the injection, each rat was placed in a glass metabolic cage and $^{14}CO_2$ was collected for 2 hours by drawing the expired air through a 2% aqueous solution of sodium hydroxide. Radioactivity in the solution was periodically determined as previously described.[27] At the end of the collection period the rats were decapitated and blood was collected in heparinized beakers. Plasma was separated by centrifugation and stored at −70° C until analyzed. Livers were quickly excised, weighed, and frozen in liquid nitrogen. Plasma glucose, hepatic glycogen, fatty acids, and proteins were isolated and radioactivity was determined by previously described procedures.[28,29]

Experiment 2—At 9, 18, and 36 hours after surgery, SO (n = 5) and HX rats (n = 5) were decapitated, and the livers excised and cooled in ice-cold saline. Ten percent (wt/vol) liver homogenates were prepared in 0.25 M sucrose solution using an all-glass homogenizer. An aliquot of the homogenates was centrifuged at 105,000 × g in a refrigerated ultracentrifuge for 30 minutes. Enzyme assays were performed at 31° C on the diluted homogenate or on a clear supernatant fluid. The following methods were applied to determine enzyme activities: fructose-1,6-diphosphatase (FDPase, EC 3.1.3.11), the method of Weber and Cantero[30]; phosphoenolpyruvate carboxykinase (PEPase, EC 4.1.132), the method of Nordlie and Lardy[31]; acetyl-CoA carboxylase (EC 6.4.1.2), the method of Maherus et al.[32]; fatty acid synthetase (EC 6.2.1.3), the method of Hsu et al.[33] Protein was determined by the method of Lowry et al.[34]

Experiment 3—A group of SO rats was divided into two subgroups. One subgroup was allowed to eat ad libitum and the second subgroup was pair-fed with HX rats. Nine, 18, and 36 hours postoperatively (n = 5), the activity of hepatic FDPase and PEPase was determined according to the procedures described in experiment 2.

Experiment 4—Nine, 18, and 36 hours postoperatively, 2 SO and 2 HX rats in each time group were injected intraperitoneally with a saline solution of 1-[2-^{14}C]alanine (0.2 ml containing 10 uCi/100 g body weight, specific activity 8 mCi/mmole). One hour later the rats were decapitated, livers were quickly excised and cooled in ice-cold saline. Hepatic protein was isolated by the previously described methods.[35] The protein was hydrolyzed for 24 hours with 10 volumes of 6 N HCl in the presence of carrier quantities of glutamic acid. The hydrolysate was decolorized with charcoal, filtered and concentrated under reduced pressure at 50° C to a small volume. The solution was passed through a column of Amberlite IR-45 resin. Glutamic and aspartic acids were absorbed by the resin, whereas all other amino acids passed through the column. The two acidic amino acids were eluted from the resin with dilute HCl. Glutamic acid hydrochloride was crystallized from a solution of concentrated HCl. Glutamic acid was obtained by neutralization with pyridine and precipitated with ethanol. The glutamic acid was degraded by the procedure of Mosbach et al.,[36] as modified by Koeppe and Hill.[37] The $^{14}CO_2$ liberated during the degradation was absorbed in Hyamine® and radioactivity was determined in a Packard liquid scintillation spectrometer (model 460; Packard Instruments, Des Plaines, IL). For total ra-

Table 1 Effect of partial hepatectomy on changes in body and liver weights, food intake and alanine conversion into glucose, glycogen, fatty acids and proteins.

	Hours after operation					
	9		18		36	
	SO	HX	SO	HX	SO	HX
Change in body weight, g	−3.8 ± 0.6	−8.7 ± 0.9[a]	−1.3 ± 0.3	−5.1 ± 0.4[a]	4.9 ± 0.4	−1.2 ± 0.3[a]
Food intake, g	6.1 ± 0.3	1.9 ± 0.4[a]	8.3 ± 0.7	3.4 ± 0.5[a]	16.6 ± 0.4	14.1 ± 0.3
			Plasma			
Glucose, mg/dl	107 ± 6	90 ± 4[a]	109 ± 5	105 ± 7	102 ± 7	108 ± 3
Glucose, DPM/mg	612 ± 36	1354 ± 70[a]	573 ± 48	810 ± 56[a]	620 ± 68	690 ± 91
			Liver			
Weight, g	7.92 ± 0.14	2.31 ± 0.12[a]	8.10 ± 0.18	3.1 ± 0.10[a]	8.42 ± 0.36	3.78 ± 0.20[a]
Glycogen, mg/g	39.8 ± 1.3	3.4 ± 0.3[a]	37.6 ± 0.9	6.1 ± 0.7[a]	52.3 ± 2.6	10.3 ± 0.9[a]
Glycogen, DPM/mg	27 ± 4	148 ± 10[a]	33 ± 5	89 ± 14[a]	38 ± 6	48 ± 10
Fatty acids, mg/g	41.2 ± 1.3	59.8 ± 2.4[a]	43.2 ± 2.7	74.9 ± 3.0[a]	39.7 ± 1.9	98.6 ± 3.6[a]
Fatty acids, DPM/mg	37 ± 7	121 ± 10[a]	39 ± 4	115 ± 8[a]	33 ± 6	101 ± 5[a]
Protein, mg/g	141 ± 10	147 ± 12	130 ± 8	142 ± 15	135 ± 9	159 ± 11
Protein, DPM/mg	98 ± 6	136 ± 8[a]	110 ± 5	209 ± 8[a]	104 ± 10	298 ± 12[a]

Values are mean ± SEM, $n = 5$.
SO = sham operated.
HX = partially hepatectomized.
[a] Indicates significant difference from SO values, same time interval. $P < 0.05$.

dioactivity, a sample of the purified glutamic acid was combusted in a Packard tissue oxidizer (model 306; Packard Instruments) and radioactivity was determined as above.

All data were evaluated by the analysis of variance. Differences at $P < 0.05$ were considered significant. Differences between means were further evaluated by the Newman-Keuls test.[38]

Results

As shown in Table 1, both the SO and HX rats lost weight at 9 hours after surgery. At 18 hours neither group had regained the original body weight. However, at 36 hours the SO rats exceeded and the HX rats approached the initial body weight. At 9 and 18 hours the HX rats consumed less food than the SO rats. At 36 hours no difference in food intake between the two groups was observed.

At 9 hours the plasma level of glucose was slightly lower in the HX rats than in the SO group. At 18 and 36 hours there was no difference in the level of plasma glucose between the two groups. At 9 and 18 hours the specific activity of plasma glucose was twice as high in the HX group as in the SO group. At 36 hours no difference in glucose specific activity between the two groups was observed.

The regenerating liver rapidly increased its mass. Compared to the 9 hour group, the liver mass increased by 34 and 64%, respectively, at 18 and 36 hours.

The glycogen content decreased markedly at 9 hours after partial hepatectomy. However, at 18 and 36 hours glycogen levels increased slightly, but remained below the level of the SO rats. The specific activity of glycogen (DPM/mg) at 9 and 18 hours was about five and three times, respectively, higher than in the SO group. In contrast, the specific activity of glycogen at 36 hours was similar to that of the SO group.

The concentration of fatty acids in the 9-hr HX group was significantly higher than in the SO group. A further increase was observed at 18 and 36 hours. The specific activity of fatty acids (DPM/mg) was about three times higher in the HX groups than in the SO group.

At 9 hours the protein specific activity (DPM/mg) was increased by almost 30% over the controls, and by about 74 and 117%, at 18 and 36 hours, respectively.

Compared to the SO group, oxidation of alanine was markedly decreased at 9 and 18 hours (Table 2). Fifteen minutes after alanine administration, the 9 and 18-hour rats respectively expired about 64 and 55% less radioactivity than the corresponding SO rats. This effect persisted throughout the entire 2-hour collection period. In contrast, the rate of alanine oxidation in the 36-hour group was similar to that of the SO rats.

Compared to the SO group, there was a 3-fold increase in the activity of hepatic FDPase at 9 hrs, and about a two-fold increase at 18 and 36 hours (Table 3). The activity of PEPase followed a similar pattern. Partial hepatectomy had no effect on the activity of acetyl CoA or fatty acid synthetase. Enhanced activity of FDPase and PEPase was not due to decreased food intake observed after surgery, since there was no difference in the activities of the two enzymes between the ad lib and pair-fed SO rats (Table 4).

The effect of partial hepatectomy on the labeling pattern of glutamic acid is presented in Table 5. Compared to the SO group, hepatectomy had essentially

Table 2 Effect of partial hepatectomy on alanine oxidation.

Minute after injection	Hours after operation					
	9		18		36	
	SO	HX	SO	HX	SO	HX
15	1,545 ± 90	547 ± 64[a]	1,335 ± 87	734 ± 87[a]	1,492 ± 98	1,340 ± 116
30	2,884 ± 120	1,269 ± 85[a]	2,724 ± 98	1,521 ± 119[a]	3,076 ± 210	2,910 ± 170
60	4,717 ± 140	2,310 ± 93[a]	4,639 ± 165	2,530 ± 148[a]	4,320 ± 185	3,840 ± 230
120	5,638 ± 230	3,194 ± 160[a]	5,827 ± 260	3,912 ± 320[a]	5,480 ± 250	5,230 ± 190

SO = sham operated.
HX = partially hepatectomized.
Values are means ± SEM DPMs expired, $n = 5$.
[a] Indicates significant difference from SO values, same time interval, $P < 0.05$.

Table 3 Effect of partial hepatectomy on the activity of key gluconeogenic and lipogenic enzymes.

Enzyme	9[a]		18[a]		36[a]	
	SO	HX	SO	HX	SO	HX
Fructose-1,6-diphosphatase	47 ± 8	127 ± 10[b]	41 ± 5	103 ± 14[b]	42 ± 7	99 ± 12[b]
Phosphoenolpyruvate carboxykinase	37 ± 9	108 ± 12[b]	48 ± 12	94 ± 8[b]	39 ± 10	87 ± 14[b]
Fatty acid synthetase	21 ± 4	24 ± 6	20 ± 7	28 ± 5	25 ± 3	28 ± 4
Acetyl CoA carboxylase	8 ± 1.2	12 ± 0.9	10 ± 0.6	8 ± 0.9	11 ± 2	9 ± 1.5

SO = sham operated.
HX = partially hepatectomized.
Values are means ± SEM nanomoles substrate/min/mg protein, $n = 6$.
[a] Hours after operation.
[b] Indicates significant difference from SO values, same time interval, $P < 0.05$.

Table 4 Effect of partial hepatectomy and food intake on the activity of fructose-1,6-diphosphatase and phosphoenolpyruvate carboxykinase.

Hours		SO		HX
		Ad lib	Pair-fed	Ad lib
9	Food intake, g	7.1 ± 0.4	2.3[a]	2.3 ± 0.2[a]
	Fructose-1,6-diphosphatase	53 ± 7	59 ± 4	116 ± 14[a]
	Phosphoenolpyruvate carboxykinase	42 ± 7	35 ± 6	101 ± 18[a]
18	Food intake, g	9.4 ± 0.8	3.9[a]	3.9 ± 0.5[a]
	Fructose-1,6-diphosphatase	47 ± 8	51 ± 6	104 ± 12[a]
	Phosphoenolpyruvate carboxykinase	46 ± 5	53 ± 8	103 ± 15[a]
36	Food intake, g	15.4 ± 0.8	14.8	14.8 ± 1.2
	Fructose-1,6-diphosphatase	51 ± 8	56 ± 4	97 ± 14[a]
	Phosphoenolpyruvate carboxykinase	38 ± 5	42 ± 3	91 ± 11[a]

SO = sham operated.
HX = partially hepatectomized.
Enzyme activity = nanomoles substrate/min/mg protein ± SEM, $n = 5$.
[a] Indicates significant difference from ad lib-fed group, same time interval, $P < 0.05$.

Table 5 Effect of partial hepatectomy on the labeling pattern of hepatic glutamic acid.

Hours	Treatment	Rat	% Total radioactivity[a]					
			C-1	C-2	C-3	C-4	C-5	Total
9	SO	1	12.6	16.4	39.5	2.1	25.3	95.9
		2	14.8	15.1	37.7	3.8	24.2	95.6
	HX	3	15.3	27.3	44.1	3.4	6.8	96.9
		4	16.7	24.9	45.8	2.6	7.9	97.9
18	SO	5	11.3	18.7	40.3	2.4	24.1	96.8
		6	10.1	13.9	42.1	3.5	26.3	95.9
	HX	7	14.9	25.1	52.6	2.5	4.7	99.8
		8	10.3	27.9	51.3	3.4	5.3	98.2
36	SO	9	11.9	15.8	41.2	2.8	23.5	95.2
		10	12.4	16.8	42.0	2.9	24.5	98.6
	HX	11	10.3	26.2	52.6	3.8	5.4	98.3
		12	12.8	24.8	51.9	3.3	4.9	97.7

SO = sham operated.
HX = partially hepatectomized.
[a] Individual values from 2 rats/treatment group.

no effect on the distribution of radioactivity in C-1 and C-4 of glutamic acid. However, a striking drop of radioactivity in C-5, and a concomitant increase of radioactivity in C-2 and C-3 were observed at 9, 18, and 36 hours after operation.

Discussion

Hepatic regeneration after partial hepatectomy is associated with dynamic changes in the activity of several major metabolic pathways of alanine. Prominent among these are a markedly increased incorporation of carbons from alanine into plasma glucose, hepatic glycogen, fatty acids, and enhanced incorporation of alanine into hepatic proteins. In contrast, alanine oxidation is drastically reduced for at least 18 hours after surgery.

The initial step in the synthesis of glucose from alanine involves transamination of the amino acid to form pyruvate which can enter the tricarboxylic acid (TCA) cycle via acetyl-CoA or oxalacetate. The labeling pattern of glutamic acid indicates the pathway by which labeled pyruvate enters the TCA cycle.[24,39] High activity in C-5 relative to C-2 and C-3 suggests the predominance of the acetyl-CoA pathway. The reverse situation implies an increased flow of carbons via the oxalacetic pathway.[39] Our study demonstrates that HX rats metabolize pyruvate primarily via the oxalacetate pathway, whereas SO rats preferentially utilize the acetyl-CoA pathway. The shift from the acetyl-CoA pathway to the oxalacetic pathway enables the regenerating liver to form glucose and nonessential amino acids which can be used for the synthesis of new proteins or other metabolites. Enhanced glucose formation is evidenced by the high specific activities of plasma glucose and hepatic glycogen, and by elevated activities of FDPase and PEPase. The enhanced gluconeogenesis observed after partial hepatectomy is not due to an inadequate food intake. The process is apparently stimulated by the synergistic action of several hormones. Corticosterone production has been shown to peak at 4 hours after partial hepatectomy[40] and peaks in cyclic AMP have been observed at 2 and 12 hours.[41] Glucagon levels increase about eleven-fold 6 hours after operation, while the levels of growth hormone and thyroxine sharply decrease, and the level of insulin remains unchanged.[42,43] Among the hormones, glucagon enhances the activity of FDPase and PEPase.[44,45] The increased rate of hepatic fatty acid synthesis observed in HX rats may also be related to enhanced citrate formation via the condensation of oxaloacetate with acetyl-CoA. The citrate can diffuse into the cytosol, the site of fatty acid formation, where it can be cleaved to form acetyl-CoA once again. Thus, we propose that increased availability of cytosolic acetyl-CoA enhances fatty acid synthesis during liver regeneration. Diversion of acetyl-CoA into the fatty acid pathway will then reduce the amount entering the ketogenic pathway. Indeed, reduced ketogenesis after partial hepatectomy has been reported.[46] In addition, it has been suggested that an increase in the rate of NADPH production by increased cycling of pyruvate via NAD-malate dehydrogenase and malic enzyme may explain high rates of lipid synthesis in regenerating liver.[47] High rates of lipogenesis in liver remnants determined by ^3H incorporation from ^3H_2O into fatty acids have been previously reported.[48,49]

It has been well established that an increase in protein content in regenerating liver is due to enhanced protein synthesis and a markedly decreased rate in protein degradation.[11,12,50] There are many difficulties inherent in the interpretation of protein synthesis from a labelled amino acid in a metabolically active single tissue. These difficulties are magnified enormously when an attempt is made to interpret a similar set of data from in vivo studies. During the 36-hour experimental period, a variety of compensatory changes may have taken place which could have complicated the intepretation of the observed changes. Such compensatory changes may include the pool size of free ala-

nine, its specific activity, and alanine turnover. Our data demonstrate that enhanced protein synthesis during the early phase of liver regeneration is accompanied by decreased rates of alanine oxidation. The contribution of individual organs or tissues to the overall $^{14}CO_2$production is unknown. In addition, the recovery of total radioactivity may have been affected by the rate of ventilation, the acid-base status of the tissues and other metabolic factors. A decrease in the oxidation of phenylalanine and threonine in partially hepatectomized rats has been reported.[51] It appears that a reduction in amino acid oxidation during the proliferation phase of liver regeneration is a mechanism by which the organism preserves the pool of free-amino acids for protein synthesis. This may be in response to the diminished supply of dietary amino acids during the first 24 hours after surgery. Available evidence indicates that hepatic protein synthesis is affected by the amino acid supply.[52,53] Indeed, a correlation has been reported between hepatic levels of alanine and rates of protein synthesis.[54] The mechanisms that regulate the alanine production and/or utilization may, therefore, indirectly regulate protein synthesis in regenerating liver.

In summary, our results provide evidence for significant changes in the activities of alanine metabolic pathways in the early stages of liver regeneration. The mechanism by which this is achieved is apparently related to the specific metabolic requirements of the organism.

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