Gluconeogenesis in Liver Slices from Partially Hepatectomized Rats[1] (35406)

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The biochemical changes in the liver remaining after partial hepatectomy have been extensively studied (1–3). However, the capacity of the remaining tissue to synthesize glucose does not appear to have been evaluated. In the present work, partially hepatectomized rats and sham-operated controls were compared during a 24-hr period with respect to blood glucose concentration, gluconeogenesis in liver slices, and activities of two key enzymes involved in glucose synthesis, glucose-6-phosphatase and fructose-1,6-diphosphatase. Incorporation of L-alanine-^{14}C and bicarbonate-^{14}C into the glucose produced by liver slices and net glucose production from alanine were used to estimate gluconeogenesis.

Materials and Methods. Male Wistar rats weighing 180–230 g were used. They were supplied a standard diet and tap water *ad libitum* until surgery. Partial hepatectomy was carried out under ether anesthesia as described by Higgins and Anderson (4). About 70% of the liver was removed. After surgery the animals were kept without food but were given access to water. Rats submitted to sham-operation and kept under the same conditions were utilized as controls. The animals were decapitated 6, 14, and 24 hr after surgery. Blood was collected for glucose determinations by the method of King and Garner (5). Liver for measurements of gluconeogenesis and enzymes activities was removed rapidly and cooled in ice.

Gluconeogenesis. Liver slices were prepared in the cold by use of a Stadie-Riggs type apparatus. About 300 mg of tissue were placed in 3 ml of Krebs–Henseleit bicarbonate buffer, pH 7.4, containing 30 μmoles of L-alanine-U-^{14}C or 72 μmoles of sodium bicarbonate-^{14}C. The sp act of the alanine and bicarbonate were 50 and 20 μCi/mole, respectively. In the experiments with bicarbonate, the buffer also contained 90 μmoles of glycerol, 120 μmoles of pyruvate, and 60 μmoles of acetate. The tissue preparations were incubated 3 hr with shaking under 5% CO_2 in O_2. At the end of incubation, the glucose in the medium was converted into pentacetate as described by Jones (6). The ^{14}C in the glucose pentacetate was estimated in a liquid scintillation counter (Tricarb, Packard). Glycogen was isolated from the tissue as described by Abraham and Hassid (7). The ^{14}C in the glycogen was determined in a low-background gas flow counter (Nuclear, Chicago). For estimation of net glucose production, two 500-mg samples of liver slices were placed in 5 ml of Krebs–Henseleit bicarbonate buffer containing 10 μmoles of L-alanine. One sample was immediately removed for initial glycogen determination by the method of Good *et al.* (8), the other was incubated 2 hr. At the end of the incubation, the tissue was assayed for glycogen and the incubation medium for glucose by the method of Somogyi–Nelson (9).

Glucose-6-phosphatase and fructose-1,6-diphosphatase. For determination of enzyme activities, the liver was homogenized in ice-cold 0.25 *M* sucrose (1:3 w/s) with a Teflon pestle homogenizer. The homogenate was centrifuged for 10 min at 10,000*g* at 0°. Portions of the supernatant liquid containing 2–4 mg of protein were assayed for the glucose-6-phosphatase and fructose-1,6-diphosphatase as described by Fitch *et al.* (10) and Weber and Cantero (11), respectively, with the exception of the P_i (inorganic

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phosphate) determination, which was carried out according to the procedure of Lohman and Jendrassik (12). The protein content of the supernatant liquid was determined by the method of Lowry *et al.* (13).

The Student's *t* test was used to determine the significance of the difference between means.

Results. The means of the blood glucose levels and the standard errors of the means for 6 sham-operated animals at 6, 14, and 24 hr after surgery were 90 ± 4.0, 80.5 ± 4.5, and 75 ± 3.0 mg of glucose/100 ml of blood, respectively. The corresponding values for 6 partially hepatectomized rats were 73 ± 3.7, 76 ± 5.5, and 72 ± 3.5. The means for the control and experimental animals differ significantly only at 6 hr ($p<0.05$).

Table I summarizes the data on the incorporation of alanine-^{14}C and of bicarbonate-^{14}C into the glycogen of the liver slices and into the glucose produced by the slices and transferred to the medium. In both experimental groups, the incorporation of labeled carbon from both precursors into liver glycogen was very small compared to the recovery of ^{14}C from the medium glucose, indicating that the hexose synthesized by the slices was rapidly transferred to the medium. At all time intervals, the rate of glucose synthesis in the liver remaining after partial hepatectomy was higher than that in the control tissue, as shown by the increased incorporation of label from both alanine and bicarbonate into the medium hexose. The experimental and control groups did not differ significantly with respect to incorporation of ^{14}C from both substrates into liver glycogen except at 6 hr, when a higher recovery of glycogen-^{14}C was obtained in the liver slices from partially hepatectomized rats incubated with alanine.

The results of measurements of liver glucose-6-phosphatase and fructose-1,6-diphosphatase activities are given in Table II. When expressed as units of P_i liberated from substrate per milligram of protein with liver extract the activities of both enzymes in the remnant liver were not significantly different from the activities for the sham-operated animals. When expressed as activity per gram of liver, the levels of the two

TABLE I. Gluconeogenesis in Liver Slices Obtained from Partially Hepatectomized and Sham-Operated Rats at Several Time Intervals After Surgery.[a]

After surgery (hr)	Alanine-^{14}C incorporation (μmole/100 g of wet wt/hr)				$H^{14}CO_3$ incorporation (μmole/g of wet wt/hr)			
	Medium glucose		Tissue glycogen		Medium glucose		Tissue glycogen	
	Partially hepatecto.	Sham-operated	Partially hepatecto.	Sham-operated	Partially hepatecto.	Sham-operated	Partially hepatecto.	Sham-operated
6	341 ± 12[b] (8)	282 ± 12 (8)	20 ± 0.6[b] (12)	6.0 ± 0.2 (12)	118 ± 16[b] (6)	61 ± 2 (6)	2.3 ± 0.5 (12)	2.4 ± 0.2 (11)
14	377 ± 52[b] (8)	196 ± 19 (9)	4.6 ± 0.1 (8)	3.3 ± 0.1 (9)	134 ± 35[b] (6)	55 ± 6 (5)	1.6 ± 0.5 (8)	0.95 ± 0.02 (8)
24	507 ± 27[b] (8)	322 ± 16 (8)	4.5 ± 0.3 (12)	5.3 ± 0.1 (11)	181 ± 22[b] (5)	74 ± 4 (6)	2.4 ± 0.1 (6)	2.2 ± 0.4 (6)

[a] Values are means and SE with no. of animals in parentheses.
[b] Different from sham-operated control values at $p < 0.05$.

TABLE II. Glucose-6-phosphatase and Fructose-1,6-diphosphatase in the Liver of Rats at Several Time Intervals After Partial Hepatectomy or Sham-Operation.[a]

After surgery (hr)	Glucose-6-phosphatase				Fructose-1,6-diphosphatase			
	(μmole of P_i/mg of protein[b]/hr)		(μmole of P_i/g of wet liver/hr)		(μmole of P_i/mg of protein/hr)		(μmole of P_i/g of wet liver/hr)	
	Partially hepatecto.	Sham-operated	Partially hepatecto.	Sham-operated	Partially hepatecto.	Sham-operated	Partially hepatecto.	Sham-operated
6	4.15 ± 0.38 (7)	3.73 ± 0.38 (7)	438 ± 33	491 ± 36	7.56 ± 0.54 (7)	6.91 ± 0.33 (7)	777 ± 68[c]	920 ± 44
14	3.17 ± 0.2 (12)	3.53 ± 0.1 (12)	348 ± 18[c]	556 ± 18	8.33 ± 0.59 (6)	7.68 ± 0.71 (6)	988 ± 38	1078 ± 53
24	3.7 ± 0.25 (12)	3.73 ± 0.15 (12)	338 ± 43[c]	469 ± 35	6.12 ± 0.13 (6)	5.71 ± 0.3 (6)	676 ± 29[c]	779 ± 31

[a] Values are means and SE with no. of animals in parentheses.
[b] Protein in liver extract tested for enzyme activities.
[c] Different from sham-operated control values at $p < 0.05$.

enzymes were lower in the partially hepatectomized rats, except for glucose-6-phosphatase activity at 6 hr and fructose-1,6-diphosphatase activity at 14 hr. This tendency toward lower enzyme levels, when activities are calculated on a wet weight basis, probably reflects the higher water and fat content of the residual tissue (14).

Net glucose production from alanine by liver slices obtained 24 hr after the operation is given in Table III. In liver slices from partially hepatectomized rats, the extremely low levels of glycogen did not change during incubation. Therefore the very uniform amount of hexose found in the incubation medium represents only newly synthesized glucose. Net glucose production from alanine was significantly higher in slices from partially hepatectomized rats.

Discussion. The results obtained show that the liver remaining in rats subjected to the removal of two-thirds of the organ exhibits, per unit weight, an increased rate of gluconeogenesis, which can be detected as early as 6 hr after the operation. This increase apparently accounts for the ability of the partially hepatectomized animals to maintain essentially normal blood sugar levels despite complete anorexia and the large reduction in hepatic tissue. During the first few hours after the surgery, *i.e.*, before gluconeogenesis increases, the breakdown of preformed reserves of liver glycogen is presumably essential for the prevention of hypoglycemia. This period is particularly critical for partially hepatectomized rats because their liver glycogen reserves are reduced to one-third of those in control animals. This seems to be the main reason why the blood sugar levels are lower at 6 hr after the operation than later, though at this time gluconeogenesis is already more active.

In the present experiments, the increase in gluconeogenesis in the remaining hepatic tissue was not accompanied by increases in the concentration of glucose-6-phosphatase or fructose-1,6-diphosphatase, as judged by the activities of both enzymes in liver homogenates. Since the higher gluconeogenesis rate which appears under conditions of hormonal imbalance, such as excess glucocorticoids

TABLE III. Glucose Production by Rat Liver Slices Obtained 24 hr After Partial Hepatectomy or Sham-Operation.

Group	Net change in glycogen (μmole of glucose/g of wet wt/2 hr)	Medium glucose (μmole/g of wet wt/2 hr)	Net glucose production[b] (μmole/g of wet wt/2 hr)
Sham-operated	−4.4 ± 1.0	17.0 ± 1.3	12.6 ± 0.5
Partially hepatectomized	0	17.6 ± 0.1	17.6 ± 0.1[c]

[a] Values are means ± SE of 6 animals.
[b] Calculated as: medium glucose—net change in glycogen (initial glycogen—final glycogen).
[c] Significantly different from control ($p < 0.01$).

and insulin deficit, are characteristically accompanied by enhanced glucose-6-phosphatase and fructose-1,6-diphosphatase activities (15, 16), it seems that such factors play no essential role in the development of increased gluconeogenesis after partial hepatectomy.

The increased rate of CO_2 incorporation into glucose exhibited by the residual liver of the partially hepatectomized rats conceivably indicates changes in the activities of the two enzymes involved in the conversion of pyruvate to phosphoenolpyruvate. The activity of the enzymes, phosphoenolypyruvate carboxykinase, has been reported to increase in the developing rat liver (17) and in the liver of cold-acclimated rats (18). The increases are accompanied by enhanced gluconeogenesis and little or no change of the levels of glucose-6-phosphatase or fructose-1, 6-diphosphatase.

Removal of two/thirds of the liver induces circulatory changes which lead to an increase in the blood flow of the remaining tissue (19). Since an elevated blood flow conceivably could increase the concentration of gluconeogenic substrates within the cell and thus stimulate glucose synthesis, it is possible that an increased blood flow contributed to the enhanced gluconeogenesis observed in the partially hepatectomized rats.

Another factor which may account for the present results is the increase in lipid content of the residual liver that occurs during the first 24 hr after partial hepatectomy (2). The increase in lipids is attributable to a higher rate of the FFA (free fatty acids) delivery to the remaining liver (20). Since an elevation of the FFA level stimulates hepatic glucose production by liver slices (21), perfused rat liver (22–24), and rat liver *in vivo* (25), the increased gluconeogenesis observed after partial hepatectomy could be due, at least in part, to an increased supply in FFA.

The present study does not provide direct information about the agent responsible for the increased gluconeogenesis exhibited by the residual liver. However, it appears likely that two factors are involved: (a) increased hepatic blood flow, and (b) a greater influx of FFA into the remaining liver.

Summary. During the 24-hr period after 70% hepatectomy, rats kept without food were found to maintain blood glucose levels comparable to those of sham-operated controls. As indicated by measurements of L-alanine-^{14}C and $H^{14}CO_3$ incorporation into glucose and net glucose production, the rate of gluconeogenesis in liver slices was higher for the partially hepatectomized rats than for sham-operated controls. The enhanced gluconeogenesis exhibited by the residual tissue from the hepatectomized animals was not accompanied by increase in the activity of either glucose-6-phosphatase and fructose-1, 6-diphosphatase.

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