

Liver Protein Synthesis after Partial Hepatectomy and Acute Stress*

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SUMMARY

Partial hepatectomy and acute stress (intraperitoneal injection of Celite) cause increases in the rates of synthesis of serum albumin and fibrinogen, proteins that are formed mainly or entirely by the liver. The rises in protein synthesis after removal of part of the liver differ kinetically from those after acute stress. In addition, only the response to partial hepatectomy is independent of the adrenal glands. Both responses seem to be related to an increase in the ability of liver polyribosomes to form protein.

It has been known that liver microsomal (1-4) and polyribosomal (5) preparations from partially hepatectomized rats incorporate amino acids into protein at an increased rate. Meaningful studies *in vivo* have, however, not been reported. A major obstacle to work with the intact animal stems from the kinetics of the incorporation of a labeled amino acid into liver protein. The rate of incorporation has been found to be linear with time for about 1 min¹ to 3 min (6) after the intravenous injection of a radioactive amino acid. Especially because of the time required to remove the liver samples, such kinetics make comparative studies difficult.

The effect of excision of part of the liver on hepatic protein synthesis *in vivo* has now been studied by measuring the formation of serum albumin and fibrinogen. These proteins are made largely or entirely by liver (7-10), and the rates of their syntheses can be measured with some accuracy.

Partial hepatectomy was found to cause rises in the rates of albumin and fibrinogen synthesis. However, any acute stress, including surgery that did not involve the liver, also enhanced the formation of these proteins. The question arose, therefore, whether the increased rates of albumin and fibrinogen formation after partial hepatectomy resulted simply from the stress of the operation.

The major purpose of this report is to describe some of the properties of the rises in the rates of albumin and fibrinogen synthesis after partial hepatectomy and acute stress. The etiologies of

the responses are shown to be different, the response to partial hepatectomy being independent of the adrenal glands, whereas the response to acute stress is completely adrenal-dependent. Evidence is also presented to show that both responses are related to an increased efficiency of the liver polyribosomes.

EXPERIMENTAL PROCEDURE

Materials—Male albino rats, obtained locally, received food and water *ad libitum* except as indicated and were used when they weighed 80 to 100 g. ¹⁴C-L-Leucine (uniformly labeled, 230 μC per μmole) was from New England Nuclear, Celite was a product of Johns-Manville, New York, New York, and hydrocortisone sodium succinate (Solu-Cortef) was from The Upjohn Company, Kalamazoo, Michigan.

Methods—Partial hepatectomy refers to the removal of about 70% of the liver (left lateral and median lobes) (11). After bilateral adrenalectomy, drinking water was replaced with 0.15 M NaCl. Ligation of the branch of the hepatic portal vein to the 70% part of the liver was as described by Steiner and Martinez (12).

To measure the rates of albumin and fibrinogen synthesis, 1 μC (1 ml) of ¹⁴C-leucine was injected into the tail vein, and cardiac blood samples (about 2 ml) were removed 1 hour later. Clotting was prevented by the addition of 0.1 ml of heparin (1000 units per ml, Upjohn), and plasma was prepared by centrifugation.

The isolation of albumin depended upon its solubility in mixtures of trichloroacetic acid and ethanol (13-15). To 0.2 ml of plasma, 9.8 ml of 1% trichloroacetic acid in 95% ethanol were added. After 10 min at room temperature, insoluble material was removed by centrifugation, and albumin was precipitated from the supernatant solution by the addition of 1 ml of 5% phosphotungstic acid in 2 M HCl. The precipitate, allowed to form for 10 min at room temperature, was collected by centrifugation and resuspended in 4 ml of 5% trichloroacetic acid containing Celite (20 mg per ml). The suspension was transferred to a filter disc of Whatman No. 1 filter paper (2.5 cm in diameter) which had previously been coated with a thin layer of Celite. After washes with 30 ml of 5% trichloroacetic acid followed by 10 ml of chloroform-methanol (1:2), the samples were air dried. The dried disc was then suspended in 0.5 ml of 1 M Hyamine in methanol, and 10 ml of a phosphor solution (0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis[2-(5-phenyloxazolyl)]benzene in toluene) were added. Radioassay was in a liquid scintillation spectrometer.

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¹ K. Tsukada and I. Lieberman, unpublished observations.

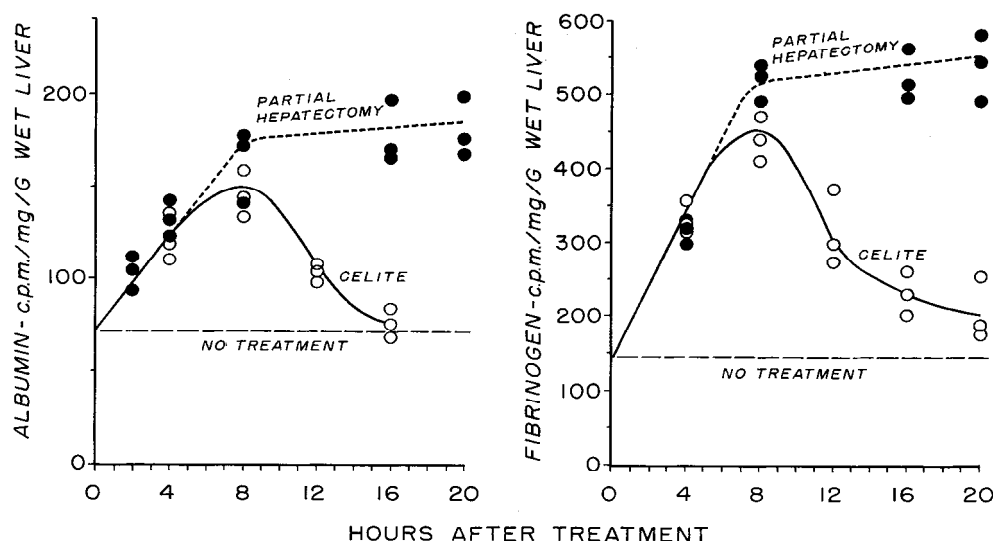


FIG. 1. Rates of synthesis of albumin and fibrinogen as a function of time after partial hepatectomy and acute stress (Celite). Partial hepatectomy and the injection of Celite (intraperitoneally, 5 mg/100 g of body weight) were at zero time. At the times indicated, ^{14}C -leucine (1 μC) was injected intravenously, and

To determine the protein content of the albumin sample, duplicate portions of plasma were processed exactly as for the estimation of radioactivity. After the wash with chloroform-methanol, the dried disc was suspended in 3 ml of NaOH (0.5 M), and protein was estimated on the soluble fraction by the method of Lowry *et al.* (16). The recovery of albumin was 15 to 18 mg per ml of plasma.

Fibrinogen was isolated as fibrin, essentially as described by Miller *et al.* (10). To 0.25 ml of plasma in a glass homogenizer were added, in order, 4.75 ml of NaCl (0.15 M), 0.25 ml of protamine sulfate (2.5%), 0.1 ml of CaCl_2 (1 M), and 0.5 ml of thrombin (100 NIH units (17) per ml, Parke, Davis and Company, Detroit, Michigan). After 30 min at room temperature, the fibrin was dispersed by homogenization and 4 ml of Celite in water (20 mg per ml) were added. The suspension was now transferred to a filter disc and washed exactly as for albumin, except that ethanol was substituted for the mixture of chloroform-methanol. The radioactivity of the fibrin was measured as for albumin, and protein determinations were made on duplicate samples that had been dissolved in 0.5 M NaOH (2 ml). The recovery of fibrin was 2 to 3 mg per ml of plasma.

The microsomal fraction was prepared from liver homogenized in 2 volumes of Medium A (18). The homogenates were centrifuged for 20 min ($10,000 \times g$), and the microsomes were then sedimented from the supernatant fluid at $105,000 \times g$ (1 hour).

Liver polyribosomes were isolated without detergent (19) from rats that had been fasted overnight.

The rates of amino acid incorporation *in vitro* were measured in reaction mixtures (0.5 ml) that contained 40 mM Tris-HCl (pH 7.8), 3 mM MgCl_2 , 0.4 mM GTP, 1 mM ATP, 10 mM sodium phosphoenolpyruvate, 0.01 mg of crystalline pyruvate kinase, 2 mM mercaptoethanol, a mixture of 19 amino acids (each 0.3 mM), 0.04 mM ^{14}C -L-leucine (5 mC per mmole), $105,000 \times g$ supernatant fluid (about 1 mg of protein) from a normal rat liver, and the microsome or polyribosome preparation. After 10 min at 37° , 5% trichloroacetic acid containing 0.2% carrier leucine was added. After extensive washes with trichloroacetic

acid, ethanol, and ether, the samples were counted in a Packard Tri-Carb liquid scintillation spectrometer. Under the conditions of the assay, the rate of amino acid incorporation was linear with time and proportional to the amount of ribosomal preparation used.

RESULTS

Rates of Synthesis in Vivo of Albumin and Fibrinogen as Function of Time after Partial Hepatectomy and Acute Stress (Celite)—Partial hepatectomy caused gradual rises in the rates of albumin and fibrinogen synthesis² for about 8 hours after the operation, when the rates become constant at the elevated levels (Fig. 1). As the figure shows, similar rises occurred after the intraperitoneal injection of Celite (5 mg/100 g of body weight), but after 8 hours the rates declined to normal.

Not shown in the figure are the observations that were made when acute stress was induced by laparotomy (8 cm incision)³ or by the intramuscular injection of turpentine (0.1 ml/100 g of body weight). The results were the same as those with Celite. Ether anesthesia, on the other hand, even for extended periods of time (30 min), had no effect on albumin and fibrinogen synthesis as measured after 8 hours.

Rates of Appearance of Labeled Albumin and Fibrinogen in Blood—As shown by Peters (6), the injection of a labeled amino acid is not followed immediately by the appearance of radioactive albumin in the serum. Rather, he found a lag of 10 to 15 min before the tagged protein began to appear in the blood. At the end of the lag period, the counts in circulating albumin were seen to rise sharply to approach a plateau at about 35 min after the injection.

² The possibility was considered that the increased incorporation of radioactivity into albumin and fibrinogen reflected a decrease in the pool sizes of free leucine in blood serum or liver. No pool size changes, however, follow partial hepatectomy or acute stress (Reference 20; also, K. Okazaki and I. Lieberman, unpublished observations).

³ Smaller incisions (1 to 2 cm) yielded little or no increase in the rates of formation of albumin and fibrinogen.

These observations have been confirmed for albumin, and have been shown to apply as well to fibrinogen (Fig. 2). The mechanism responsible for the gradual appearance of the labeled proteins in the blood is not understood.

Not shown in the figure are the results on the appearance in the blood of radioactive albumin and fibrinogen as tested 8 hours after partial hepatectomy and the intraperitoneal injection of Celite. The rates of appearance of the tagged proteins did not

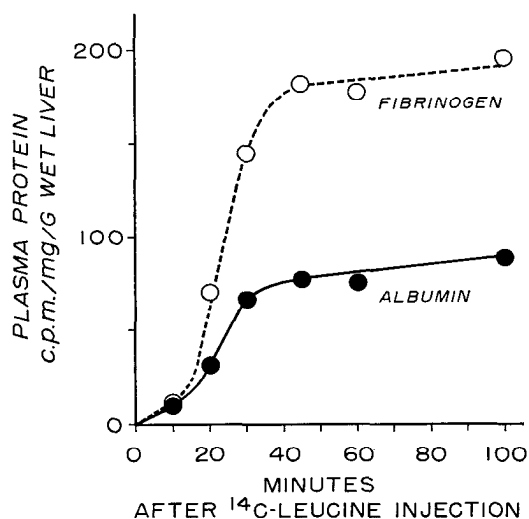


FIG. 2. Rates of appearance of labeled albumin and fibrinogen in the blood. Cardiac blood samples were taken from normal rats at the indicated times after the injection of ^{14}C -leucine ($1\ \mu\text{C}$). The specific activities of albumin and fibrinogen were estimated as described under "Experimental Procedure," and each point represents the average of the results obtained with six rats.

TABLE I

Effect of puromycin on appearance of labeled albumin and fibrinogen in blood

^{14}C -Leucine ($1\ \mu\text{C}$) was given intravenously 7 hours after the removal of 70% of the liver or the intraperitoneal injection of Celite (5 mg/100 g of body weight). Puromycin (10 mg/100 g of body weight) was given intravenously either 5 min before or 5 min after the administration of the amino acid. Cardiac blood samples were taken 1 hour after the injection of the labeled substrate, and the specific activities of albumin and fibrinogen were determined as described under "Experimental Procedure." Each value is the average of the results obtained with four rats, and the ranges of the individual analyses are shown in parentheses.

Treatment and time of puromycin injection ^a	Albumin	Fibrinogen
	<i>c.p.m./mg protein/g liver, wet wt</i>	
None	72 (69-74)	158 (142-174)
-5	4 (2-7)	25 (21-27)
+5	55 (51-57)	136 (134-141)
Partial hepatectomy	121 (113-141)	422 (417-444)
-5	7 (6-7)	60 (53-67)
+5	114 (92-125)	318 (311-349)
Celite	118 (105-128)	352 (332-432)
-5	4 (3-6)	30 (26-39)
+5	98 (87-109)	252 (236-278)

^a Expressed as minutes before (-) or after (+) the administration of ^{14}C -leucine.

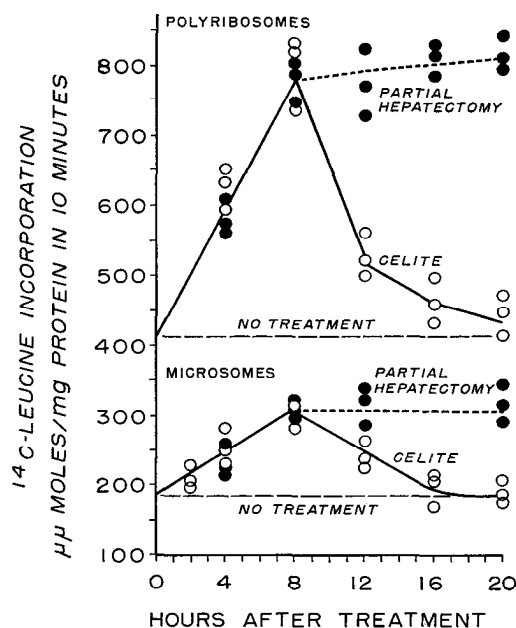


FIG. 3. Amino acid incorporation *in vitro* by liver microsomes and polyribosomes as a function of time after partial hepatectomy and acute stress (Celite). The rats were treated as for Fig. 1, except that those to be used as a source of liver polyribosomes were fasted for 15 hours before the experiment. Liver samples were taken at the times indicated, and the preparation and assay of microsomes and polyribosomes (prepared without detergent) were as described under "Experimental Procedure." In the calculation of amino acid incorporation, the dilution of the ^{14}C -leucine by the amino acid of the liver supernatant fraction was considered. Each point represents the result obtained with a single preparation. Each microsome preparation was from the livers of one rat or three partial hepatectomy rats, and for polyribosomes, the livers of two rats or six partial hepatectomy animals were pooled. The lines describing the results with normal animals (no treatment) were from seven separate determinations, and the ranges of the individual analyses, in micromicromoles of ^{14}C -leucine incorporated per mg of protein, were 152 to 196 (microsomes) and 373 to 456 (polyribosomes).

differ from those with untreated control rats (Fig. 2). The increased labeling of albumin and fibrinogen after removal of part of the liver and acute stress would seem, therefore, to result from rises in protein synthesis rather than from an altered rate of secretion of the proteins.

Effect of Puromycin on Appearance of Labeled Albumin and Fibrinogen in Blood—To provide additional evidence that the increased radioactivity in the circulating proteins stemmed from an enhanced rate of synthesis, control and treated animals were given puromycin (10 mg/100 g of body weight) shortly before or after the injection of ^{14}C -leucine (Table I). The table shows, first, that the formation of the labeled proteins was largely complete at 5 min after the injection of the ^{14}C -amino acid. Second, during the 5-min period, the partially hepatectomized and Celite-treated rats formed more albumin and fibrinogen than did the control animals.

Amino Acid-incorporating Activity in Vitro of Liver Microsomes and Polyribosomes as Function of Time after Partial Hepatectomy and Acute Stress (Celite)—The ability of liver microsomes and polyribosomes (prepared without detergent) to incorporate ^{14}C -leucine *in vitro* increased linearly for about 8 hours after partial hepatectomy or the intraperitoneal injection of Celite (Fig. 3). Just as with the response of the liver *in vivo* (Fig. 1), after the

raises the activities of the fractions from the partially hepatectomized animals remained at the elevated level, whereas those from the stressed rats returned to normal.

Responses in Vivo of Adrenalectomized Animals to Partial Hepatectomy and Acute Stress (Celite)—With adrenalectomized animals, the rates of albumin and fibrinogen synthesis *in vivo* were not increased by the injection of Celite, whereas the response to partial hepatectomy was the same as with normal rats (Table II).

As the table shows, hydrocortisone alone stimulated the formation of the plasma proteins in the adrenalectomized animals, but it had little or no effect on the normal rats. This discrepancy may be related to an increased uptake of the hormone by the livers of the adrenalectomized rats. As suggested by Kenney and Flora (21), who measured tyrosine- α -ketoglutarate transaminase, the effect of the hormone may be determined, in part, by its ability to enter the liver cell. In terms of their observations, in the normal rat, Celite may be required both to cause the release of adrenal hormones and to potentiate their uptake by the liver.

Amino Acid-incorporating Activity in Vitro of Liver Polyribosomes from Adrenalectomized Animals after Partial Hepatectomy and Acute Stress (Celite)—As with the rises in protein synthesis *in vivo* (Table II), Celite caused no enhancement in the activity of liver polyribosomes from adrenalectomized rats (Table III). After partial hepatectomy, on the other hand, the increase occurred in both adrenalectomized and untreated animals.

Effect of Ligation of Branch of Portal Vein on Amino Acid Incorporation by Polyribosome Fraction from Nonligated and Ligated Liver Lobes—Before it enters the liver, the portal vein of the rat divides in two, one of its branches feeding 70% of the liver (left lateral and median lobes), the other, 30% (right lateral and caudate lobes). Despite the availability of blood from the

TABLE II

Responses in vivo of adrenalectomized rats to partial hepatectomy and acute stress (Celite)

Bilateral adrenalectomy was performed 3 days before the experiment. Partial hepatectomy and the administration of Celite (intraperitoneally, 5 mg/100 g of body weight) and hydrocortisone (intravenously, 10 mg/100 g of body weight) were at zero time. ^{14}C -Leucine was injected intravenously at 7 hours, and cardiac blood samples were taken 1 hour later. The specific activities of albumin and fibrinogen were determined as described under "Experimental Procedure." The values presented are the averages of the results with 8 to 18 rats, and the ranges of the individual analyses are shown in parentheses.

Operative treatment and injected material	Albumin	Fibrinogen
	<i>cpm/mg protein/g liver, wet wt</i>	
None.....	76 (64-89)	163 (142-189)
Celite.....	155 (141-208)	444 (421-505)
Hydrocortisone.....	78 (61-100)	145 (134-178)
Celite + hydrocortisone.....	152 (132-171)	453 (366-542)
Partial hepatectomy.....	146 (129-192)	565 (503-585)
Adrenalectomy + partial hepatectomy.....	121 (114-142)	500 (498-631)
Adrenalectomy.....	74 (57-90)	167 (131-192)
Celite.....	78 (72-93)	146 (129-180)
Hydrocortisone.....	140 (111-177)	274 (234-396)
Celite + hydrocortisone.....	176 (141-193)	271 (228-314)

TABLE III

Amino acid incorporation in vitro by liver polyribosomes from adrenalectomized animals after partial hepatectomy and acute stress (Celite)

The rats were treated as in Table II, except that they had been fasted for 15 hours before partial hepatectomy or the injection of Celite or hydrocortisone. Liver samples were taken at 8 hours. Each polyribosomal preparation was from the livers of two rats or six partial hepatectomy rats, and the averages of the results of two experiments are shown.

Operative treatment and injected material	^{14}C -Leucine incorporation
	$\mu\text{moles/mg protein}$
None.....	379
Celite.....	728
Adrenalectomy.....	412
Celite.....	409
Celite + hydrocortisone.....	703
Partial hepatectomy.....	721
Adrenalectomy + partial hepatectomy..	714

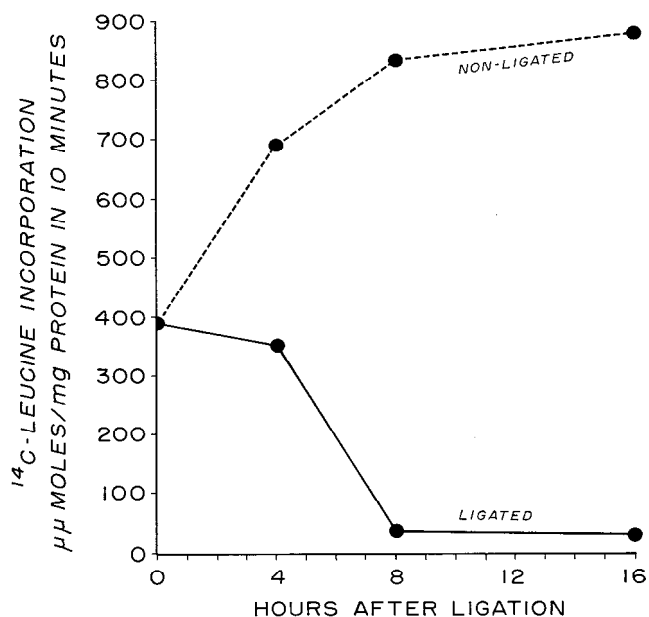


Fig. 4. Amino acid incorporation *in vitro* by the polyribosome fraction of liver as a function of time after ligation of the branch of the portal vein to the 70% part of the liver. To avoid the effects of acute stress, the rats were adrenalectomized 2 days before the experiment. The animals were fasted overnight, and the portal branch vessel was ligated at zero time. Liver samples were taken at the indicated times, and each polyribosome fraction was prepared from the pooled livers of six rats. Each point shows the average of the results of two experiments.

hepatic artery, ligation of the portal branch to the 70% part of the liver has been shown to lead to a decrease in the size of the ligated lobes and a compensatory increase in the nonligated lobes (12). The kinetics of DNA formation (as measured by the incorporation of ^3H -thymidine) in the nonligated liver lobes was shown to be essentially the same after ligation of the portal branch as after partial hepatectomy (22).

In view of the stimulating effect of portal branch ligation on DNA synthesis, it was of interest to measure the amino acid-incorporating ability of the polyribosome fraction from the non-

ligated liver lobes (Fig. 4). As can be seen from the figure, the same enhancement in activity occurred as after partial hepatectomy. The figure also shows that the loss of the portal blood supply caused a marked reduction in the amino acid-incorporating activity of the polyribosome fraction of the ligated liver lobes.

DISCUSSION

The effect of partial hepatectomy on liver protein formation has received attention, at least in part, to shed light on the mechanisms that control mammalian protein synthesis. This attention has concerned itself with cell-free systems, and it seems to have been implied that the changes *in vitro* would be reflected in the intact animal as well. It was of some interest to test this assumption.

The removal of a part of the liver has now been found to cause an enhancement in the rate of synthesis *in vivo* of albumin and fibrinogen (and presumably of all proteins made by the liver). Initially, efforts to study the nature of the relaxed controls were hampered by the similar effects of a variety of acute stresses (laparotomy and injections of Celite and turpentine).⁴ The stimulatory effects of partial hepatectomy and acute stress have, however, been shown to have different etiologies. The two can be readily dissociated in the adrenalectomized rat, in which only the response to partial hepatectomy can occur.

Despite the difference in etiology, the intracellular changes responsible for the enhanced protein synthesis may be the same after partial hepatectomy and acute stress. In both cases, the rises in protein synthesis appear to result from an increased efficiency of the liver polyribosomes. At least in the case of partial hepatectomy, and contrary to the observations of Webb, Blobel, and Potter (26), the enhanced protein formation does not seem to be correlated with a significant increase in the number or size of liver polyribosomes. In agreement with their studies, neither could an increase in membrane-bound liver ribosomes be detected after partial hepatectomy.¹

The exact nature of the polyribosomal change is not known. It appears to be associated with an enhanced resistance of the polyribosomes to disaggregation at 37° and by some activity present in phospholipase A preparations from the venom of *Naja naja* (5).

Why the removal of part of the liver should lead to a polyribosomal alteration is also unknown. One possibility is that it is caused somehow by the altered blood flow that is a necessary

⁴ Accumulation of some blood proteins considered to be formed by liver has been shown to follow such stresses as electric shock (23), myocardial infarction (24), and injury (25).

consequence of partial hepatectomy. Although it does not constitute proof, the identical effect of removal of 70% of the liver and of diverting all the portal blood to the 30% part of the liver is consistent with this possibility.

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