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Effect of Partial Portal Vein Ligation on Hepatic Regeneration

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Abstract *To evaluate the effect of portal hypertension and diminished portal venous blood flow to the liver on hepatic regeneration, male rats were subjected to partial portal vein ligation and subsequently to a two-thirds partial hepatectomy. The levels of ornithine decarboxylase activity at 6 h after partial hepatectomy were greater ($p < 0.001$) in the rats with prior partial portal vein ligation than in those without portal hypertension. The rats with prior partial portal vein ligation also had greater ($p < 0.005$) levels of thymidine kinase activity at 48 h after partial hepatectomy than did those without portal hypertension. Hepatic sex hormone receptor activity was not affected by prior partial portal vein ligation either before or after partial hepatectomy. The reductions in both estrogen and androgen receptor activity observed in the hepatic cytosol after partial hepatectomy were similar to those observed in control animals. These data indicate that animals with portal hypertension having a diminished hepatic portal blood flow have a normal capacity to regenerate hepatic mass following a hepatic resection.*

Keywords: hepatic regeneration, portal hypertension, cirrhosis, liver growth, protal blood flow.

The origin and nature of the factors that control hepatic regeneration remain unresolved. Portal blood has been shown to be hepatotrophic as compared to peripheral blood.[1] However, controversy continues to surround the relative importance of the qualitative changes (hormonal factors) and the quantitative changes (blood flow parameters) in portal blood that occur after partial hepatectomy as they relate to the hepatic regeneration that occurs after a partial hepatic

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resection.[1–8] The pancreatic hormones, insulin and glucagon, have been shown to modulate, at least in part, the regenerative response that occurs after partial hepatectomy.[1–5] These data, however, do not negate an important role for hepatic blood flow, particularly portal venous blood flow, in the regulation of hepatic regeneration following partial hepatectomy.[6–8]

The role of hepatic blood flow in modulating liver regeneration was first suggested by the observation that hepatic atrophy occurs after an Eck fistula (end-to-side portal caval shunt).[6] This hepatic atrophy was thought to be the result of a reduced hepatic blood flow, particularly the portal venous component of total hepatic blood flow. However, it has been shown subsequently that animals with an end-to-side portacaval shunt (Eck fistula) retain their capacity to regenerate liver tissue, albeit at a reduced rate, in response to partial hepatectomy.[6–8] Thus, a reduction in portal venous inflow alone cannot explain the reduction in hepatic mass that occurs following prolonged portal caval anastomosis (surgically induced) or shunting (spontaneous), both of which deprive the liver of its portal venous blood inflow.

Intrinsic liver disease such as cirrhosis is frequently associated with portal systemic shunting and a reduced hepatic, particularly portal venous, blood flow. This reduction in portal venous flow to the liver and its shunting around the liver are thought to be responsible, at least in part, for some of the pathophysiological changes that are present in individuals with chronic liver disease.[9] Cirrhosis is also associated with an increased incidence of hepatoma; furthermore, a successful outcome after a hepatic resection is unlikely in cirrhotics because of the reduced regenerative response present in cirrhosis which may in part be due to portal venous shunting. To investigate the effect of diminished portal blood flow, as opposed to a total absence of portal blood flow, on the hepatic regeneration that occurs after partial hepatectomy the following studies were performed.

Materials and Methods

Animals and Chemicals

Four-week-old male inbred Wistar rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Pyridoxal phosphate, unlabeled ornithine, Tris base, adenosine triphosphate, diethylstilbestrol, sodium molybdate, nicotinamide adenine dinucleotide, calf thymus DNA, and bovine serum albumin were obtained from Sigma Chemical Company (St. Louis, MO). New England Nuclear (Boston, MA) provided the [^{14}C]ornithine (57.6 mCi/mmol), [^{3}H]estradiol (99 Ci/mmol), [^{3}H]R1881 (87 Ci/mmol), and unlabeled R1881. Absolute ethanol and DEAE-cellulose paper were purchased from U.S. Industrial Chemicals Company (Tuscola, IL) and BioRad (Richmond, CA), respectively. Tritiated thymidine (5 Ci/mmol) and ACS scintillation fluid were obtained from Amersham (Arlington Heights, IL). Fisher Chemical Company (Pittsburgh, PA) was the source for all other chemicals.

Surgical Procedures

The rats were randomly allocated to have either a partial portal vein ligation or a sham operation. The technique of partial portal vein ligation in the rat utilized in

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these experiments has been described previously and was performed by exposing the portal vein via a midline abdominal incision.[10] A 20-gauge needle was held alongside the length of the portal vein and two 3-O silk ligatures were tied around the needle and the portal vein. The needle was then carefully slipped out of the ligatures allowing the portal vein to open to a diameter equal to that of a 20-gauge needle and the abdomen was closed. The sham operation consisted of a similar operation with mobilization of but not ligation of the portal vein.

Typically eight weeks later, animals having undergone partial portal vein ligation have a doubling of their portal venous pressure that can be measured directly and develop major portal systemic shunts that can be demonstrated angiographically.[10]

Eight weeks after partial portal vein ligation on the sham operation, both groups of animals were subjected to a standard two-thirds partial hepatectomy.[11] All surgical procedures were performed under light ether anesthesia between 09:00 and 11:00 h to minimize the influence of any diurnal rhythms on the subsequent hepatic regenerative response.

At various times up to 72 h after partial hepatectomy, the animals were anesthetized with ether, weighed, and sacrificed. The remnant livers were removed, weighed, and homogenized in four volumes of ice-cold buffer consisting of 0.25 M sucrose, 1.5 mM EDTA, 10 mM mercaptoethanol, and 10 mM Tris–HCl (pH 7.4) using a Brinkman Polytron homogenizer. The homogenate was centrifuged at 103,000*g* for 1 h at 4 °C and the supernatant used for all cytosolic assays.

Ornithine Decarboxylase Activity

The release of $^{14}CO_2$ from [^{14}C]ornithine was used to determine the level of ornithine decarboxylase activity within the liver.[12] A 0.4-mL aliquot of cytosol was preincubated for 5 min at 37 °C with a mixture containing 0.2 mM pyridoxal phosphate, 5 mM dithiothreitol, and 1.5 mM L-ornithine in 10 mM Tris–HCl (pH 8.0). The reaction was started by the addition of 0.5 μCi α-L-[1-^{14}C]ornithine to the mixture and used 250 μL ethanolamine/ethylene glycol (2/1), placed in a center well, as a carbon dioxide trap. After sealing the assay flask, the mixture was allowed to incubate for 1 h at 37 °C. Thereafter 0.1 mL saturated trichloroacetic acid solution was added to the assay flask to terminate the reaction. The reaction mixture was maintained at 37 °C for an additional 1 h. The ethanolamine/ethylene glycol was removed from the center well and placed into a glass scintillation vial containing 10 mL ACS scintillation fluid. The radioactivity in the ethanolamine/ethylene glycol mixture was measured in a Packard Tri-Carb 460 CD liquid scintillation system.

Thymidine Kinase Activity

Thymidine kinase activity was determined by measuring the in vitro conversion of thymidine to thymidine phosphate.[13] A 0.1-mL aliquot of cytosol was incubated for 10 min at 37 °C with 850 μL of incubation buffer consisting of 5 mM adenosine triphosphate and 3.6 mM $MgCl_2$ in 50 mM Tris–HCl (pH 8.0), and 50 μL, 1 μM, [^{3}H]thymidine. The reaction was terminated by immersing the assay tubes in boiling water for 2 min. The tubes were allowed to cool in an ice

bath and the denatured protein removed by centrifugation at 1500*g* for 5 min at 4 °C. Thereafter 0.1 mL of supernatant was spotted onto a 3.8-cm square of DEAE-cellulose paper and the paper washed twice with 1 mM ammonium formate for 5 min followed by distilled water for 3 min. The paper squares were placed into glass scintillation vials and a 0.1 M HCl/0.2 M KCl mixture was added to elute the radioactivity into solution. After 15 min. 10 mL ACS scintillation fluid was added to each vial and the radioactivity in solution was counted in a Packard Tri-Carb 460 CD Liquid Scintillation System (Downers Grove, IL).

Estrogen and Androgen Receptor Assays

The specific binding of a saturating concentration of labeled estradiol was used to determine the cytosolic estrogen receptor activity.[14] The hepatic cytosol was diluted 1:1 with a buffer consisting of 40 mM sodium molybdate, 1.5 mM EDTA, and 10 mM Tris–HCl (pH 7.4) to stabilize the estrogen receptor. To determine the total binding of the [^{3}H]estradiol, 200 μL of the diluted cytosol was mixed with 25 μL, 30 mM, [^{3}H]estradiol and 25 μL ethanol. To determine the nonspecific binding, parallel assays were performed in which the ethanol was replaced with 25 μL, 3 μM, unlabeled DES dissolved in ethanol. The mixture was allowed to incubate for 2 h at 4 °C. The reaction was terminated by adding 0.4 mL 1% dextran-coated charcoal to the mixture to remove unbound steroid and the mixture was centrifuged at 1500*g* for 5 min at 4 °C. The supernatant was carefully removed and placed in a scintillation vial with 8 mL ACS scintillation fluid. The radioactivity was measured in a Packard Tri-Carb 460 CD Liquid Scintillation System (Downers Grove, IL).

The assay for cytosolic androgen receptor activity was similar to that described above for the estrogen receptor with minor variations.[15] Total binding of the androgen receptor was measured by adding tritiated R1881, a synthetic androgen, and ethanol to the cytosol. Nonspecific binding was determined by adding unlabeled R1881 to the mixture in place of the ethanol. To block the binding of the R1881 to glucocorticoid receptors 5 μM triamcinolone acetonide was added to each tube utilized for measurement of the androgen receptor. After an overnight incubation at 4 °C, the reaction was terminated by adding 1% dextran-coated charcoal. The mixture was centrifuged at 1500*g* for 5 min at 4 °C and the supernatant carefully transferred to a scintillation fluid. The radioactivity was measured in a Packard Tri-Carb 460 CD Liquid Scintillation System (Downers Grove, IL).

Miscellaneous Methods

Cytosolic protein concentration was determined using the method of Lowry with bovine serum albumin being used as the standard.[16]

All data are presented as mean values ± SEM. Statistical analysis of the data was performed using a Student's *t* test. A *p* value of 0.05 or less was considered to represent a significant difference.

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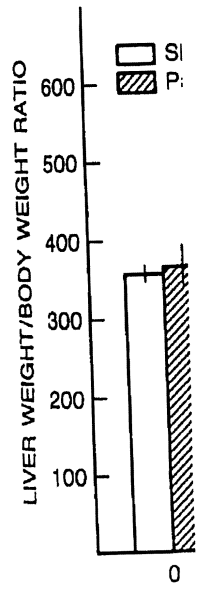

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Results

Animals with partial portal vein ligation had a portal venous pressure of 18 ± 11.6 cm saline at the time of sacrifice, while those undergoing a sham ligation had a portal venous pressure of 9.0 ± 1.2 cm saline.

The effect of partial portal vein ligation on the rate of hepatic growth after partial hepatectomy is shown in Figure 1. The liver weight/body weight ratios (LW/BW) at 6 h in both groups were approximately one-third of the baseline values, confirming that a two-thirds partial hepatectomy had indeed been performed. An approximately twofold increase in the LW/BW ratio occurred between 6 and 72 h after partial hepatectomy in both groups of animals.

The baseline levels of ornithine decarboxylase activity in the livers prior to partial hepatectomy are shown in Figure 2 and were similar in the two groups of animals. A 10-fold or greater increase in hepatic ornithine decarboxylase activity was observed at 6 h in the animals subjected to a partial hepatectomy following a previous sham partial portal vein ligation procedure ($p < 0.001$). The hepatic ornithine decarboxylase activity at 6 h in the animals with a prior partial portal vein ligation followed by partial hepatectomy was significantly greater than that present in the animals with sham portal vein ligation ($p < 0.001$). At subsequent time points 24, 48, and 72 h after partial hepatectomy, the levels of ornithine decarboxylase activity in both groups of animals were increased significantly ($p < 0.005$) above baseline values but there were no differences between the two groups (Fig. 2).

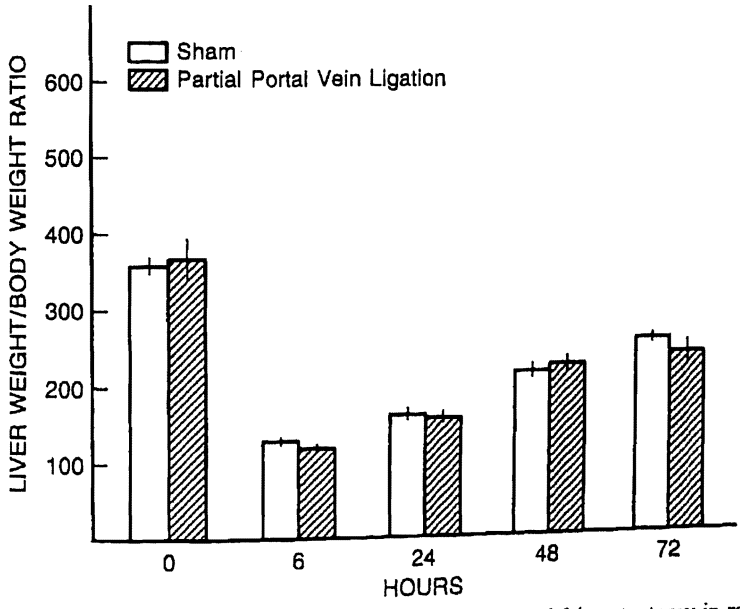


Figure 1. Change in liver weight/body weight ratios after partial hepatectomy in rats previously subjected to partial portal vein ligation and sham operation. Mean ± SEM. (n = 4–6 animals per group).

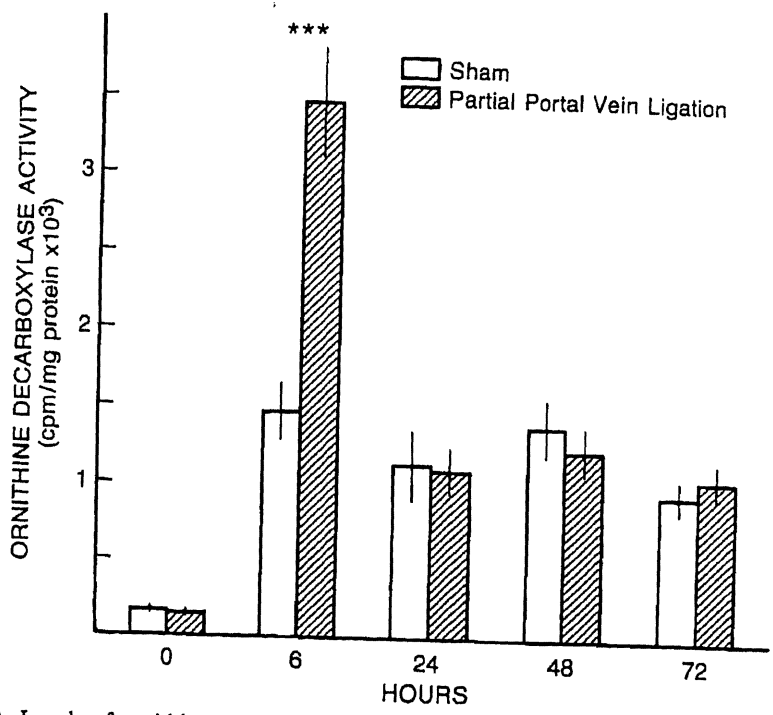
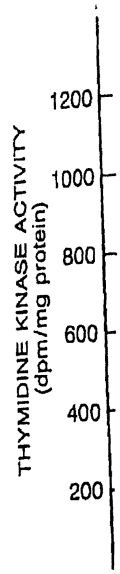


Figure 2. Levels of ornithine decarboxylase activity (cpm/mg protein) in the liver after partial hepatectomy in rats previously subjected to partial portal vein ligation and sham operation. Mean ± SEM (n = 4–6 animals per group) (***$p < 0.001$).

The changes in thymidine kinase activity seen after partial hepatectomy in the two groups of animals studied are shown in Figure 3. Levels of thymidine kinase activity in the liver at the time of partial hepatectomy were similar in the two groups of animals studied. A significant 6- to 10-fold increase in thymidine kinase activity ($p < 0.01$) was observed at 24, 48, and 72 h after partial hepatectomy in both groups, with the levels being significantly greater ($p < 0.005$) in the rats subjected to partial portal vein ligation at the 48-h time point.

Significantly lower levels of cytosolic estrogen receptor activity were observed in the livers at 6 and 24 h after partial hepatectomy in both groups of animals studied ($p < 0.001$) with the receptor activity returning to baseline levels by 48 h (Fig. 4). The estrogen receptor activity in the hepatic cytosol before and after partial hepatectomy in the rats with previous partial portal vein ligation was similar to that in the control animals at each of the time points studied.

The amount of androgen receptor activity in the hepatic cytosol prior to partial hepatectomy was similar in the two groups of animals. Reduced levels of androgen receptor activity were observed in the liver of both groups after partial hepatectomy, with the activity being undetectable in some specimens. As was the case with the estrogen receptor activity in the hepatic cytosol, there were no differences in androgen receptor activity between the animals having undergone a prior partial portal vein ligation and the sham-operated control animals after partial hepatectomy.



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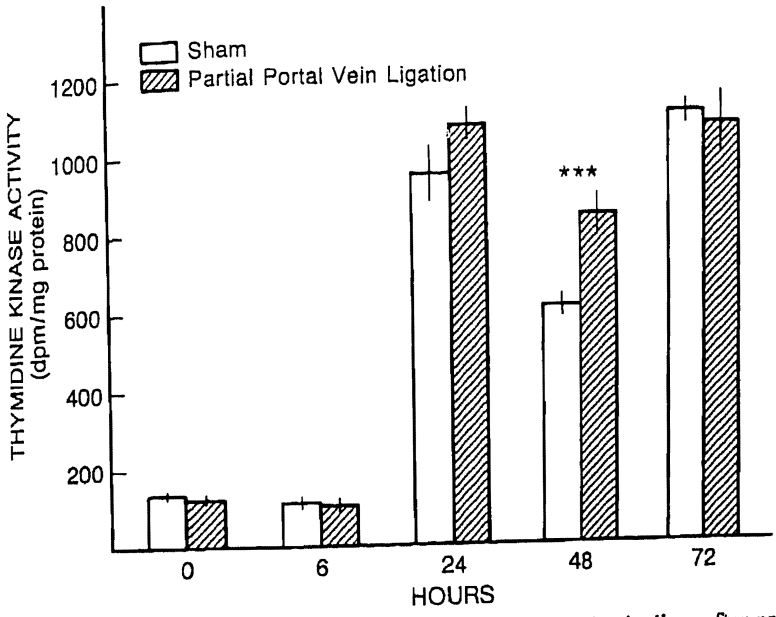

Figure 3. Levels of thymidine kinase activity (dpm/mg protein) in the liver after partial hepatectomy in rats previously subjected to partial portal vein ligation and sham operation. Mean ± SEM (n = 4–6 animals per group) (***$p < 0.001$).

Discussion

The results of this study reveal that partial portal vein ligation and the resultant portal venous shunting do not affect adversely, but actually potentiate, the hepatic regenerative response measured biochemically after partial hepatectomy in rats. Moreover, this study confirms earlier studies that report a lower level of estrogen receptor activity in the hepatic cytosol after partial portal vein ligation.[17] The changes in androgen receptor activity produced by partial portal vein ligation have not been reported previously. The changes in estrogen and androgen receptor activity in hepatic cytosol observed after partial hepatectomy were qualitatively similar to those previously described in normal animals.[15]

Ornithine decarboxylase, the initial enzyme in polyamine synthesis, is induced prior to organ growth and proliferation and is considered to be essential for regeneration.[18,19] Thymidine kinase, the enzyme that phosphorylates thymidine prior to its incorporation into deoxyribonucleic acid, is also induced in proliferating tissue[20,21] and has been used widely as a biochemical index of regeneration.[13] Both ornithine decarboxylase activity at 6 h and hepatic thymidine kinase activity at 48 h after partial hepatectomy were greater in the animals having had a prior partial portal vein ligation procedure than in those without this procedure.

The results presented therefore are consistent with biochemical evidence of an increased hepatic regenerative response after partial hepatectomy in rats subjected to partial portal vein ligation which was not expressed as an enhanced phenotypic regenerative response. It is possible that the minor hepatic injury experienced as a result of a diminished portal venous blood flow in the rats with a

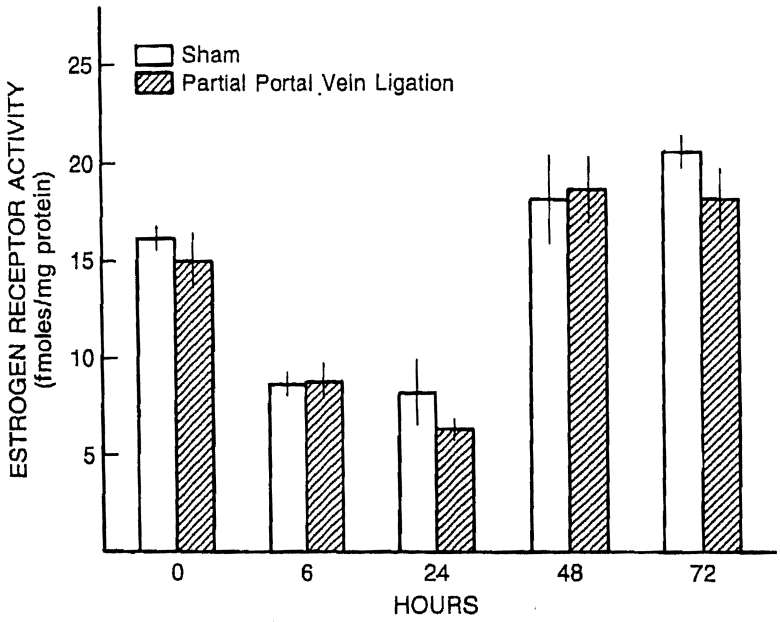

Figure 4. Levels of estrogen receptor activity in the hepatic cytosol (fmol/mg protein) after partial hepatectomy in rats previously subjected to partial portal vein ligation and sham operation. Mean ± SEM (n = 4–6 animals per group).

partial portal vein ligation accounted for the enhanced biochemical regenerative response seen after partial hepatectomy. In contrast, animals with a total diversion of their portal venous blood, as occurs with an end-to-side portacaval shunt, have been reported to have a diminished biochemical and phenotypic regenerative response after partial hepatectomy.[7,8] Thus, the results herein reported confirm the presence of hepatotrophic factors in portal venous blood and suggest that their presence, even in amounts less than occurs with complete portal venous flow to the liver, but more than occurs with a complete portal caval shunt, modulates the hepatic regenerative response seen after a partial hepatectomy.

Several hepatic functions have been shown to display a sexual dimorphism in mammals.[15,22] In male rats, hepatic regeneration is associated with a loss of certain male-specific hepatic characteristics.[15,22] The hepatic changes seen after a partial hepatectomy and during hepatic regeneration include an increased plasma level of estradiol, an increased hepatic estrogen receptor activity, decreased plasma levels of testosterone, as well as a reduced level of hepatic androgen receptor activity.[15] Although the total hepatic estrogen receptor activity increases after partial hepatectomy, cytosolic estrogen receptor activity actually decreases as the receptors shift from the cytosolic compartment to the nucleus.[15] It is believed by some that this transfer of the cytosolic estrogen receptor to the hepatic nucleus initiates the regenerative response following a partial hepatectomy. The results herein presented demonstrate arithmetically reduced basal levels of estrogen receptor activity in the hepatic cytosol before partial hepatectomy in rats having experienced prior partial portal vein ligation. Moreover, the findings in the present study are compatible with previous reports that have shown a significant increase in estrogen receptor activity present in the nuclear compartment of the

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liver with no change in the total level of estrogen receptor activity after partial portal vein ligation.[17] This net increase of estrogen receptor activity in the nucleus in animals having had a prior partial portal vein ligation could account for the greater biochemical regenerative response seen after partial hepatectomy in the animals studied.

These data have important clinical implications. They suggest that patients with a diminished hepatic portal blood flow but having a normal anatomic liver probably have a normal capacity to regenerate hepatic mass following a hepatic resection. Such a situation might occur in patients with extrahepatic portal venous obstruction, who maintain some degree of portal venous blood delivery to the liver via collaterals. In contrast, patients with chronic liver disease, who also have spontaneous portal caval shunting, at least to some degree, who are known to have an impaired regenerative response to hepatic resection, probably have an impaired regenerative response as a result of their intrinsic liver disease rather than a partial loss of the portal venous blood flow to the liver.

Acknowledgments

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