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THE EVALUATION OF LIVER FUNCTION AFTER PARTIAL HEPATECTOMY IN THE RAT: SERUM CHANGES

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Summary.—In serial studies of hepatic function in rats after 70% partial hepatectomy, quantitative changes were found in several of the serum components used clinically to assess liver status. The activities of the following enzymes were found to increase: γ-glutamyl transpeptidase and lactic dehydrogenase were maximal 6 h postoperatively, while glutamic oxaloacetic transaminase and alkaline phosphatase reached peak values at 24 and 48 h respectively. Albumin levels were found to be relatively constant during the study; however, total protein concentration was lowest 6–12 h postoperatively, paralleling a decrease in globulin concentration. Bilirubin levels were elevated to 4× normal within 12 h after surgery. After partial hepatectomy calcium and phosphorus concentrations were significantly decreased at 24 and 12 h respectively. With the exception of alkaline phosphatase, the activities of all serum components measured returned to normal levels by 1 week after surgery; the alkaline phosphatase concentration continued to be elevated 2 weeks postoperatively.

A SURVEY of the literature reveals extensive studies of the intracellular changes occurring in the liver after partial hepatectomy in the rat (Ludewig, Minor and Hortenstine, 1939; Sulkin and Gardner, 1948; Bucher and Malt, 1971); however, little information is available on the effect of partial hepatectomy on the serum concentrations of those substances used to assess liver function. In this report we describe the serial quantitative changes in 12 serum components at times from 6 h to 14 days after two-thirds partial hepatectomy.

MATERIALS AND METHODS

Sprague–Dawley male rats weighing 190–210 g were housed in an area with a 12-h light–dark cycle (light 6 a.m.–6 p.m.). The animals were allowed food (Purina Rat Chow, Ralston-Purina Co., Inc., St. Louis, Mo.) and water *ad libitum*. The rats were partially hepatectomized by a standard method (Higgins and Anderson, 1931) under ether anaesthetic. Control animals were subjected to a sham operation in which the liver was gently palpated. All operations were performed between 9 a.m. and 11 a.m.

At 6, 12, 24, 36 and 48 h and at 7 and 14 days after surgery, under light ether anaesthesia the animals were killed by decapitation and mixed arterial–venous blood collected directly. This method of blood collection was compared in control experiments with collection by cardiac puncture; no significant differences in any of the measured serum components were found.

The collected blood was allowed to clot at 4°. The serum was separated by centrifugation using "Sure-Sep" serum–plasma separators (General Diagnostic Co., Morris Plains, N.J.); it was analysed for the following components: albumin, globulin; total protein; total bilirubin; alkaline phosphatase, E.C. 3.1.3.1; lactic dehydrogenase, E.C. 1.1.1.27; γ-glutamyl transpeptidase, E.C. 2.3.2.-; glutamic oxaloacetic transaminase, E.C. 2.6.1.1; total lipids; cholesterol; calcium; and phosphorus. All analyses were performed on a Technicon SMA 12/60 automatic clinical analyser (Technicon Instrument Co., Tarrytown, N.Y.) by Medi-Comp Laboratories Inc. (Cleveland, Ohio) using standard Technicon methodology.

As controls for the alterations in growth rate resulting from partial hepatectomy, additional unoperated animals were included in some of the

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experiments and killed at 1 and 2 weeks with the operated animals. One week after partial hepatectomy the average weight of the partially hepatectomized, sham-operated, and intact rats were 210, 210 and 257 g respectively. After 2 weeks these weights were 250, 293 and 294 g respectively. No differences were found in any of the serum values of the intact rats at the various times and weights described. The results were expressed as the mean ± 1 s.d. Groups ranged from 5 to 11 animals in size, with the exception of the 2 groups killed at 48 h after surgery, which included 4 animals each. Significant differences between the means were determined using Student's t test (Huntsberger, 1967), the significant level being $P < 0{\cdot}05$.

RESULTS AND DISCUSSION

Serum enzymes

The serum enzyme activities after partial hepatectomy are shown in Fig. 1.

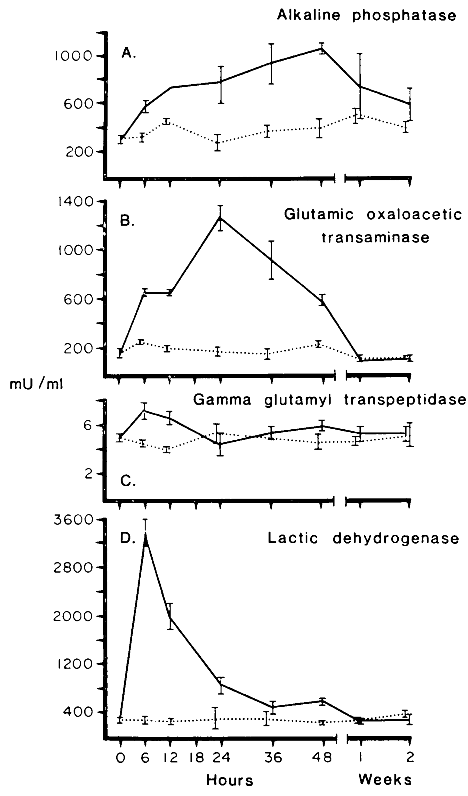

FIG. 1.—Enzyme activities in rat sera at various times after partial hepatectomy; ··· control, —— partially hepatectomized. Bars represent one standard deviation.

γ-Glutamyl transpeptidase and lactic dehydrogenase (Figs 1C, 1D) reach maximum levels 6 h after partial hepatectomy, while alkaline phosphatase and glutamic oxaloacetic transaminase peak at 48 and 24 h respectively (Figs 1A, 1B). γ-Glutamyl transpeptidase levels have returned to normal by 24 h after surgery; lactic dehydrogenase and glutamic oxaloacetic transaminase are normal by 1 week. The decrease in alkaline phosphatase to normal levels is more protracted, the plasma level remaining elevated 2 weeks after operation. Oppenheimer and Flock (1947) have reported that the alkaline phosphatase level is elevated in the plasma after 70% partial hepatectomy; in that study, the highest values occurred 2 days after surgery and decreased gradually to normal in 9 days.

Several mechanisms may account for increases in serum enzyme activities (Zimmerman, 1974); these include enzyme release due to cell necrosis, enzyme release due to increased cell-membrane permeability without cell necrosis, enzyme induction and release, or a decreased disposition of an enzyme.

The clearance of liver enzymes from the circulation has been studied by several groups of investigators. Strandjord, Thomas and White (1959) injected lactic dehydrogenase into dogs and found that the enzyme was cleared in 7–10 h. The normal disappearance curves found in nephrectomized or hepatectomized dogs, in conjunction with data obtained after intraportal injection of enzyme, indicated that the liver and kidney were not responsible for clearing lactic dehydrogenase from the circulation. Frankl and Merritt (1959) reported that i.v. administration of lactic dehydrogenase in dogs was not associated with increased biliary excretion of these enzymes. Dunn, Martins and Reissman (1958) and Wakim and Fleisher (1963*a*) measured the clearance of an i.v. injection of glutamic oxaloacetic transaminase in dogs; 75% of the injected enzyme disappeared from the circulation within 6 h and the remaining 25% was cleared within 20–72 h. The rapid disappearance phase was found to be due to diffusion of the enzyme into interstitial fluid. Wakim and Fleisher (1963*b*) showed that when zymosan was injected before the enzyme there was a marked acceleration of enzyme clearance, suggesting that glutamic oxaloacetic transaminase is removed by the reticuloendotheilal system. Since a substantial portion of the reticuloendothelial system exists as the Kupffer cells of the liver, the clearance of substances by these cells might be influenced by the reduced number of reticuloendothelial cells resulting from partial hepatectomy.

Possibly enzyme activities are increased as the result of i.p. absorption of enzymes released by necrosis of the small amount of hepatic tissue remaining distal to the site of ligation. Studies of enzyme release after acute toxic liver injury (Rees and Sinha, 1960; Curtis, Moritz and Snodgrass, 1972; Schmidt *et al.*, 1974) have shown that there is a pattern in which the intracellular enzymes are released into the circulation; cytoplasmic enzymes increase in the serum within a few hours, enzymes found in both the cytoplasm and mitochondria increase next, and finally the mitochondrial enzymes appear. The early appearance of cytoplasmic enzymes was suggested (Rees and Sinha, 1960) to be a reflection of cell-membrane injury or permeability change before the onset of frank necrosis. However, inconsistencies in the appearance of intrahepatic enzymes after toxic injury are known (Curtis *et al.*, 1972) and the general complexity of the balance of enzyme release, synthesis and degradation make firm interpretations hazardous.

In the present experiments lactic dehydrogenase, a cytoplasmic enzyme, and γ-glutamyl transpeptidase, which has both cytoplasmic and microsomal locations (Szewczuk, 1966), peak at 6 h after partial hepatectomy; glutamic oxaloacetic transaminase, which is found in both the cytoplasm and the mitochondria, is maximal at 24 h. However, the activity of alkaline phosphatase, a membrane-associated enzyme, is highest 36 h after partial hepatectomy. Kaplan and Righetti (1970) have reported that liver alkaline phosphatase in the serum increases about $2\frac{1}{2}$-fold by 12 h after bile-duct ligation; this enzyme increase was prevented by cycloheximide, suggesting that the increase represented enzyme induction. Although our data (Fig. 1A) show a similar rise in serum alkaline phosphatase, and might be interpreted to indicate some degree of biliary stasis, no stasis was present upon light microscopic examination of histological specimens. Stenger and Confer (1966) have commented on the presence, visible on electron microscopy, of dilated and irregular bile canaliculi in regenerating rat liver, while Mori, Novikoff and Quintana (1975) have described comparable changes in the bile canaliculi and have reported an associated increased canalicular alkaline phosphatase activity by histochemical measurement.

Overall, the enzyme data appear compatible both with some early release of enzyme by damaged cells or cells with altered permeability, and with longer-term effects perhaps involving increased synthesis and release of enzyme.

Protein

The serial changes in serum total protein and globulin concentrations are shown in Figs 2A and 2B respectively. Serum albumin concentration was fairly constant, ranging from 2·5 to 3·6 mg/100 ml, the average concentration being 2·8 mg/100 ml over the 2 weeks of the study. Albumin levels were essentially identical in the sham-operated and partially hepatectomized animals. Roberts and White (1949) reported that after partial hepatectomy albumin levels undergo a transient rise which is followed by an abrupt drop, the lowest concentration occurring 24 h after surgery. We have not observed this decrease in albumin concentration (data not shown). An abrupt decrease in albumin synthesis would not be expected to cause a rapid drop in concentration, since the

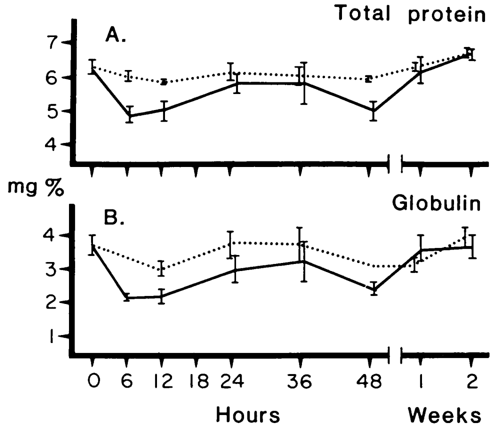

FIG. 2.—Total protein and globulin concentrations in rat sera after partial hepatectomy; ···· control, —— partially hepatectomized.

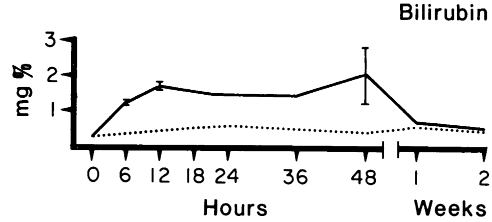

FIG. 3.—Bilirubin concentrations in rat sera after partial hepatectomy; ···· control, —— partially hepatectomized. No standard deviations are shown if the bilirubin concentrations were identical within a group of rats.

half-life of albumin in the circulation is 17–21 days (Spiro, 1977).

The decrease in total proteins appears to be primarily due to a decrease in the concentration of globulins. Lowrance and Chanutin (1942) noted that the globulins decrease immediately after partial hepatectomy, and are at their lowest concentration 6–12 h postoperatively; by 36 h after partial hepatectomy this decreased concentration is not statistically significant when compared to the concentrations found in the sham-operated animals. It should be noted that a slight decrease in globulin concentration also occurs immediately after surgery in the sham-operated animals (Fig 2b). Chandler and Snider (1970) have found that after partial hepatectomy there is an increase in the relative rates of synthesis of albumin and the seromucoid fraction of the serum; this increase extends over a period of 14 days after surgery and would tend to mask the early decrease in globulins if only total protein is measured. In their study, laparotomy had no effect on albumin synthesis, but caused an increase in the seromucoid fraction.

Bilirubin

Total bilirubin is increased to a maximum of approximately 4× the normal concentration within 12 h after partial hepatectomy (Fig. 3); this increase remains constant for the next 36 h, but by 1 week after surgery the level has returned to normal. Mild cholestasis might account in part for the raised bilirubin (and alkaline phosphatase) levels, but morphological evidence for cholestasis is lacking in our studies, whereas others have described increases in tortuosity and size of bile canaliculi as mentioned above. In functional studies Leong, Pessotti and Brauer (1959) measured bile flow in preparations of regenerating rat-liver remnants isolated at various times after partial hepatectomy. Their data indicated that, at constant perfusion rates, regenerating liver remnants had increased bile flow per unit liver weight up to a maximum increase of 50% by 3 days after operation. During the first 24–48 h the smaller increase they report, coupled with the reduced liver mass, would yield a bile flow considerably below normal, indicating a relative functional deficiency due to loss of liver mass. However, the situation *in vivo* may not be strictly comparable and additional factors may be involved.

Calcium and phosphorus

Rixon and Whitfield (1972) have measured the concentration of plasma calcium at varying times after partial hepatectomy. They reported a decrease in calcium concentration evident at 1 h after surgery, with the concentration reaching the lowest value at 6 h. In our series no determinations of calcium concentration were made before 6 h after partial hepatectomy. We found a statistically significant decrease in calcium concentration to occur in the sera of the partially hepatectomized animals at 24 h; the calcium concentration was $10{\cdot}5 \pm 0{\cdot}3$ mg/100 ml for the sham-operated animals and $8{\cdot}5 \pm 0{\cdot}6$ mg/100 ml for the partially hepatectomized animals. The calcium level returned to normal within 36 h after surgery.

Measurement of the serum phosphorus levels revealed a significant decrease at 12 h after partial hepatectomy; the concentration in the sham-operated animals was $9{\cdot}1 \pm 0{\cdot}3$ mg/100 ml, while the concentration in the partially hepatectomized animals was $7{\cdot}8 \pm 0{\cdot}4$ mg/100 ml. There was no difference in the phosphorus concentration in the 2 groups of rats 24 h postoperatively.

Lipids

Figures 4A and 4B show the concentration of total lipids and cholesterol respectively after partial hepatectomy. Triglyceride and cholesterol ester levels were not determined separately in these experiments. The fluctuation in total lipid concentration parallels that occurring with cholesterol. There is no statistically significant difference in either of the serum components measured between the partially hepatectomized and the sham-operated rats. It is difficult to interpret the changes occurring in the serum lipids in these experiments, although it has been shown (Redgrave, 1970) that ether anaesthesia increases the plasma concentrations of both cholesterol and triglycerides over the short term. One interpretation of the profiles shown would be an immediate increase of lipids after anaesthesia, followed by a gradual return to normal levels, with reduced feeding the night after surgery and increased feeding the night after that. Fisher and Fisher (1963) have reported food intake to be 5% of normal in the first 12 h and about 25% of normal during the second 12-h postoperative period, intakes being increased during the second and third postoperative days. Clearly, the increase of total plasma lipids 4–16 h after feeding can be much greater in normal rats (Dunn, Wilcox and Heinberg, 1975) than the increases (Fig. 4) after *ad libitum* feeding on the second postoperative night.

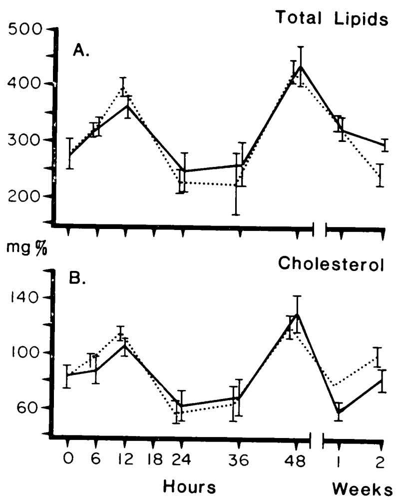

FIG. 4.—Total lipid and cholesterol concentrations in rat sera after partial hepatectomy; ···· control, —— partially hepatectomized.

In conclusion, the present study shows that after partial hepatectomy changes occur in the serum concentrations of several markers used to assess liver function; the serial changes appear to be somewhat different from those which occur during cell necrosis.

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