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Bile Duct Obstruction and Regeneration of the Liver.* (26483)

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It was recently observed in this laboratory that ligation of the common bile duct of the rat stimulated proliferation of liver cells in the absence of liver cell necrosis(1). The present work was carried out to study further the effect of biliary obstruction on hepatic regeneration, and to compare regeneration due to biliary obstruction with that following partial hepatectomy. Incorporation of tritiated thymidine (H^3-thymidine) into deoxyribonucleic acid (DNA) was quantitated in autoradiographs, and mitoses were counted in histologic sections to detect and quantitate regeneration(2).

Higgins and Anderson(3) performed partial hepatectomies after bile duct ligation in rats: Wet and dry weights were correlated with the histologic appearance and with hepatic mitoses; it was found that weight was restored as well or better than in normal rats subjected to partial hepatectomy. On the basis of gross and histologic observations, however, they interpreted restoration of weight in obstructed liver to be due to engorgement with bile and blood, and they concluded that regeneration was impaired after bile duct ligation. Cameron(4) studied survival of slices of normal and obstructed liver placed as autotransplants in the omentum of rats. He found that in liver from animals with bile duct obstruction liver cells did not survive as long as from normal rats. He concluded that bile duct ligation inhibited regeneration. Ferguson, Rogers and Vars(5) studied total liver weight, protein content, and mitotic activity and found that in rats that had undergone bile duct ligation at the time of partial hepatectomy, regeneration was as great or greater than regeneration in partially hepatectomized normal liver. Weinbren(6) studied rat liver subjected to partial hepatectomy after bile duct ligation. He compared gain in weight of remaining liver lobes, and found no difference between obstructed and normal liver; he concluded that bile duct ligation did not inhibit regeneration.

Materials and methods. Forty male rats of the Sprague-Dawley strain,‡ separated by litter-mates into 24-hour and 48-hour experimental groups were used. Animals were obtained at 21 days of age, and were maintained in this laboratory until 119 to 124 days of age, at which time they weighed 315 to 415 g. They were housed individually in an air-conditioned room, and were fed laboratory chow and tap-water *ad libitum.* Animals were divided into groups as follows: 12 underwent ligation of the common bile duct midway between liver and duodenum, followed immediately by partial hepatectomy of approximately 50% of the liver(7). Twelve rats were subjected to partial hepatectomy alone; 8 to ligation of the bile ducts alone, and 8 to a sham operation consisting of

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‡ Purchased from Charles River Breeding Laboratories, Boston, Mass.

TABLE I. Uptake of Tritiated Thymidine and Mitotic Activity of Rat Liver Cells Following Ligation of Common Bile Duct and Partial Hepatectomy.

Operative procedure	Hr after operation	No. rats	Tritium labeled hepatic nuclei*	Mitoses*
Partial hepatectomy	24	6	24,688 ± 9,669†	695 ± 409†
Bile duct ligation & partial hepatectomy	24	6	3,151 ± 859	54 ± 66
Bile duct ligation	24	4	22 ± 10	4 ± 19
Sham operation	24	4	27 ± 9	0
Partial hepatectomy	48	6	3,761 ± 1,331	288 ± 180
Bile duct ligation & partial hepatectomy	48	6	8,863 ± 3,380	698 ± 254
Bile duct ligation	48	4	4,958 ± 3,362	298 ± 134
Sham operation	48	4	8 ± 3	0

* Expressed per 100,000 hepatic cell nuclei.
† ± stand. dev.

laparotomy and manipulation of the liver and bile ducts.

Twenty of the rats were sacrificed at 24 hours, and the remaining twenty 48 hours after operation as shown in Table I. Four hours prior to sacrifice H^3-thymidine§ was administered under ether anesthesia *via* one femoral vein, in a dose of one-half microcurie per gram body weight. Animals were sacrificed by etherization and decapitation. Slices of liver were fixed in 10% neutral buffered formalin. Histologic sections were stained with hematoxylin and eosin and stripping film autoradiographs were prepared as described elsewhere(2). Kodak AR-10 film was used, with an exposure time of 4 weeks. In autoradiographs, tritium labeled hepatic nuclei were counted in 100 consecutive 400 × fields containing parenchymal cells; in histologic sections mitotic figures in hepatic cells were counted in 50 consecutive 400 × fields. Results were expressed per 100,000 hepatic nuclei.

An attempt was made to carry out similar studies 3 weeks after bile duct obstruction and partial hepatectomy, but because of a high mortality of rats with ligation and division of bile ducts, apparently due to liver failure and to infection, this part of the experiment was discontinued.

Results. All operated rats lost 10 to 30 g during the one or the 2 days between operation and sacrifice. Rats which had undergone partial hepatectomy had fibrinous adhesions of intestine, stomach or omentum to the stumps of resected lobes. In rats with bile duct ligation and partial hepatectomy the livers were bile stained in addition. Bile ducts in animals with ligation showed little or no dilatation 24 hours later; 48 hours after operation the ducts proximal to the ligation were dilated and contained an estimated 0.5 ml of bile. Sham operated rats showed no intraperitoneal changes.

Histology. The livers of sham operated rats were normal; in animals that had undergone partial hepatectomy, liver cells were enlarged, with finely vacuolated cytoplasm(7). In rats with bile duct ligation alone the changes were similar to those described by Cameron and Oakley(8). At 24 hours and more markedly at 48 hours bile ducts were increased in number in portal areas; cells of bile duct epithelium were enlarged and contained large, pale nuclei. In portal areas connective tissue appeared edematous, with more fibroblasts than usual, and there were increased numbers of lymphocytes and polymorphonuclear leukocytes. Liver cell nuclei were enlarged and pale staining. As noted previously(1) necrosis was not a prominent feature of these livers; there were scattered degenerated liver cells with pyknotic nuclei, occasional "hyaline bodies" composed of cytoplasm of degenerated liver cells, and in 2 livers there were rare "Gombault infarcts." (8). The histologic findings were similar in

§ Schwarz Laboratories, Mount Vernon, N. Y., specific activity 1.9 curies per millimole.

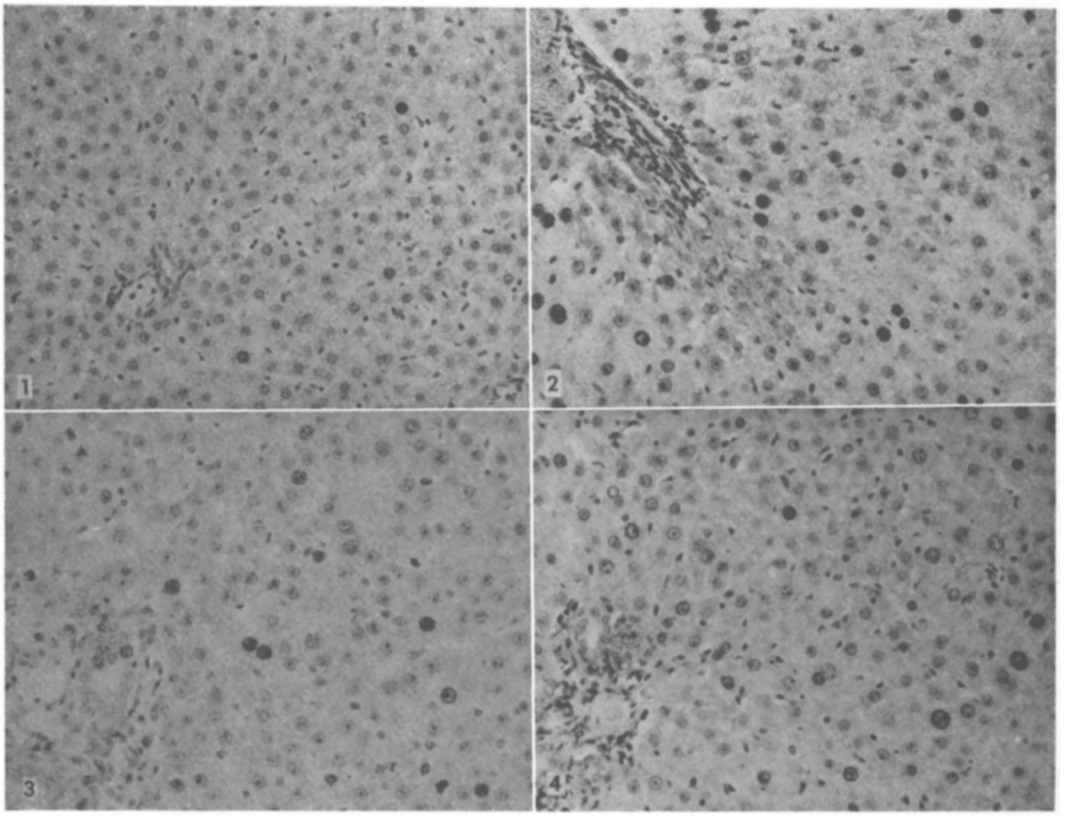

FIG. 1. Autoradiograph of liver of normal rat, inj. with H^3-thymidine 4 hr prior to sacrifice. One labeled liver cell nucleus is present in upper right portion. Hematoxylin × 200.

FIG. 2. Autoradiograph of liver of rat subjected to partial hepatectomy and inj. with H^3-thymidine 24 hr later. Approximately 40 labeled liver cell nuclei. Hematoxylin × 200.

FIG. 3. Autoradiograph of liver of rat subjected to ligation of common bile duct and partial hepatectomy, and inj. with H^3-thymidine 24 hr later. Approximately 20 labeled liver cell nuclei. Hematoxylin × 200.

FIG. 4. Autoradiograph of liver of rat subjected to ligation of common bile duct and inj. with H^3-thymidine 48 hr later. Approximately 20 labeled liver cell nuclei. Hematoxylin × 200.

rats that had undergone both bile duct ligation and partial hepatectomy.

Autoradiographs and Mitotic Counts. Twenty-four hours after operation, rats that had undergone partial hepatectomy and common bile duct ligation showed significantly less H^3-thymidine uptake and mitotic activity of hepatic cells ($P<0.01$) than rats subjected to partial hepatectomy alone. (Table I) H^3-thymidine uptake and mitoses in livers of rats with ligation of the common bile duct were not significantly different from normal.

To confirm the findings at 24 hours, this portion of the experiment was repeated with an additional 17 rats; the results were essentially the same.

At 48 hours after operation, rats with partial hepatectomy and bile duct ligation showed significantly more H^3-thymidine uptake and mitoses ($P<0.05$) than rats with partial hepatectomy alone. Rats with bile duct ligation alone showed markedly increased H^3-thymidine uptake and mitotic activity of liver cells, comparable to the 48 hour values of rats that had undergone partial hepatectomy.

H^3-thymidine uptake by bile duct epithelium was increased beginning 24 hours after operation in rats with bile duct ligation and in those with combined bile duct ligation and partial hepatectomy.

Comment. This work suggests that liver cell proliferation occurring as a result of bile duct obstruction may be due to sublethal,

histologically inapparent damage to liver cells. The regenerative response on the part of liver cells begins 40 to 48 hours after common duct obstruction; in time of onset, it is thus different from the regeneration that occurs after partial hepatectomy, which is advanced at 24 hours. Further, there is an early, brief interference of biliary obstruction with regeneration after partial hepatectomy, and there is a long interval of 48 hours between obstruction of the bile ducts and appearance of increased DNA synthesis and mitoses in liver cells, during which time injury, and response to injury, may occur. Substances such as serotonin(9) and thiourea(10) have been shown to cause increased liver cell mitoses in the absence of necrosis, probably due to sublethal damage to cells which is followed by proliferation.

Summary. DNA synthesis and mitosis in liver cells are partially inhibited 24 hours after operation in rats subjected to both partial hepatectomy and bile duct obstruction; at 48 hours regeneration is greater than after partial hepatectomy alone, although not as marked as that seen at 24 hours after partial hepatectomy. Liver cell proliferation occurs 40 to 48 hours after obstruction of the common bile duct, and is presumably the result of histologically inapparent injury to liver cells.

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Lens Sodium and Potassium Concentrations During Galactose Induced Cataractogenesis.* (26484)

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The electrolyte composition of the ocular lens of the rat is greatly modified during formation of galactose-induced cataracts. Of particular interest is a reduction in potassium and elevations in sodium and calcium concentration(1,2). The relationship between the changes in these cations and lens opacification is not known. Certain biochemical studies suggest that cataractogenesis is the consequence of an inhibition of lens metabolism by galactose(3,4). It has also been demonstrated that maintenance of the lens content of sodium and potassium is energy dependent(5) and the changes observed in these cations during cataractogenesis may reflect the effect of galactose on lenticular metabolism. The proposal has been made that the concentration of these cations in the lens during cataractogenesis could serve as an index to the progression of the lesion(6).

The present studies were made to determine the time course of the modification of lens sodium and potassium in relation to development of galactose-induced lens opacification. Large differences are known to exist in susceptibility of several strains of albino rats to the cataractogenic property of galac-

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