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Histology and Regenerative Capacity of Liver Following Multiple Partial Hepatectomies.* (23371)

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Repeated partial hepatectomies were performed in the rat in order to study in the remaining portion of the liver (a) maintenance of regenerative capacity, (b) histological alterations, and (c) possible appearance of neoplasia as a result of prolonged stimulus to regeneration. The regenerative capacity was not exhausted by as many as 12 partial hepatectomies and neoplasia was not seen.

Procedure. The surgery was carried out at the University of Chicago and the histological studies were done at the University of Michigan. The procedure for repeated partial hepatectomy in the rat has been described(1). Thirty male rats having initial weights of 240-260 g were given Archer Dog Pellets to eat *ad libitum* during the experiments. Twelve of the rats were subjected to sham operations which consisted of a laparotomy once each month. Eighteen rats underwent repeated partial hepatectomies, once each month. The procedure was to remove all of the liver but the median lobe at the first operation, a part of the median lobe was excised at the second operation and thereafter liver was aspirated by negative pressure applied to a pipette inserted in a slit in the now thickened capsule of liver. The post-operative care of the rats has been described(1).

At the termination of the experiment, the rats were killed by exsanguination under ether anesthesia. The liver was fixed in ice-cold Carnoy's fluid and left in the refrigerator overnight. On the next day the Carnoy's fluid was replaced with absolute alcohol and the livers shipped to Ann Arbor. A large piece of the liver was prepared for microscopic study. Sections were stained with periodic acid-leucofuchsin(PAS) and counter-stained with methylene blue in a citric acid-phosphate buffer (pH 5.6).

Results. All of the sham-operated rats survived for the duration of the experiment. All of the hepatectomized rats survived 8 operations. During the following 4 months 5 rats died as the result of self-inflicted wounds after the incision of the last operation had healed. Two rats died from post-operative hemorrhage due to breaking of adhesions. There were no deaths due to failure to control hemor-

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TABLE I. Body Weights and Liver Weights of Sham Operated Rats and Rats Subjected to 12 Partial Hepatectomies during 1 Year. Averages and stand. errors.

Group	No. rats	g body wt		g liver wt	
		Initial	Final	Total removed	Final
Sham operated	12	250	534 ± 15.3		17.5 ± 1.15
Partial hepatectomy	6	250	507 ± 20.8	70.77 ± 1.54	13.8 ± 1.22

rhage from the liver itself. The remaining complications represented abscesses and diffuse infections which were no longer controlled by penicillin and streptomycin. After 8 months all of the rats developed peritonitis on one or more occasions following liver surgery. All of the rats which were killed because of impending death had multiple abscesses in the remaining liver and elsewhere in the peritoneal cavity and occasionally in the lungs. Gastric ulcers were found in 3 of these rats.

As previously noted(1) the rat subjected to repeated partial hepatectomy shows various signs of hepatic insufficiency during the first week following each operation. In addition to lowered resistance to infection these rats develop hypoglycemic shock unless given readily utilizable carbohydrate. Blood clotting is impaired and the tiny broken or cut vessels of adhesions or scars may continue to leak slowly for hours. The rats usually became jaundiced for a few days following operation.

Six hepatectomized rats survived for the duration of the experiment (1 year) and in them the liver was reduced 12 times. Data on these rats and the sham-operated controls are in Table I. The body weight of these animals was maintained rather well. One rat did lose 121 g during the 12th month after gaining weight at a normal rate during most of the year. At autopsy it was found to have severe bronchiectasis. The capacity of liver to regenerate was also maintained in spite of the removal of large amounts of liver during the year although the final average weight was somewhat less than normal.

The histology of the regenerated liver from the 6 rats which lived throughout the experiment was similar with respect to several points (Fig. 1 and 2). No mitosis was observed in parenchymal cells. This was to be expected because the most active proliferation occurs within a few days after the operation (2). A normal relationship between parenchymal cells and sinusoids was maintained. However, the pattern of orientation of liver cords within lobules was not as regular as in livers from sham-operated rats. In all cases there were areas of parenchymal cell necrosis and of fibrous scars which usually contained proliferated bile ducts. In one case, septa of connective tissue fibers divided the liver substance into compartments. Abscesses were not found in the livers of these rats, suggesting again that the capacity to resist infection was an important factor in determining duration of survival after repeated partial hepatectomy.

Cytologically there was considerable variation between the individual animals. Where-

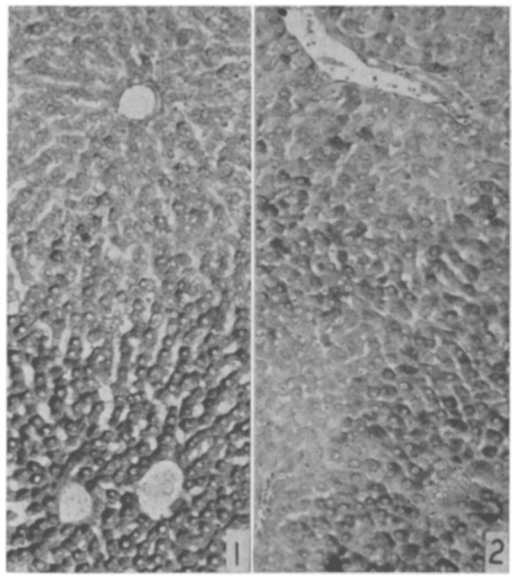

FIG. 1. Liver from sham-operated control. Cytoplasmic material appearing black is glycogen; most of the gray bodies are ribonucleic acid. Portal canal is at the top and central veil below. PAS and methylene blue. ×125.

FIG. 2. Liver from rat which was partially hepatectomized 12 times. PAS and methylene blue. ×125.

as in the specimens from sham-operated rats, cytoplasmic ribonucleic acid (as shown by staining with methylene blue) was generally in the form of dense clumps, in 3 of the hepatectomized rats it was diffusely distributed throughout the cytoplasm as a delicate stippling. This pattern seemed to be correlated with the presence of enlarged parenchymal cells and variability in nuclear structure. Some nuclei were enlarged and often contained either multiple nucleoli or hypertrophied nucleoli of variable shape. In one case, parenchymal cells were extensively vacuolated, indicating that fat had accumulated. The glycogen content did not follow a consistent pattern, in at least 3 cases being comparable in amount to that in the sham-operated controls.

Histologic study of the livers of the rats which died or were killed before termination of the experiment confirmed the frequency of infection in these rats by the presence of abscesses in the fibrotic scarred areas and by mononuclear cellular infiltration into the connective tissue of the portal canals of some rats. Otherwise the histologic changes varied only in degree from those described for the rats which lived throughout the experiment.

In no case was histologic evidence of neoplasia observed.†

Summary. The capacity of liver to regenerate was maintained in rats which were subjected to partial hepatectomy 12 times within a period of one year. At the end of the experiment, only minor cytological changes were observed in the regenerated liver and there was no neoplasia.

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Intraperitoneal Insemination of the Heifer.* (23372)

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Anatomically, there is no barrier to prevent sperm from reaching the ovary following intraperitoneal injection of semen in species such as the cow, ewe, mare(1), rabbit(2), and to some extent the sow(1) whereas bursae such as those of the rat(2), bitch(1), and mouse(3) could be a barrier. Physiologically, little is known of the compatibility of the peritoneal surfaces and fluid with sperm. Brown(4) found peritoneal fluid of the human being compatible with living sperm. Van Drimmelen(5) obtained fertilization in the chicken following intraperitoneal insemination. Black *et al.*(6) failed to obtain fertilization when 9 superovulated calves were inseminated intraperitoneally. Skjerven(7) recently reported conception in a heifer following intraperitoneal deposition of semen by puncturing the vaginal wall.

It is the object of this paper to report the results of intraperitoneal insemination of heifers.

Materials and methods. Four sexually mature Holstein-Friesian heifers were inseminated a total of 6 times near the end of estrus by injection of Holstein-Friesian semen, in some cases diluted, in other cases undiluted and of variable quality, into the peritoneal cavity by puncturing the abdominal wall in the region of the right paralumbar fossa with a 16-gage, 4 inch hypodermic needle. An in-

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