Regeneration of Mouse Liver after Partial Hepatectomy*

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The rapidly growing liver tissue after partial hepatectomy has been used advantageously by investigators to study changes during accelerated growth and proliferation of cells (4, 7, 12, 15). Previous reports have been chiefly on rats. In many instances, pooled samples were taken for chemical data. In the present investigation a comprehensive study was made on individual samples of regenerating (restoring) mouse liver by several methods of approach: (a) quantitative morphological and cytological studies of cell and tissue growth (22), (b) histochemical and cytochemical localization of substances (28), and (c) quantitative biochemical analyses (20).

MATERIALS AND METHODS

A total of 165 white mice of strain A was used in the preliminary and final experiments. Three-month-old male animals weighing 24-27.5 gm. were fed ad libitum, because fasting, a procedure used on rats by some investigators, tended to increase the mortality rate in mice.

Partial hepatectomy was performed by a single person to reduce possible variations in technic. The method of Brues, Drury, and Brues (4) was followed with slight modifications. The left lateral and median lobes were ligated at the base and excised under ether anesthesia, with special attention being given to tying the ligature at the proper level to avoid obstruction of the hepatic portal vein and the bile duct. Hemostats were not used. The percentage of total liver tissue removed was determined in a preliminary experiment by operating on 29 male mice by the standard procedure and weighing both the excised and remaining portions immediately. Results showed that 65 per cent (standard deviation ±4) of the total liver was removed by partial hepatectomy (Table 1). Thus, the weight of the excised portion gives an indication of total liver weight. In the final experiments only those animals with an excised liver weight of more than 0.84 gm. were used, with a range in weight from 0.84 to 1.00 gm. in 90 per cent of the animals. This was done to select a reasonably uniform group of experimental animals.

After partial hepatectomy, changes in body weight were followed as an indication of postoperative health. Regenerating livers were selected from animals showing the least body weight loss soon after hepatectomy and a steady gain in weight in later stages. The residual lobes were always removed between 9:00 and 10:30 A.M., and all analyses made on individual animals. For microscopic studies pieces of tissue were collected immediately from the posterior lobe of the right lateral lobe for freezing-drying (16) and chemical fixation. The remainder of the right lateral lobe and caudate lobe was used for biochemical analyses. Deoxyribonucleic acid, nitrogen, and other chemical constituents were determined by the methods previously described (19).

Observations were made on the regenerating liver at the following intervals after partial hepatectomy: 1, 2, 3, 4, 5, 6, 7, 8, 10, 14, 21, 28, 38-45 days, and 2, 4, and 6 months. Six to nine animals were used in each group, except after 45 days when four to five animals were examined. Two types of control material were used: (a) livers from normal healthy males of 3 months (ten animals), 5 months (four animals), and 9 months (five animals) for comparison with early and late stages of restoration after partial hepatectomy; and (b) for some animals, the excised portions were analyzed to serve as their own controls.

A brief study was made comparing the two methods available for obtaining nuclear counts: (a) that of counting the nuclei from microscopic sections (4); and (b) using a diluted sample of the fresh homogenate in a counting chamber (18). The former method proved to be extremely tedious and time-consuming, with more possible sources of error than the latter. The counts from sections were usually slightly higher than those obtained from fresh homogenates. The latter method was adopted for our experiments. A homogenate was prepared for each liver in an all-glass homogenizer (Scientific Glass Apparatus Co.) with 9 ml. of slightly alkaline 0.85 per cent sodium chloride solution per gram of tissue for quantitative chemical analyses. For counting purposes, 1 ml. of this sample was diluted further with 10 ml. of saline. This was mixed with an equal part of a staining solution, which consisted of 80 mg. crystal violet in 100 ml. of 0 per cent acetic acid. Nuclei can be distinguished readily with this staining mixture.

Samples were agitated on a variable speed shaker (Eberbach & Sons) and counted within 2 hours after preparation. Repeat counted made at intervals within this period showed no change. In comparing different dyes at various concentrations it was observed that homogenates prepared with saline gave distinctly darker staining reactions than those with 0.88 M sucrose.

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Counts were made with the hemocytometer, using five 1-mm. squares from two chambers. The original counts in this volume of fluid (1 c. mm. in the ten squares) ranged from 275 to 600 nuclei, the lower figures for some of the regenerating livers and the higher numbers for control livers. Each sample was counted by two persons whose average was accepted when their individual results were within 5 per cent of each other's counts. When not in agreement, the counts were repeated until agreement was obtained. Nuclei belonging to all types of cells were contributing to the quantitative values of desoxyribonucleic acid.

**Tissues fixed in Zenker-acetic fluid were cut at 4 μ after paraffin imbedding. These were stained with hematoxylin-eosin for histological observations. Material from control animals as well as the excised control and regenerated portions was examined.**

The percentage of parenchymal cells in mitotic division was determined as described in the following paper (22). The percentages of binucleate parenchymal cells and other cellular elements in the liver tissue were obtained at the same time the mitotic counts were made. Results from the 4-μ sections compared favorably with those taken from 12-μ sections, except for slight discrepancies in instances where the liver cells were much enlarged, as in some regenerating livers. Here, as expected, the relative number of parenchymal nuclei tends to be higher in the thinner section. Since it was not feasible to make separate counts from 12-μ sections for the entire experiment, all counts to be reported were obtained from 4-μ sections. No correction factor for section thickness was introduced in calculations of percentages.

### TABLE 1

<table>
<thead>
<tr>
<th>Liver weight (gm.)</th>
<th>Percentage liver/body weight</th>
<th>No. nuclei per gm. X 10^-4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regenerating liver:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2 day</td>
<td>0.67</td>
<td>2.7 (2.5-2.8)</td>
</tr>
<tr>
<td>1 day</td>
<td>0.70</td>
<td>3.0 (2.7-3.6)</td>
</tr>
<tr>
<td>2 days</td>
<td>0.81</td>
<td>3.5 (2.8-4.1)</td>
</tr>
<tr>
<td>3 days</td>
<td>0.89</td>
<td>3.9 (2.8-4.0)</td>
</tr>
<tr>
<td>4 days</td>
<td>1.08</td>
<td>4.7 (3.6-5.5)</td>
</tr>
<tr>
<td>5 days</td>
<td>1.12</td>
<td>4.9 (3.8-5.9)</td>
</tr>
<tr>
<td>6 days</td>
<td>1.23</td>
<td>5.4 (4.0-5.8)</td>
</tr>
<tr>
<td>7 days</td>
<td>1.30</td>
<td>5.9 (5.2-5.9)</td>
</tr>
<tr>
<td>8 days</td>
<td>1.45</td>
<td>6.4 (5.7-7.5)</td>
</tr>
<tr>
<td>9 days</td>
<td>1.58</td>
<td>6.8 (4.7-4.7)</td>
</tr>
<tr>
<td>10 days</td>
<td>1.55</td>
<td>6.0 (4.8-6.4)</td>
</tr>
<tr>
<td>14 days</td>
<td>1.66</td>
<td>6.8 (4.6-7.6)</td>
</tr>
<tr>
<td>21 days</td>
<td>1.50</td>
<td>5.1 (5.2-5.9)</td>
</tr>
<tr>
<td>28 days</td>
<td>1.55</td>
<td>6.0 (4.8-6.0)</td>
</tr>
<tr>
<td>35-45 days</td>
<td>1.54</td>
<td>5.5 (4.5-7.8)</td>
</tr>
<tr>
<td>2 months</td>
<td>1.58</td>
<td>5.5 (4.7-6.4)</td>
</tr>
<tr>
<td>4 months</td>
<td>1.70</td>
<td>6.5 (4.4-6.0)</td>
</tr>
<tr>
<td>6 months</td>
<td>1.75</td>
<td>6.5 (4.4-6.6)</td>
</tr>
</tbody>
</table>

| **Controls:** | | |
| Before hepatectomy | 1.45 | 5.5 (4.1-4.6) | 140 |
| After hepatectomy | 0.50 | 2.0 (1.4-2.5) | 215 (204-231) |
| Normal controls 3-mo. males | 1.45 | 5.5 (4.0-5.8) | 174 (159-192) |
| Normal controls 5-mo. males | 1.49 | 5.5 (4.5-7.3) | 193 (192-207) |
| Normal controls 9-mo. males | 1.40 | 5.1 (4.5-5.8) | 231 (192-227) |

### OBSERVATIONS

Partial extirpation of the mouse liver results in a rapid growth of the remaining lobes in a manner similar to those of other rodents. Although an effort was made to control many variable factors, mice do not respond to partial hepatectomy in an entirely uniform manner with respect to any of the criteria observed. Although some attempt will be made to indicate the degree of individual variation in some of the data, generally the mean group trends will be discussed. Changes in body weight, liver weight and cell population, and some of the chemical results will be presented here. Further information on biochemical, histochemical, and quantitative cytological studies will appear in subsequent papers (20, 22, 23).

**Liver and body weight changes.**—Following partial hepatectomy there is a loss in body weight exceeding the immediate loss of 3.6 per cent due to removal of the two lobes of liver. On the first day the average body weight drops to 8 per cent below that immediately after hepatectomy, with little change through the tenth day. Although a few animals begin gaining weight on the fourth day, it is not until the fourteenth day that the average weight shows an increase. A steady gain in weight is observed thereafter, and complete recovery to the pre-operative range occurs at 21 days.

The right lateral and caudate lobes show a rapid increase in weight soon after hepatectomy, so that the original total liver weight is regained by the eighth day (Table 1). This quick growth, coupled with a loss in body weight during this period of early regeneration, tends to bring the percentage of liver to body weight back within the normal range much sooner than if the body weight were to remain normal. The largest gain of 26 per cent in this ratio occurs during the first 18 hours; by the end of the first day there is an increase of 33 per cent over that of the hepatectomized controls. The normal pre-operative ratio is restored as early as the fourth day in some animals, but the average ratio is not back to normal until the sixth day. Since the increase in weight of the liver remnants continues while the restoration of the body weight lags, the ratio is higher than normal between the eighth and 28th days.

Much of the early gain in liver weight is due to mobilization of lipids into parenchymal cells and increase of fluid in the liver as evidenced by histochemical and chemical observations. The total nitrogen and desoxyribonucleic acid content increases only slightly during the first day when synthesis is presumably not occurring at a fast rate. Restoration of these constituents progresses rapidly from the second to the eighth day, their...
curves paralleling that of liver weight (Chart 1). The second day must necessarily be the period of most active premitotic synthesis of substances concerned in the duplication of chromosomes and production of daughter cells as will be discussed below.

Changes in number of nuclei.—The number of cells begins to increase slowly from the second day when some individuals first show increased mitotic activity. A peak in mitotic activity of parenchymal cells is reached on the third day, at which time an average of 6.7 per cent of these cells is found to be in some stage of mitosis (2%). A noticeable increase in the number of cells is therefore expected to occur on the fourth day. Actually, the total number of nuclei increases from 121 to 145 million (17 per cent) between the third and fourth days. This is the largest daily percental increase during the period of early regeneration. The marked increase in liver to body weight ratio at this time thus represents an actual increase in number and growth of cells. After the third day mitotic division declines gradually, and by the seventh day rapid proliferation is essentially over. Following this, the number of cells apparently continues to increase slowly by some mitotic activity, which, however, is so low that only a few are observed in the size samples used. The total number of nuclei reaches 87 per cent of the normal value at 28 days after hepatectomy (Chart 1). At 4, 6, and 9 months, however, the numbers are 88, 92, and 101 per cent, respectively, when compared to control animals of 5 and 9 months of age. The number of nuclei per gram of tissue is low through the 21st day (Table 1), indicating that the average size of cells in the regenerating liver is somewhat larger than in the controls.

Mitotic activity of the lining cells and bile duct cells occurs particularly during the first several days of regeneration, although counts were not made for these. Increase of bile duct cells is accompanied by multiplication of the underlying connective tissue cells.

The percentage of parenchymal cell nuclei in liver tissue was obtained from sections at the same time that counts were made for mitotic cells. In the normal mouse liver an average of only 56 per cent of the total nuclei belong to parenchymal cells, the remainder belonging to bile duct, Kupffer, connective tissue, smooth muscle, and mesothelial cells and also to macrophages and white blood cells. The percentage of nuclei other than those of hepatic parenchymal cells at various stages of regeneration are presented in Charts 2 and 3. The range for each group has also been indicated. There is an increase of the nonparenchymal elements from the third through the 45th day of liver restoration. This increase may be due to one of three conditions or a combination of all these factors: (a) a more rapid proliferation of cells other than parenchymal cells, (b) a decrease in the number of parenchymal cells due to some focal necrosis, and (c) an infiltration of leukocytes and macrophages accompanying focal necrosis.

The numbers of liver parenchymal nuclei were calculated from total nuclear counts, using the percentages of parenchymal nuclei obtained from microscopic sections. This revealed that at 7, 14, 21, and 28 days of regeneration the liver parenchymal nuclei recovered are 48, 54, 58, and 66 per cent, respectively, of the normal controls. At 6 months the number returns to 99 per cent of the original. Correction of these numbers for binucleate cells gives the total number of liver parenchymal cells recovered at various intervals. At 7, 14, 21, and 28 days the numbers of liver cells are, respectively, 58, 63, 68, and 76 per cent of the control livers. This rises to 95 per cent at 4 months and 114 per cent at 6 months. These represent relative gains only, since the percentages are derived from calculations of several sets of data.

Histological findings.—Individual variations are found in control livers as to the amounts of leukocytic infiltration, glycogen content, and necrotic cells. Of the ten normal animals of 3 months, three showed slight leukocytic infiltration around the portal or central veins. Since the animals were not fasted, there were varying degrees of glycogen storage, the areas occupied by glycogen in the liver showing cells appearing as unstained areas in hematoxylin-eosin slides. These observations on glycogen were substantiated by histochemical tests and chemical determinations. One control sample contained a few enlarged nucleoli, which have been designated by some authors as nuclear inclusion.
bodies. The physiological significance of these abnormally enlarged nucleoli has not yet been elucidated.

The excised control portion for each animal was examined closely for indications that might explain the wide individual variations in response after partial hepatectomy. In addition to the differences observed above for control animals, this survey of excised livers revealed that liver tissue from 14 per cent of the animals contained small areas of focal necrosis or isolated necrotic cells. This observation, however, does not necessarily mean that these particular animals were predisposed to develop necrosis in the regenerating portions. During the first 10 days, focal necrosis was evident in only five regenerating livers from eleven animals which originally showed necrotic cells, either singly or in small groups. Larger numbers of regenerating livers showing focal necrosis were from animals whose excised portions were normal in appearance.

At 18 hours after partial hepatectomy, some livers showed evidence of engorgement and dilatation of the sinusoids and blood vessels as well as hemorrhage and thrombi in sinusoids. Some focal necrosis was observed principally in the mid-zonal areas. The occurrence and extent of these necrotic areas varied considerably among individuals. This change was followed by some infiltration of leukocytes. The parenchymal cytoplasm appeared vacuolated during the first few days, due to large amounts of lipids. These vacuoles stained positively for lipids with oil red O in formalin-fixed material.

The occurrence of focal necrosis at ¼ and 1 day appeared to be due to some difficulty in the vascular channels, since there was evidence of engorgement in larger blood vessels. Following this there was a period of repair, so that at 2 days all nine animals showed either areas of resorption of necrosis or no involvement. At 3 days, however, one-third of all the regenerating livers again contained some groups of necrotic cells. The period in which the largest number of animals showed recent hemorrhage, necrosis, and fibrosis was between the fifth and tenth days. Some necrosis was still evident at 28 days in two out of six individuals. However, at 2, 4, and 6 months this process had ceased completely.

DISCUSSION

The restoration of mouse liver proceeds at a variable rate in different individuals. Apparently, the time required for readjustment of the remaining tissue prior to cell proliferation and actual growth depends on physiological factors which are difficult to control in these experimental animals. Animals in this experiment were selected as to sex, age, body weight, weight of excised liver, and also on the basis of good postoperative health. Tissues were collected within the same time period in the morning. These factors did not eliminate the wide range in variations of results. Animals which responded quickly showed mitotic activity at 48 hours or earlier, while some showed no mitosis at this time. Fasting the animals prior to operation would probably not have diminished the variations, since Brues and Marble (5), using fasted rats, obtained similar variations in mitotic activity. It is clear from the present study that the length of time elapsed after partial hepatectomy is not necessarily indicative of the degree of restoration occurring in individual livers. Unfortunately
cells was in division, the remainder of cells being liver is essentially restored to the original mass by average of only 6.7 per cent of the parenchymal cells which are in different stages at any one time. That shows an average activity of large numbers of processes accompanying rapid proliferation of cells. The initial adjustment to the great loss of functional tissue is that of mobilization of lipids and other constituents into the liver with concomitant loss in body weight, indicating a heavy drain on body reserves. The remarkable increase in lipid content during the first 2 days is in agreement with histochemical and chemical observations on the rat liver after partial hepatectomy (15, 18). This increase in lipids is followed by an influx of other substances in preparation for synthesis of new cells (20).

The removal of the gall bladder with the median lobe at the time of hepatectomy probably causes a greater degree of disturbance in the biliary system of the mouse than in the rat, which lacks a gall bladder. Whether this causes an actual delay in regeneration is not known.

In this experiment the young adult mouse liver showed a peak in mitotic activity on the third day of regeneration. In rats this has been reported to occur at 24 hours (5), at 30 hours (15), and on the second and third days (12). A direct comparison cannot be made between these different groups of animals, since, among other factors, the age of the animal affects the rate of regeneration (6, 8, 9). There is an earlier and greater restoration of mass and number of cells in young animals compared to adult and old individuals. There may also be differences in response between different strains of the same species.

The early changes in the first few days are of chief interest to investigators concerned with processes accompanying rapid proliferation of cells. The premitotic influx of various substances, the period of synthesis (cell division), and the postmitotic reorganization and synthesis of material for normal function occur within a relatively short time. A study of this sort, however, is at best one that shows an average activity of large numbers of cells which are in different stages at any one time. At the height of mitotic activity, for example, an average of only 6.7 per cent of the parenchymal cells was in division, the remainder of cells being either in premitotic or postmitotic periods or in a normal functional state.

In terms of liver to body weight ratio, the mouse liver is essentially restored to the original mass by the sixth day. The original total liver weight, nitrogen, and deoxyribonucleic acid, however, do not reach the control levels until the eighth day. Presumably, most of the functional cytoplasm restored between the seventh and tenth days. The total number of nuclei, on the other hand, is slow to recover. There may be several factors which affect the number of nuclei obtained. First, it has been observed in this study and others (6, 13, 17, 22) that the number of binucleate cells is reduced during regeneration. This indicates mitotic activity of binucleate cells which form single metaphase plates with production of mononucleate polyploid daughter cells (3). Such cell divisions would then produce no net increase in the number of nuclei. There is ample evidence of increase in polyploid nuclei in regenerating livers (17, 21, 22). Second, some nuclei of the regenerating livers may have been fragmented in the process of homogenization and thus not included in the counts. Third, the functional tissue may have been restored by means of larger cells, without necessarily regaining the original number of cells.

The normal young adult mouse liver contains a large percentage of nonparenchymal cells. On the basis of nuclear counts on sections, only 56 per cent of all the nuclei in the liver tissue belong to parenchymal cells, although the volume occupied by these cells is 88 per cent (22). Siess and Stegmann (14) reported somewhat similar ratios of parenchymal to endothelial cells. Swift (11) reported that in beef liver the percentage of parenchymal nuclei is higher (70 per cent). Unpublished observations in this laboratory show similar results for the rat liver.

The relative as well as absolute number of parenchymal nuclei is reduced during regeneration, with a reciprocal rise in the number of nuclei belonging to nonparenchymal cells. Abercrombie and Harkness (1) observed a significant increase of litoral cells on the seventh day of regeneration in the rat liver.

The occurrence of necrotic cells in normal mouse liver has previously been reported by Wilson and Leduc (21) and Olitsky and Casals (10). Abnormal appearance of liver cell cytoplasm in early regenerating liver has been observed by Price and Laird (12), who described cytoplasmic inclusion bodies in the rat liver which appeared as early as 6 hours after partial hepatectomy and tended to disappear at 48 hours. Aterman (9) reported the presence of glycogen-free, fat-free vacuoles in rat liver as early as 5 minutes after partial hepatectomy. He believed that this "watery vacuolation" after hepatectomy is identical with that observed in the liver in anoxic states.
SUMMARY

Extirpation of 65 per cent of the mouse liver results in rapid growth of the remaining lobes, so that the original mass, in terms of percentage of liver to body weight, is essentially restored by the sixth day. However, the original total liver weight, nitrogen, and deoxyribonucleic acid are not completely recovered until the eighth day. The total number of nuclei increases more slowly and reaches 87 per cent of the normal value at 28 days after hepatectomy.

Parenchymal nuclei comprise only 56 per cent of the total nuclei of normal mouse liver, the remainder belonging to bile duct, Kupffer, connective tissue, smooth muscle, and mesothelial cells and also to macrophages and leukocytes. These nonparenchymal elements are increased between the third and 45th days of regeneration.

Substantial individual variation is observed in the response of mice to partial hepatectomy. The time interval after operation is not a satisfactory, uniform criterion of the degree of restoration of the liver remnant in individual mice.

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