The Enhancement of Ductal Proliferation by Deprivation of the Portal Blood Supply in the Rat Liver

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SUMMARY

By inducing hepatic parenchymal atrophy through deviation of the portal blood flow and, at the same time, bile-duct proliferation by common bile-duct obstruction, proliferating bile ducts were found to occupy almost the whole of the lobe affected by both these procedures. The lobe, therefore, appeared to be composed almost completely of bile-duct cells, with only very few surviving hepatic cell plates. Bile-duct proliferation was, therefore, increased when the tissue was deprived of portal blood.

This preparation, consisting substantially of bile-duct epithelium, might be useful for assessing the biochemical and enzymic characteristics of these cells.

Attempts have been made in recent years to find experimental hepatomas whose enzyme characteristics closely resemble those of normal liver (7, 8). This work has directed attention to the enzymic constitution of the normal liver cells which serve as a reference base for the tumors. It is not possible, however, to ascribe particular enzymes to specific cell types, since bile-duct epithelium and hepatic parenchymal cells are too intimately related to be separated accurately for the relevant biochemical technics. In general, analysis of the enzymic pattern of the liver probably yields information mainly about parenchymal cells, since these form the bulk of the normal organ.

Although some tumors have been found to differ only slightly from the normal, both morphologically and in respect to enzyme content (6, 8), it seems possible that certain other tumors, which appear to deviate markedly, may, in fact, bear a close resemblance to bile-duct epithelium (8). A particular example is the Novikoff hepatoma, which possesses an enzyme, deoxyctydyllic acid deaminase, considered to be characteristic of proliferating bile ducts and not detected in normal liver (4, 8). It seems reasonable to suppose that if tumors which develop from bile-duct cells could be compared with bile-duct tissue, then a more accurate assessment of the abnormalities in the enzymic pattern might be made.

It is difficult, however, to acquire a mass of bile-duct tissue sufficient for biochemical analysis. One way of overcoming this difficulty might be to try to transform part of the liver into tissue consisting predominantly of bile-duct cells. It seemed possible that this might be effected by surgical procedures.

It is well established that a lobe of the liver atrophies when deprived of its portal blood supply (1, 10). The main component involved in this atrophic process has been found to be the hepatic parenchymal cells (12). It has also been established that bile ducts, supplied by the hepatic artery (3), proliferate after ligation of the common bile duct (11). A combination of these two procedures might provide a lobe of the liver consisting predominantly of bile-duct cells, the net result of parenchymal cell atrophy and bile-duct cell proliferation. This preparation might serve as a model for analyzing the characteristics of the bile-duct cells. At the same time we wished to assess the part played by the portal blood supply in proliferation of the bile ducts.

MATERIALS AND METHODS

Male, white rats were used throughout the investigation. They were bred in our own laboratories, weaned after 3 weeks, and kept on a diet of "Research" rat cubes and water. The rats weighed between 110 and 200 gm. at the time of operation.

Total common bile-duct obstruction.—The method used was similar to that described by Cameron and Oakley (2).

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Ligation of portal vein branches.—The technic was similar to that described previously (12).

Collection of specimens.—The rats were killed by ether inhalation, and the liver was removed while the animal was still under anesthesia, by first clamping the portal vein and then dividing the hepatic vein. Before this procedure, in those rats in which serum bilirubin was to be estimated, 2 ml. of blood was withdrawn from the aorta. In the livers with a distended stump of the common bile duct, the latter was completely dissected and removed before the liver was divided into its different lobes for recording their individual weights. In rats with portal vein obstruction, 0.2 ml. of a 10 per cent solution of India ink was injected into the spleen to confirm that total portal obstruction to the lobe was present. The rats were not fasted before the operation nor before killing.

The portal veins to the median lobes were ligated in all. The common bile duct was ligated after 4 months in thirteen rats, and the remaining seven animals were subjected only to laparotomy after 4 months and were used as controls. The rats were killed 5–7 days after the second surgical procedure.

A group of twenty normal rats was killed without previous operation, and the liver lobes were dissected and weighed for control values.

RESULTS

Weight changes (Table 1).—The mean weight of the median lobe in the normal controls was 1.49 gm ± S.E. 0.055/100 gm body wt.

In the rats of Group I (portal vein ligated and common bile duct obstructed) the weight was reduced to 1.17 ± 0.070 in the 1 to 3-day period; 0.87 ± 0.070 after 4–6 days; 0.81 ± 0.028 after 7–9 days; 0.58 ± 0.057 after 10–12 days; 0.42 ± 0.040 after 13–15 days; 0.36 ± 0.070 after 16–18 days; 0.29 ± 0.098 after 19–21 days.

In rats of Group II (portal vein obstruction to median lobe, common bile duct intact), the reduction in weight of the median lobe was more conspicuous. After 1–3 days the mean weight of the median lobe was 0.81 ± 0.115 gm/100 gm weight; 0.28 ± 0.023 after 4–6 days; 0.23 ± 0.028 after 7–9 days; 0.20 ± 0.030 after 10–12 days; and values varying between 0.15 and 0.23 gm/100 gm body wt. from the 15th to the 40th day.

In Group III (common bile-duct obstruction only), the mean weight of the median lobe increased for 30 days, reaching 1.76 ± 0.103 after 4–6 days; 2.26 ± 0.177 after 7–9 days; 2.18 ± 0.103 after 10–12 days; and values varying between 0.15 and 0.23 gm/100 gm body wt. from the 15th to the 40th day.

In Group IV (long-term experiments—twelve rats), the weight of the median lobe increased from 0.040 after 16–18 days. Thereafter there was an increase in weight, reaching 0.58 ± 0.098 between 31 and 40 days.

In rats of Group II (portal vein obstruction to median lobe, common bile duct intact), the reduction in weight of the median lobe was more conspicuous. After 1–3 days the mean weight of the median lobe was 0.81 ± 0.115 gm/100 gm body weight; 0.28 ± 0.023 after 4–6 days; 0.23 ± 0.035 after 7–9 days; 0.20 ± 0.030 after 10–12 days; and values varying between 0.15 and 0.23 gm/100 gm body wt. from the 15th to the 40th day.

In Group III (common bile-duct obstruction only), the mean weight of the median lobe increased for 30 days, reaching 1.76 ± 0.103 after 4–6 days; 2.26 ± 0.177 after 7–9 days; 2.18 ± 0.202 after 10–12 days; 2.14 ± 0.103 after 15–15 days; 2.18 ± 0.263 after 16–18 days; 2.46 ± 0.166 after 19–21 days.

### Table 1

<table>
<thead>
<tr>
<th>Days after operation</th>
<th>PVO</th>
<th>PVO+CBDO</th>
<th>CBDO</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>No. rats</td>
<td>M.Wt. (%)±S. E.</td>
<td>No. rats</td>
</tr>
<tr>
<td>0</td>
<td>20</td>
<td>1.40±0.055</td>
<td>20</td>
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<tr>
<td>1–3</td>
<td>7</td>
<td>0.81±0.115</td>
<td>10</td>
</tr>
<tr>
<td>4–6</td>
<td>6</td>
<td>0.22±0.023</td>
<td>3</td>
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<tr>
<td>7–9</td>
<td>9</td>
<td>0.23±0.035</td>
<td>9</td>
</tr>
<tr>
<td>10–12</td>
<td>7</td>
<td>0.20±0.080</td>
<td>6</td>
</tr>
<tr>
<td>13–15</td>
<td>7</td>
<td>0.22±0.029</td>
<td>5</td>
</tr>
<tr>
<td>16–18</td>
<td>6</td>
<td>0.22±0.030</td>
<td>7</td>
</tr>
<tr>
<td>19–21</td>
<td>9</td>
<td>0.18±0.016</td>
<td>8</td>
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<tr>
<td>22–24</td>
<td>6</td>
<td>0.13±0.010</td>
<td>8</td>
</tr>
<tr>
<td>25–30</td>
<td>12</td>
<td>0.20±0.015</td>
<td>10</td>
</tr>
<tr>
<td>31–40</td>
<td>3</td>
<td>0.21±0.021</td>
<td>3</td>
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PVO = portal vein obstruction; CBDO = common bile duct obstruction.

Estimation of water content of liver tissue.—This was carried out as described previously (11).

Microscopy.—The usual microscopic procedures were carried out after fixation in formol saline and paraffin wax embedding.

Bilirubin.—The method of Malloy and Evelyn (5) was used, for both serum and bile samples, the bile being initially diluted 1:10 with water.

The rats were divided into four groups.

Group 1: 66 rats.—Ligation of portal vein branches to median lobe, and a simultaneous ligation and division of common bile duct.

Group 2: 78 rats.—Ligation of portal vein branches to median lobe. Intact common bile duct.

Group 3: 35 rats.—Ligation and division of common bile duct. Intact portal blood supply.

Groups of animals were killed between 1 and 40 days after the operation.

Group 4: Long-term experiments—twenty rats.—
In Groups I, II, and III the "surgically unmanipulated" lobes (left, right, and caudate) increased in weight, the greatest increase being found in the rats of Group I (portal vein obstruction and common bile-duct obstruction).

In the long-term experiments (Group IV), the weight of the median lobe decreased to 0.90 ± S.E. 0.021 gm/100 gm body wt. 4 months after portal vein ligation in such lobes, but increased to 0.37 ± 0.023 5–7 days after the common bile duct was obstructed.

The water content of the median lobe increased from 70.9 to 80 per cent in Group I, to 76.7 per cent in Group II, and to 76 per cent in Group III.

**Macroscopic appearances.**—In general, after the combined operation the median lobe showed the features usually associated with parenchymal atrophy, eventually becoming a smooth-surfaced tag, whereas the other lobes showed increasing granularity (Figs. 1, 2). The common bile duct developed the great distension usually found after duct obstruction. Serum bilirubin was always raised in cases of bile-duct obstruction and varied between 5 and 14 mg/100 ml.

**Microscopic appearances.**—An increase in the amount of bile-duct tissue was found in all rats subjected to common bile-duct obstruction. This was most conspicuous in the median lobes deprived of portal blood supply. Mitoses in the ductular epithelium were found as early as 1 day after the operation and more frequently at 3 days. At the same time, the parenchymal cells in such lobes appeared to be smaller than normal, and by 6 days there was conspicuous extension of proliferating bile-duct tissue toward the hepatic vein radicle. The degree of bile-duct proliferation in the lobes with intact blood supply was less marked (Figs. 3, 4). By 10–15 days the median lobe hepatic vein radicles in Group I were surrounded by proliferating bile ducts with the parenchyma represented by surviving islets or single cells.

By 16–21 days, the median lobe in Group I (duct obstructed, deprived of portal blood) appeared to consist almost entirely of bile-duct tissue. Hepatic vein radicles were often completely surrounded by duct cells. Less marked duct proliferation was found in all the lobes in the group subjected to common duct obstruction only. In the hypertrophied lobes of Group I, there was also duct proliferation, but this was significantly less than in the lobe deprived of portal blood supply.

By 22–30 days, the portal-deprived median lobe in Group I consisted of vast sheets of bile-duct tissue surrounding occasional parenchymal cells; in Group III (uncomplicated duct obstruction) the amount of ductal tissue was distinctly less, with significant intervening parenchymal tissue (Figs. 5, 6). In the unmanipulated lobes of Group I, the ductal tissue, although increased above normal, was not so conspicuous.

After 31–40 days, these changes were still present, but did not appear to be more advanced.

**DISCUSSION**

Liver lobes, deprived of portal blood supply, rapidly decreased in size up to 6 days, after which time there was only a gradual reduction until about the 18th day. After this time there was no further significant atrophy. If the common bile duct was obstructed at the same time (Group I: portal vein obstruction and common bile-duct obstruction) such lobes weighed more than the corresponding tissue in Group II controls (portal vein obstruction only).

In the long-term experiment, lobes subjected to portal deprivation 4 months previously increased in weight 5–7 days after the common bile duct was obstructed.

It seems clear from these data and from the microscopic appearances that bile-duct tissue proliferates to a greater extent after common duct obstruction, when the lobe is deprived of portal blood flow.

The factors responsible for this are not clear. It is possible that the bile ducts proliferate more actively when parenchymal cells are not proliferating, that in fact there may be an inverse relationship between growth of these two elements. It is possible that the intravisceral pressure is reduced in lobes atrophying liver cells, and that therefore the biliary structures are mechanically more free to proliferate. One might expect a reduced bile duct growth in lobes undergoing parenchymal hypertrophy. This has not been clearly observed in these experiments, but the degree of hypertrophy is not great when induced by atrophy of only one-third (median lobe) of the liver. This concept is difficult to prove, since measurements of intravisceral pressure are not accurate.

Although it is not possible to determine accurately what the mechanism for the increased ductal proliferation is in these experiments, the transformation of the liver lobe into one consisting almost entirely of bile duct tissue is regularly attained by this combination of surgical procedures. The cells have the morphological characteristics of bile duct cells and, although they have developed as a result of rapid growth, it is possible that the chemical and enzymic characteristics may parallel the morphological features sufficiently well to provide a model for analyzing the enzymatic and other characteristics of the mass of bile-duct tissue.
REFERENCES


Fig. 1.—Ventral view of normal rat liver. M = cleft median lobe weighing approximately one-third of total liver. L = left lateral lobe (one-third of total liver). R = right lobe (one-fourth-one-fifth of total liver). C = caudate lobe (one-tenth of total liver).

Fig. 2.—Dorsal view of liver 23 days after combined operation. Note smooth small median lobe.

Fig. 3.—Section of liver 6 days after common bile-duct obstruction. The liver parenchymal cells are darkly stained and proliferating ductules pale. P.T.A.H. X110.

Fig. 4.—Section of median lobe of liver 6 days after common bile-duct obstruction and portal vein obstruction. Note the relatively greater quantity of bile-duct tissue, as compared with that in Fig. 3. P.T.A.H. X125.

Fig. 5.—Section of liver 32 days after common bile-duct obstruction. P.T.A.H. X105.

Fig. 6.—Section of median lobe of liver 32 days after common bile-duct obstruction and portal vein obstruction. The tissue consists almost entirely of palely-stained bile-duct epithelium. P.T.A.H. X125.
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