Effect of Partial Hepatectomy on DNA Synthesis and Mitosis in Heterotopic Partial Autografts of Rat Liver*

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SUMMARY

Functioning, heterotopic, partial hepatic autografts without portal blood supply were used to investigate factors controlling regeneration of liver after partial hepatectomy in rats. Following partial hepatectomy DNA synthesis and mitosis in grafts were virtually identical to those occurring in residual livers of the same animals. These results are consistent with a regulatory mechanism controlling hepatocytic proliferation, active throughout the body and distributed through the systemic circulation (blood-borne). The nature, origin, and manner of action of the blood-borne mediator are not indicated by this study.

This study also confirms that portal blood and increased hepatic blood flow are unnecessary for proliferation of hepatocytes.

In the rat the deficit in mass of hepatic tissue resulting from partial hepatectomy is rapidly replaced by proliferation of remaining cells. The mechanism by which residual hepatocytes "recognize" the deficit and proceed to form a compensating number of new cells is unknown (4). Hepatocytes in all parts of the residual liver, not just those in areas contiguous to the excision wound, proliferate. It is of interest to know whether autochthonous hepatocytes located in distant parts of the body and not directly connected to the residual liver or its blood supply respond similarly. Proliferation of the distantly located hepatocytes would indicate that the mechanism for recognition of deficiency of hepatocytes after hepatectomy is widely disseminated throughout the body by the systemic circulation (blood-borne).

Attempts to solve this problem by use of parabiotic animals have yielded equivocal results (4, 11). There are several reasons why parabiotically connected animals are not ideally suited for this purpose: (a) frequently poor and variable cross-circulation of blood between partners (1); (b) potential immunologic reactions between partners (11); (c) potential differences in functional status of livers in each partner (11); and (d) difficulty in removing enough liver from one partner (11) to cause a maximal proliferative response (4). Each of these factors may seriously affect the manner and extent to which hepatocytes react to stimuli.

Functioning partial autografts of liver are free of these

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liabilities (7). Grafts are located subcutaneously and are separated from the liver by tissues of the abdominal wall. Livers and grafts are connected only by systemic vasculature; blood reaching grafts is identical to that leaving livers except for dilution resulting from mixture with blood from other parts of the body and for possible subtle alterations occurring during transit through the lungs. Hepatocytes in grafts are cytologically normal; function of grafts, as judged by bile flow, is equivalent to that of livers. Presence of grafts will not significantly affect the proliferative response of residual liver, since their size is very small in relation to liver size. In addition, nonspecific factors such as variation in food intake, hormonal stimulation, etc., would have identical influences on both liver and graft of individual rats.

In this study the effect of partial hepatectomy on DNA synthesis in partial hepatic autografts in rats was determined. After partial hepatectomy hepatocytes in grafts responded with a burst of DNA synthesis and mitosis almost identical to that occurring in hepatocytes in residual livers.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 200–300 gm. were caged and fed, and the median lobe of the liver was transplanted to the anterior abdominal wall as previously described (7). To allow cellular changes that occur in newly grafted liver to stabilize (7), grafts were performed 1–3 months before they were used for this study. All grafts used were demonstrated to be free of direct portal venous blood supply by inspection at autopsy, by injection of CrP32O4 or other colloid into the portal vein, or by a combination of these methods (7). Colloid was not
taken up by grafts when injected intraportally but was taken up when injected systemically (7). Adequacy of bile drainage was demonstrated by cannulation of the duct to the graft or by the characteristic histologic appearance of obstructed grafts (7, 15). Location of grafts in the midline and presence of abdominal adhesions from previous surgical procedures made adequate exposure of the liver for partial hepatectomy difficult. Usually a single incision in the right upper quadrant about 1.5 cm. lateral to the midline provided adequate access to the liver, but occasionally it was necessary to make bilateral upper quadrant incisions. An attempt was made to remove the left and right lateral lobes (which constituted about 70 per cent of the liver remaining within the abdomen), but the difficulty of the operative approach, together with absence of the median lobe, resulted in removal of less liver than is usually removed with the standard operation (8); 54.3 ± 4.3 per cent (mean ± standard deviation) of the liver was removed from each animal. Care was taken not to disturb grafts during these procedures. Another group of animals was subjected to the same operation, except that no liver or less than 10 per cent of the liver was removed. All operations were performed at times that allowed DNA synthesis and mitosis in hepatocytes to be measured during early morning hours when they reach diurnal peaks (9, 13). Partially hepatectomized and sham-hepatectomized rats with grafts were separated into groups, each containing six of the former and three of the latter. At 24, 48, and 72 hours after operation DNA synthesis was measured in the animals of one group; 100 μc. of thymidine-H³ (specific activity, 1.9 c/mmole) was injected intravenously into each animal, and they were killed 2–4 hours later. Labeled cells were visualized in autoradiographs of sectioned tissue prepared as previously described (6). To determine the basal level of DNA synthesis in livers and grafts similar studies were made in five animals with grafts that had not been subjected to either partial hepatectomy or sham hepatectomy.

The magnitude of DNA synthesis in residual hepatocytes is dependent on the amount of liver excised (4, 10). In the rat, removal of an amount of liver below a certain threshold (10–20 per cent by wet weight) elicits no significant increase in DNA synthesis in residual cells; after removal of portions of liver greater than the threshold amount, the magnitude of DNA synthesis varies directly with the amount removed (4, 10). Since only about 55 per cent (in contrast to the usual two-thirds) of the liver was removed from animals with grafts, two control groups were used to determine whether grafting procedures altered the quantitative response of residual liver to hepatectomy: five animals of comparable age and weight, but without grafts, were subjected to a standard partial hepatectomy (68.3 ± 4.4 per cent of the liver removed); five similar animals had between 50 and 55 per cent of the liver excised (median lobe and major part of caudate lobe). DNA synthesis was studied in both groups 24 hours later.

To determine the effect of subcutaneous implantation alone on the response of the liver after partial hepatectomy, DNA synthesis was studied at 24 hours after hepatectomy in six rats with median lobes implanted subcutaneously but not separated from the remainder of the liver.

RESULTS

Cells synthesizing DNA or in mitosis were few in both livers and grafts in animals that had not undergone partial hepatectomy (Chart 1; Figs. 1, 2). In livers of these animals an average of 0.32 ± 0.25 per cent of hepatocytes were synthesizing DNA, and only about 0.03 per cent were in mitosis; in grafts corresponding figures were 0.18 ± 0.10 per cent and less than 0.01 per cent. Sham operation produced no increase in these base-line values at any time after surgery.

Partial hepatectomy resulted in a burst of DNA synthesis and mitosis in both residual livers and grafts (Chart 1). Reactions in grafts and in livers were similar, although at comparable times values were slightly lower in the former. At 24 hours after hepatectomy an average of 11.0 ± 2.9 per cent of hepatocytes in liver (Fig. 3) and 6.7 ± 3.2 per cent in grafts (Fig. 4) were labeled. At this time localization of labeled hepatocytes was predominantly periportal in livers (Fig. 3) but tended to be random in grafts (Fig. 4). At 48 hours 6.2 ± 3.0 per cent of hepatocytes in livers (Fig. 5) and 4.5 ± 2.0 per cent of hepatocytes in grafts (Fig. 6) were labeled. Comparable figures at 72 hours were 2.0 ± 1.0 per cent in

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CHART 1.—Per cent mitoses and per cent labeled cells among hepatocytes in livers and grafts in rats before and at different times after partial hepatectomy. Thick bars represent means, and thin lines represent two standard deviations about the means. Groups at each interval contained six animals except for the control group, which contained five animals.
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livers and 1.0 ± 0.6 per cent in grafts. Mitoses were maximal in both grafts and livers at 48 hours after hepatectomy (Chart 1) when an average of 2.9 ± 1.7 per cent of hepatocytes in livers (Fig. 8) and 2.0 ± 1.1 per cent of hepatocytes in grafts (Fig. 9) were so involved. At 24 hours mitotic figures were present in 1.0 ± 0.72 per cent of hepatocytes in livers and in 0.60 ± 0.40 per cent of hepatocytes in grafts. At 72 hours the high level of mitosis present at 48 hours had decreased to 1.1 ± 0.6 per cent of hepatocytes in livers and 0.40 ± 0.21 per cent of hepatocytes in grafts.

Twenty-four hours after two-thirds partial hepatectomy 26.4 ± 6.3 per cent of hepatocytes were labeled in residual livers of five rats without grafts; however, 24 hours after removal of 50–55 per cent of the liver in five similar animals, only 15.4 ± 4.3 per cent of hepatocytes were labeled. The latter figure is not significantly different from the per cent of hepatocytes labeled at a similar time in residual livers in animals with grafts (in which a comparable amount of liver was removed).

In six animals in which the median lobe was implanted subcutaneously but not separated from the portal blood supply, the per cent of labeled cells in livers and the per cent in implanted median lobes at 24 hours after partial hepatectomy (about 50 per cent of the liver removed) were virtually identical (12.4 ± 4.1 per cent in livers and 12.0 ± 3.7 per cent in implanted median lobes).

**DISCUSSION**

Hepatocytes in grafts responded to partial hepatectomy in essentially the same manner as did those in residual livers; patterns of labeling in both tissues were qualitatively and quantitatively similar, but not identical. The per cent of labeled hepatocytes reached a peak level at 24 hours after hepatectomy in both livers and grafts and declined rapidly at later intervals in both tissues. The location of labeled hepatocytes at the time of peak labeling was predominantly periportal in residual livers, as previously described (5, 6, 12) but tended to be more diffuse in grafts. This difference is explicable in terms of the changes that occur in grafted liver. Marked centrolobular congestion and extensive centrolobular necrosis occur in newly grafted liver, and established grafts are thought to consist mainly of hepatocytes that were originally periportal (7, 15); if this is correct there is no inconsistency in the location of labeled hepatocytes in livers and grafts.

The per cent of labeled hepatocytes was consistently less in grafts than in livers. This may have resulted from dilution of hepatic effluent blood by blood from other parts of the body before it reached grafts or from poorer blood supply to grafts than to livers, with an attendant reduction of substances reaching grafts. Comparative uptake by livers and grafts of colloidal materials injected into the systemic circulation is consistent with the latter viewpoint (7). This is further supported by the observation that in implanted median lobes with intact blood supply the per cent of labeled hepatocytes was identical to that in livers of the same animals.

Fewer labeled hepatocytes in both livers and grafts than in residual liver after classic two-thirds partial hepatectomy is explicable on the basis of less liver removed. Decrease in the amount of liver removed by 10–15 per cent from the optimal 65–70 per cent resulted in decreasing the number of labeled cells by about one-half. This change in magnitude of response with the different amounts of liver excised is consistent with that reported by others (4, 10).

Some form of regulation of cellular proliferation is necessary in order for the mass of liver to be so precisely constituted after experimentally produced deficit (4). Since the reaction of hepatocytes to a deficiency of liver tissue is proliferation, the regulatory mechanism must involve, either directly or through intermediary reactions, the synthesis of DNA. The results of this study clearly indicate that at least a component of this regulatory mechanism is blood-borne; after partial hepatectomy, hepatocytes in grafts separated from the hepatic blood supply and hepatocytes in residual livers reacted similarly—i.e., with a burst of DNA synthesis. It is not apparent from this study what the regulatory mechanism is or in what form the blood-borne component occurs. The results are consistent, however, with the concept that the liver itself generates conditions that regulate proliferation of hepatocytes. Whether these conditions result from reactions occurring entirely within the liver (19) or through metabolic interactions between liver and other tissues is not demonstrated. The regulatory mechanism may not be generated specifically for control of hepatocytic proliferation but may, instead, arise through some functional process of the liver. Whether reactions leading to DNA synthesis are specifically stimulated by partial hepatectomy or are inhibited normally and merely released from inhibition by partial hepatectomy (4) cannot be deduced.

**Figs. 1–6** are autoradiograms of sections from rats given thymidine-\(^3\)H\(^2\) 2–4 hours before they were killed. Autoradiographs were stained with hematoxylin and eosin. All magnifications are X 390.

**Figs. 1, 2.**—Liver and graft (respectively) from a sham-hepatectomized rat. There are no labeled cells in these fields.

**Figs. 3, 4.**—Liver and graft from a rat at 24 hours after hepatectomy. In both tissues labeled hepatocytes are numerous; they are grouped around portal spaces in the liver but are located diffusely in the graft.

**Figs. 5, 6.**—Liver and graft from a rat at 48 hours after hepatectomy. Labeled hepatocytes are less numerous in both tissues than at 24 hours. Labeled littoral cells are frequent.

**Figs. 7, 8.**—Liver and graft from a rat at 48 hours after hepatectomy showing numerous mitoses in both tissues. H. & E., mag. X 840.
from this study; results are consistent with either hypothesis.

Earlier studies utilizing parabiotically connected animals suggested, although in many instances the evidence was equivocal and often contradictory, the existence of humoral factors regulating hepatocytic proliferation (4). This study offers more definitive evidence for the existence of a blood-borne mediator.

Previous attempts have been made to study the effect of partial hepatectomy on partial hepatic autografts, with weight of grafts used as the endpoint (14, 15). Attrition of grafts was less rapid in partially hepatectomized animals than in control animals with intact livers. This was interpreted as evidence of a humoral factor. Our study agrees with this tentative conclusion; use of DNA synthesis rather than graft weight as the endpoint is more precise (4) and probably explains the more decisive results.

This study incidentally confirms that portal blood is unnecessary for hepatic regeneration (18). In grafts entirely lacking in portal blood, DNA synthesis was only slightly less than in residual livers or in median lobes after subcutaneous implantation (portal vein intact). Hepatic blood flow per unit of hepatic tissue increases after partial hepatectomy (2), and it has been hypothesized that this increase is important in initiating regeneration (12). Increased systemic blood flow (including that to grafts) probably does not occur after partial hepatectomy (3) and would not, therefore, be responsible for initiating DNA synthesis in grafts. Equivalent regenerative activity in both grafts and livers is compatible with the belief that increased total blood flow through residual liver has little importance in controlling regeneration after partial hepatectomy (2).

REFERENCES

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