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Research Article

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Effect of Blood Flow Reversal in Liver Autotransplants upon the Site of Hepatocyte Regeneration

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ABSTRACT We studied the role of the direction of intrahepatic blood flow upon the location of hepatocyte formation in regenerating liver. Single liver lobes in the dog were autotransplanted to the region of the neck with the blood supply reestablished in a manner to perfuse the hepatic lobule from portal tract to central vein or, in a reverse direction, from central vein to portal tract. Partial resection of the nontransplanted liver was later performed to induce regeneration in the grafts by humoral means. Tritiated thymidine was administered, and radioautographs were prepared from excised graft and nontransplanted liver. In the "straight" blood flow grafts, as well as in all nontransplanted livers, labeled hepatocytes indicating DNA synthesis were found predominantly in the vicinity of the portal tracts. In the "reverse" blood flow grafts, labeled hepatocytes were more prevalent about the central veins. Thus, the localization of hepatocyte formation in the lobule during active liver regeneration cannot be attributed to an inherently greater capacity of periportal liver cells to divide but is probably related to their preferential exposure to blood constituent changes (humoral mechanisms). Hepatocyte regeneration in the presence of abnormal directional circulation might lead to lobular disorganization resulting in consequent biochemical aberrations despite the formation of new cells.

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INTRODUCTION

Early in active liver regeneration after partial hepatectomy, hepatocytes tend to form predominantly in periportal areas. Both the number of hepatocytes in mitosis (1–4) and the number of hepatocytes synthesizing deoxyribonucleic acid in tritiated thymidine radioautographs (5, 6) are greater in the vicinity of the portal tracts than near the central vein areas of the liver lobules. This localization occurs early during regeneration and is transient. Later during the regenerative process, labeled hepatocytes are more evenly distributed throughout the lobules. Grisham (6) has speculated that this early periportal localization followed later by a more even distribution of labeled hepatocytes may be caused by hepatocytes originating near portal tracts and subsequently migrating toward the central veins. Leblond (7), in discussing Grisham's work, suggested that as new cells are added to the periphery of the lobule the first ones to be formed become positioned nearer to the central vein areas. This theory was offered as an alternative explanation to active migration by liver cells. However, regardless of what may ensue later, it appears that early in liver regeneration hepatocyte formation is segregated to the periportal regions of the liver lobules. The reason for this localization of hepatocyte regeneration has not been established. To begin an assessment of this occurrence, it appears appropriate to consider first over-all mechanisms which regulate liver regeneration.

It has been suggested that liver regeneration after partial hepatectomy is under humoral control (8–10). The existence of regulating humoral mechanisms in liver regeneration has been a controversial issue (11–14). However, more recently, we (15–18) and others (19, 20) have shown that partial liver autotransplants are effected by partial resection of the nontransplanted liver. This effect upon liver grafts is both antatrophic and regenerative, and the response of the hepatocytes in the graft is similar to that observed in the nontransplanted liver. These results are in keeping with the concept that liver regeneration is primarily under humoral control. Furthermore, we observed that induced hepatocyte regeneration in liver autografts, also, tends to be localized to periportal sites (17).

The induction of hepatocyte formation during active liver regeneration predominantly in periportal areas may be due to one of two factors. First, hepatocytes in this region may possess an inherently greater tendency to divide. Second, hepatocytes during active regeneration may have an equal potential for division, but hepatocytes located near the lobular blood inflow may be primarily affected because of their preferential exposure to changes in blood constituents (humoral mechanisms). To determine which of these two factors is responsible for the localization of new hepatocyte formation during active liver regeneration, we conducted the following experiment. Single liver lobes were autotransplanted to the neck region of dogs. The circulation in these grafts was reestablished in either a "straight" or "reverse" direction. In "straight" blood flow grafts, the entire blood inflow was directed in through the portal vein and out through the hepatic vein. In "reverse" blood flow grafts, the entire blood supply was directed in through the hepatic vein and out through the portal vein. Partial resection of the nontransplanted liver was later performed to induce regeneration in the grafts. Hepatocytes synthesizing deoxyribonucleic acid (DNA) were then located by tritiated thymidine radioautography. The primary purpose of this experiment was to establish whether the early localization of regeneration by hepatocytes was dependent upon the direction of lobular blood flow.

METHODS

Both sexes of adult mongrel dogs of indeterminate age weighing between 9.0 and 13.9 kg were used. At operation, the left lateral and left central liver lobes were resected. The left central lobe was discarded, and the left lateral lobe (about 29% of the liver) was autotransplanted to the region of the neck. The bile duct and hepatic artery of the graft were ligated. Two types of vascular connections were employed. In nine animals, the cephalic end of the carotid artery was anastomosed to the graft portal vein, and the graft hepatic vein was anastomosed to the jugular vein. This resulted in a "straight" blood flow liver graft. In nine animals, the cephalic end of the carotid artery was anastomosed to the graft hepatic vein, and the graft portal vein was joined to the jugular vein. This produced a "reverse" blood flow graft. All anastomoses were end to end. The cephalic end of the carotid artery was used to reduce perfusion pressure and, thereby, decrease graft engorgement with blood. The technical details of these two operations are reported elsewhere (21, 22). 1 wk after this procedure, all animals were submitted to a second operation. At this time, all but the right central lobe and caudate lobe minus the papillary process were resected, leaving about 30% of the original liver in the abdomen. 3 days later, tritiated thymidine (specific activity 1.9 c/mm)[1] was injected intravenously at a dose of 1 mc/kg of body weight. 2–2½ hr after this injection, wedge sections of grafted and nontransplanted liver were obtained. 2 days and 4 days after tritiated thymidine injection, tissue sections were again obtained from both liver sites. After the last biopsy procedure, the animals were killed. One animal, in poor condition, had biopsies performed on the 3rd day and then was killed. All operations including those to retrieve liver tissue were conducted under pentobarbital anesthesia.

Radioautographs were prepared by the emulsion method (23) from sections of excised tissue 5 μ in thickness. All tissue sections were randomized and coded by a technician before being given to the microscopist, so that source of material (whether parent or graft), direction of blood flow, and time of biopsy were not known. While it was usually quite easy histologically to tell graft from nontransplanted liver, it was not possible to distinguish between "straight" and "reverse" blood flow in the grafts. The microscopist also had no knowledge of the total number of dogs used or on what proportion of them each procedure had been done.

A systematic survey of the entire piece of each tissue section was made at 250 magnification, using an eyepiece reticle[2] to mark the center of the field and to keep the portal triads and central veins studied of comparable dimensions. Adjacent fields were studied, and the stage was moved so that no field overlapped the previous one. When either a portal tract or central vein was encountered, the microscope stage was adjusted slightly to

[1] Schwarz Bio Research, Inc., Orangeburg, N. Y.

[2] Edmund Scientific Co., Barrington, N. J. No. 30077, marked in concentric circles 0.5 mm apart, total diameter 23 mm.

center the structure in the field, and labeled hepatocytes were counted and recorded. The stage was then returned to the original path of the survey sweep. If a portal tract or central vein exceeded 17–19 μ in any direction, or if a portal tract or central vein appeared together in a single field, they were excluded.

Definition of structures in this study were made as follows. A portal tract was considered to be a distinguishable triad consisting of one or more bile duct structures, a branch of the hepatic artery, and a small vein. A central vein was defined as a single or branched endothelial-lined vessel with a poorly developed musculofibrous coat, unassociated with bile duct or arterial structures. A labeled hepatocyte was defined as a cell with clearly distinguishable rounded nucleus and clear cytoplasm, with arrangement of granules approximating a "target." In instances in which no labeled hepatocytes were seen on a given slide, it was noted that labeled cells of other varieties were seen, and no failure in processing was felt to have occurred.

RESULTS

Five animals with "straight" flow and four with "reverse" flow grafts either died after the operations or had necrotic transplants at the time planned for tritiated thymidine injection. All observations were made on the remaining nine dogs. In these nine animals, some of the graft biopsies were unsuitable for counting either because of small size or of areas of hemorrhage or necrosis. The histologic appearance of the nontransplanted livers was not remarkable in hematoxylin-eosin–stained sections. Tissue from both types of grafts showed centrilobular atrophy, sinusoidal congestion, and bile duct proliferation. These changes have been described previously (21, 22).

The average numbers of labeled hepatocytes counted in portal tract and central vein fields are shown in Table I. The actual numbers of portal tract and central vein fields counted are shown in Table II. In all instances, more labeled hepatocytes were seen in portal tract fields than in central vein fields in sections obtained from nontransplanted livers and the "straight" blood flow grafts. This persisting tendency did not hold for the "reverse" flow grafts. In "reverse" flow grafts, labeled hepatocytes tended to be more numerous in the central vein fields than in the portal tract fields. Of the seven biopsies obtained from five

TABLE I

Tritiated Thymidine–Labeled Hepatocytes in "Straight" and "Reverse" Blood Flow Grafts and in Parent (Nontransplanted) Liver Tissue

			Nontransplanted liver		Graft	
	Dog No.	Time*	Average No. of LHN in portal tract field	Average No. of LHN in central vein field	Average No. of LHN in portal tract field	Average No. of LHN in central vein field
Animals with "straight"	1757	2 hr	8.3	2.7	8.7	0.8
blood flow grafts	1810	2 hr	4.5	1.1	5.0	3.1
		2 days	4.1	2.4	3.3	0.3
		4 days	1.6	0.7	3.5	1.0
	1913	3 days	3.4	0.4	5.7	1.0
	1823	2 days	1.5	0.2	0.8	0.1
		4 days	0.4	0.1	5.6	2.9
Animals with "reverse"	1864	4 days	4.1	0.7	4.0	4.0
blood flow grafts	1765	2 hr	2.1	1.3	4.5	8.0
		4 days	8.5	4.0	3.0	2.4
	1820	2 hr	2.7	1.8	0.0	5.5
		4 days	3.9	1.8	1.6	16.5
	1851	2 hr	0.4	0.2	0.0	2.5
	1894	4 days	1.6	0.1	0.3	6.7

LHN, labeled hepatic nuclei.

* Time interval between injection of tritiated thymidine and biopsy.

TABLE II

Number of Portal Tract and Central Vein Fields Counted for Each Biopsy Specimen

Tissue	Average No. of portal tract fields counted	Range of portal tract fields counted	Average No. of central vein fields counted	Range of central vein fields counted
Nontransplanted liver	19	4–52	13	5–25
Graft liver	11	1–28	11	5–28

dogs, only one of the seven showed more labeled hepatocytes in the portal tract fields. In this one instance, sections obtained earlier in the study showed more labeled hepatocytes in the central vein fields. Thus, in all five "reverse" flow grafts, the number of labeled hepatocytes in central vein fields either equaled or exceeded the number in the portal tract fields at least once during the study period.

The number of labeled hepatocytes was usually greater in the grafts than in the nontransplanted liver from the same animal.

DISCUSSION

We have previously used the partial liver heterotopic transplant to investigate the role of humoral mechanisms in liver regeneration (17, 18). This work supported the concept of humoral mechanisms in the regulation of liver regeneration. In the present study, we wished to apply the same method to stimulate regeneration in aberrantly located liver tissue in order to determine the role of the direction of intrahepatic blood flow upon the location of hepatocyte formation. The experimental model which we used is relatively artificial and unstable. Consequently, the means of determining results and their interpretation must be carefully considered.

The design of this experiment was largely influenced by our previous experience with partial heterotropic liver transplants in dogs. First, we knew that the liver tissue in such grafts was not normal, and that with time the grafts became reduced in size (about half the original size in 6 wk). This was probably due to the abnormal blood supply of the grafts and the ligation of their bile ducts. Histologically, there was atrophy which was predominantly centrilobular, sinusoidal dilatation, portal fibrosis, and bile duct hyperplasia. Thus, it seemed desirable to perform the experiment as soon as feasible after transplantation in order to have the most normal-appearing graft tissue.

Second, we anticipated great interanimal variation in the number of DNA-labeled hepatocytes in regenerating liver. However, we had previously determined that there is significant correlation between the number of labeled hepatocytes in the graft and in the nontransplanted liver of the same animal (17). Consequently, an important observation was the relative proportion of labeled hepatocytes in portal tract fields compared to central vein fields in grafts and in the nontransplanted liver tissue from the same animal.

Since we would be looking for a possible increase in labeled hepatocytes about the central vein in "reverse" flow grafts, it was desirable to avoid any action which would tend to exaggerate the number of labeled hepatocytes seen in central vein fields. We had previously observed that the type of graft used in this experiment will atrophy. This atrophy is predominantly centrilobular and is more accelerated in "reverse" flow grafts than in "straight" flow transplants. There are fewer liver cells per microscopic field in the central vein part of the lobule than in the region of the portal tract. If one were to count the number of labeled hepatocytes surrounding a central vein in terms of the total number of hepatocytes present in that field, a higher value would be obtained than with a similarly counted adjacent portal tract field. This would produce an apparent increase in labeled hepatocytes in the central vein, particularly in "reverse" flow grafts. To avoid this, we reported labeled hepatocyte counts in terms of high power fields rather than total number of hepatocytes.

Although we have tried to anticipate the limitations of the methodology and have attempted to minimize their influence, still they must be considered in the interpretation of results.

The main point of our experimental results was the consistent tendency for more labeled hepatocytes to be aggregated in central vein fields than in portal tract fields of "reverse" flow grafts. This was in striking contradistinction to the labeled hepatocyte distribution in the nontransplanted livers in the same animals and to all liver tissue with a "straight" directional blood flow. An important question to be considered is whether the

central localization of labeled hepatocytes in "reverse" flow grafts was primarily due to reversal of blood flow or secondarily due to effects of reverse revascularization upon the graft.

It has been mentioned that in our previous studies the "reverse" flow grafts may have deteriorated more rapidly than "straight" flow grafts. If this is so, there may be greater necrosis in the "reverse" grafts. Death of tritiated thymidine–labeled cells may result in reutilization of the label (24–26). If this were to occur, it is conceivable that the area of most extensive necrosis and atrophy, the centrilobular region, may have contained an increase in labeled hepatocytes on this basis. Although this is reported to occur mainly as a result of leukocyte breakdown, and the extent of such reutilization in regenerating liver is only about 10% (24), this eventuality should be considered as a possible contributor to the increase in the number of labeled hepatocytes in central vein fields in the "reverse" flow grafts. All tissue sections from the "reverse" flow grafts were ranked in order of least to most necrosis. The ranked order of necrosis was compared to the number of labeled hepatocytes in the central vein fields by means of Spearman's Rank Correlation. There was no significant correlation (rank correlation was -0.54) between the extent of necrosis and the number of labeled hepatocytes in the central vein fields. Thus, the extent of central vein field localization of labeled hepatocytes did not seem to be related to the degree of necrosis.

Although the number of animals used in this study is small, some tentative conclusions regarding the effects of blood flow reversal upon liver regeneration may be made. There seems to be a definite tendency for new hepatocyte formation to be dependent upon the direction of lobular blood flow after partial hepatectomy. When the direction of flow is from portal tract toward central vein, new liver cells appear mostly in the areas nearest to portal tracts. When the direction of blood flow is from central vein toward portal tract, new hepatocytes tend to be more numerous about the central vein than around the portal tracts. This observation tends to substantiate further the existence of humoral mechanisms in the control of liver regeneration. Those liver cells first exposed to a constituent change in the circulating blood are induced to initiate DNA synthesis.

The results of this experiment indicate that periportal hepatocytes are not necessarily the progenitor cells of the liver. Regenerative capacity may reside in hepatocytes throughout the lobule. This finding is also suggested by the observation that hepatocytes throughout the lobule may divide (6). However, when a blood-borne stimulus to regeneration is supplied to the lobule, the hepatocytes nearest to the inflow will undergo the greatest response.

The dependency of new hepatocyte regeneration upon the direction of intrahepatic blood flow may have an indirect influence upon liver function. Hepatocytes in various regions of the liver lobule show differences in structure and function. It has been observed that hepatocytes in the central vein areas are most susceptible to ischemic damage (27), show a higher incidence of polyploidy (28), and are most active in accumulating lipids and releasing glycogen (29). Hepatocytes in portal tract areas are regarded as least susceptible to ischemic injury (27) and are most active in glycogenesis (29). Novikoff and Essner (30) have reported that the relative concentration of certain enzymes determined histochemically differ according to the location of the hepatocyte in the lobule. These regional differences have been attributed to adaptive processes related to establish gradients along the course of the sinusoids (27, 30).

To test the hypothesis that lobular zonation of function is an adaptation to blood constituents, we conducted experiments utilizing partial liver autografts with completely reversed blood flow. Such an alteration in the direction of blood flow would be expected to drastically change constituent gradients along the course of sinusoids. These studies revealed that the sites of predominant lipid accumulation and glycogenolysis were unchanged weeks after complete blood flow reversal (21). Similarly, the characteristic location of centrilobular injury after carbon tetrachloride administration persisted in the presence of reversed flow (31). We concluded from these findings that reversal of intralobular blood flow had no immediate effect upon established zone functions.

An alternative explanation for differences in hepatocyte function according to lobular regions may be related to the site of formation and age of hepatocytes, as suggested by Schepers (28). According to Schepers, hepatocytes drift in a cen-

tripetal direction (from paraportal to centrilobular locations) during their life span. During this process, older cells tend to become polyploid. Consequently, more of the polyploid cells are seen near the central veins. With aging, changes in some of the cell functions also occur. The prevalence of a given hepatocyte function in one lobular region over another, therefore, may be simply the expression of the state of maturity of most of the liver cells within it. If this be the case, the site of hepatocyte regeneration becomes important in determining lobular zone function. Hepatocytes originating in an aberrant locus may result in an imperfect reformation of the liver function unit. A derangement of function may result, therefore, not only from a lack of hepatocytes but also because of their disorganized interrelationships. Such an eventuality has clinical implications. It is believed that the course and direction of intrahepatic blood flow in cirrhosis of the liver is altered (32). Thus, in a cirrhotic liver after a strong stimulus to regeneration, foci of new liver cells may form in abnormal locations near inflow blood vessels. This may result in a random pattern of attempted lobular reformation. If this occurs, impairment of function may result and may ultimately cause the cirrhotic liver to fail even though new hepatocytes continue to be formed.

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