Heterotopic Partial Autotransplantation of Rat Liver: Technic and Demonstration of Structure and Function of the Graft*

J. W. GRISHAM, G. F. LEONG, AND B. V. HOLE

(U. S. Naval Radiological Defense Laboratory, San Francisco, California; and Washington University School of Medicine, St. Louis, Missouri)

SUMMARY

Previous attempts to heterotopically autograft parts of liver were reviewed. This review suggested that both adequate bile drainage and adequate blood flow were necessary for successful autografting of hepatic tissue. A two-stage procedure for the subcutaneous autotransplantation of the median lobe of the rat’s liver by pedicle transfer was modified from Seneviratne’s procedure. The modified technic, which separated the graft from the hepatic blood supply but preserved the bile drainage pathway, resulted in preservation of one-fourth to one-third of the mass of the median lobe with relatively normal structure and function for at least 1 year. This study demonstrated that hepatocytes in autografts could be maintained with normal relationship to bile ducts in the absence of portal blood, if collateral blood supply and biliary drainage were competent. Potential uses of partial autografts of liver for studying certain aspects of hepatic physiology were indicated.

Attempts to perform autologous transplantation of various amounts of liver to heterotopic sites in several mammalian species have been documented during the past 65 years (Table 1). Most efforts to maintain normal hepatic structure in the transplanted tissue failed; studies of functional integrity were rarely reported. The ultimate outcome of most attempts to autograft liver heterotopically was proliferation of bile ducts with concomitant atrophy of hepatocytes or destruction of both tissue elements. Proliferation of bile ducts has been interpreted as evidence that biliary ductal cells have a greater potential for growth in grafts than do hepatocytes (3); however, it seemed more likely to us and to others (23) that the basis of this proliferation might be biliary obstruction in the graft. To test this hypothesis we modified, in order to preserve biliary drainage, Seneviratne’s two-stage pedicle method of transplanting the median lobe of the rat’s liver to subcutaneous tissue (19). The modified procedure resulted in persistence for at least 1 year (upper time limit of our study) of an appreciable mass of cytologically and functionally near-normal hepatic parenchyma which received its blood supply from subcutaneous collateral vessels.

This communication reports the modified technic, describes the structural and functional aspects of the resulting subcutaneous hepatic autograft, and compares the results with those obtained using Seneviratne’s method.

MATERIALS AND METHODS

ANIMALS AND THEIR CARE

Male Sprague-Dawley rats weighing 200–300 gm. were used. The rats were housed five to a cage in wire-bottomed hanging cages in rooms maintained at constant temperature and humidity. They were fed Purina chow and water ad libitum. Some 250 rats were subjected to the operation to be described.

TECHNIC OF OPERATION

The basic procedure has been described by Seneviratne (19). However, since we have introduced several modifications that have proved to be essential for success, our technic will be described in detail. Subsequently in this report this method will be referred to as the modified technic, in contradistinction to the technic of Seneviratne.

The first stage of the operation consisted of implanting the median lobe of the liver to the abdominal subcutaneous tissue, leaving a pedicle attachment to the liver. Animals were anesthetized with ether. Antiseptic conditions were maintained during the operation. After the abdominal area was shaved, a midline skin incision was made from the xiphoid process to a point 3 cm. posteriorly. The skin was undermined widely in all directions around the incision. Care was taken to stop bleeding from broken subcutaneous vessels. Next an incision 3 cm. in length was made through the linea alba. Usually the xiphoid process was removed at this time, since it interfered with...
<table>
<thead>
<tr>
<th>Date</th>
<th>Investigator(s)</th>
<th>Species</th>
<th>Graft sites</th>
<th>Observation or survival time</th>
<th>Results</th>
<th>Evidence of function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1898</td>
<td>Ribbert (17)</td>
<td>Rabbit; guinea pig</td>
<td>Lymph nodes</td>
<td>4–5 weeks</td>
<td>Hepatocytes and ducts nearly normal for 1–2 weeks; after 2–3 weeks hepatocytes isolated by fibroblasts; gradual atrophy and disappearance of latter; necrosis of bile ducts or proliferation to form adenoma-like nodules</td>
<td>None</td>
</tr>
<tr>
<td>1899</td>
<td>Lubarsch (11)</td>
<td>Rabbit</td>
<td>Pulmonary veins; liver; kidney</td>
<td>3–4 weeks</td>
<td>Degeneration of intravenously injected hepatocytes; small pieces of liver usually resorbed or became fibrotic; rarely, microscopic fibro-adenoma-like foci of connective tissue and proliferated bile ducts after 3–4 weeks</td>
<td>None</td>
</tr>
<tr>
<td>1904–1905</td>
<td>Nichols (15)</td>
<td>Rabbit; guinea pig</td>
<td>Mesenteric vein; subcutaneous tissue</td>
<td>163 days</td>
<td>Uniform degeneration, atrophy and fibrosis</td>
<td>None</td>
</tr>
<tr>
<td>1924</td>
<td>Mitsuda (12)</td>
<td>Rabbit</td>
<td>Subcutaneous tissue</td>
<td>13 days</td>
<td>Necrosis and fibrosis of center of graft; initial proliferation of peripheral hepatocytes but ultimate atrophy; persistence of bile ducts</td>
<td>None</td>
</tr>
<tr>
<td>1926</td>
<td>Herxheimer and Jorns (7)</td>
<td>Rabbit</td>
<td>Subcutaneous tissue</td>
<td>39 days</td>
<td>Necrosis and fibrosis of center of graft; proliferation of peripheral hepatocytes up to 2 weeks, then isolation by fibrous tissue and atrophy of hepatocytes; continued proliferation and cystic dilatation of bile ducts; Kupffer cells not identified</td>
<td>Hepatocytes contained glycogen</td>
</tr>
<tr>
<td>1934–1935</td>
<td>Cameron and Oakley (3) Duthie (5)</td>
<td>Rat</td>
<td>Peritoneal cavity; subcutaneous tissue</td>
<td>269 days</td>
<td>Necrosis, resorption, and fibrosis of graft except for thin rim of peripheral cells, proliferation of bile ducts beginning after 1 week and continuing over next several weeks; concomitant degeneration of hepatocytes, few remaining by 5 weeks; at 5 weeks graft composed of fibrous tissue and dilated, proliferated ducts</td>
<td>None</td>
</tr>
<tr>
<td>1937</td>
<td>Bock and Popper (2)</td>
<td>Rabbit</td>
<td>Anterior chamber of eye</td>
<td>8 months</td>
<td>Regressive changes in hepatocytes and proliferation of connective tissue in portal fields near iris by 3d day; further fibrosis with necrosis of hepatocytes and proliferation of bile ducts by 5th day; hepatocytes resorbed and by 1 month graft composed of knots of proliferated bile ducts, pigmented cells, and connective tissue</td>
<td>None</td>
</tr>
<tr>
<td>1938</td>
<td>Schaefer (18)</td>
<td>Rat</td>
<td>Peritoneal cavity</td>
<td>?</td>
<td>Total necrosis and fibrosis</td>
<td>None</td>
</tr>
<tr>
<td>1949</td>
<td>Tiedemann (26)</td>
<td>Rat</td>
<td>Subcutaneous tissue</td>
<td>12 days</td>
<td>Necrosis and fibrosis with persistence of some bile ducts</td>
<td>None</td>
</tr>
<tr>
<td>1950</td>
<td>Knake (8, 9)</td>
<td>Rat</td>
<td>Mesentery; mesentery</td>
<td>5 months</td>
<td>Focal necrosis and fibrosis; ingrowth of capillaries; proliferation of remaining hepatocytes and bile ducts</td>
<td>None</td>
</tr>
<tr>
<td>1953</td>
<td>Habernehe and Diefenthal (6)</td>
<td>Guinea pig</td>
<td>Muscle; kidney; liver</td>
<td>64 days</td>
<td>Total necrosis and fibrosis</td>
<td>None</td>
</tr>
</tbody>
</table>
TABLE 1—CONTINUED

<table>
<thead>
<tr>
<th>Date</th>
<th>Investigator(s)</th>
<th>Species</th>
<th>Graft sites</th>
<th>Observation or survival time</th>
<th>Results</th>
<th>Evidence of function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1952—</td>
<td>Myren and Vinje (14); Myren (13)</td>
<td>Mouse</td>
<td>Mesotestis</td>
<td>1 year</td>
<td>Marked centrolobular congestion and necrosis after separation from hepatic blood supply; 1 month or more later, hepatocytes persisting in strands or islets surrounded by connective tissue; slight bile duct proliferation; vascular channels resembling sinusoids lined by phagocytic cells</td>
<td>None</td>
</tr>
<tr>
<td>1955</td>
<td>Seneviratne (19)</td>
<td>Rat</td>
<td>Subcutaneous tissue</td>
<td>8 months</td>
<td>Marked centrolobular congestion, necrosis and fibrosis; extensive proliferation of bile ducts and fibrous tissue; slow atrophy of hepatocytes; by 2 months graft composed of masses of cystic proliferated ducts, rare hepatocytes and fibrous tissue; little further change over next 6 months</td>
<td>None</td>
</tr>
<tr>
<td>1960</td>
<td>Thorbjarnsson et al. (25)</td>
<td>Dog</td>
<td>Peritoneal surface of spleen</td>
<td>8 months</td>
<td>Hemorrhagic infarction and massive necrosis of some grafts; persistence but slow atrophy of hepatocytes in other grafts; at 8 months grafts composed of haphazard cords of hepatocytes (some of these multinucleated), proliferated bile ducts and fibrous tissue</td>
<td>Canicular bile plugs</td>
</tr>
<tr>
<td>1960—</td>
<td>Sigel et al. (20 to 23)</td>
<td>Dog</td>
<td>Lumen of isolated loop of small intestine</td>
<td>18 months</td>
<td>Focal centrolobular congestion and necrosis followed by collapse and fibrosis; lobular architecture maintained for 3–6 months and recognizable after 1 year in spite of gradual atrophy and loss of hepatocytes; glycogen but no fat and normal localization of alkaline phosphatase in hepatocytes located in cords; slight proliferation of bile ducts</td>
<td>Storage and release of glycogen in normal manner; bile plugs</td>
</tr>
<tr>
<td>1962</td>
<td>Benichoux et al. (1) Rauber, et al. (16)</td>
<td>Rabbit; dog</td>
<td>Wall of stomach; omentum</td>
<td>More than 20 days</td>
<td>Omental grafts necrosed; intense congestion of gastric grafts with occasional hematomas; necrosis of most hepatocytes; after 20 days graft composed of thin layer of hepatocytes and cystic, proliferated bile ducts</td>
<td>None</td>
</tr>
</tbody>
</table>

subsequent manipulations of the liver. The median lobe was freed from its ligamentous attachments to the diaphragm and to the stomach.

The vascular and biliary structures in the porta hepatis were next identified. In the rat, bile ducts from the median and left lateral lobes join near the substance of the liver to form a common trunk and the ducts from the caudate and right lateral lobes enter at points successively nearer to the duodenum to form the common bile duct (Chart 1). The portal vein and the hepatic artery branch in a similar manner; however, the latter vessel is somewhat variable in its course. Both vascular channels usually are located dorsal to the common bile duct, the portal vein slightly to the right and the hepatic artery slightly to the left. In our experience the most common variation in the position of the hepatic artery from that just noted was a course closely following the ventral aspect of the common bile duct. Animals in which this variation occurred could not be used, because the artery in this position could not be included within the ligature containing the portal vein and base of the median lobe if at the same time the bile duct was excluded.

The bile duct was isolated and dissected free proximal to the point where branches from the median and left lateral lobes joined. A 000 steel suture was passed beneath the branch of the bile duct extending to the median lobe and was brought around the base of the lobe so that the lobar hepatic artery and portal vein and the substance of the base of the lobe, including the lobar hepatic vein, were contained within the loop thus formed (Chart 1). The deep fissures separating the median lobe from its neighbors facilitated this step in the procedure. A loose double knot was made in the suture so that the loop fit snugly around the base of the lobe but did not cut into the liver substance and did not restrict inflow or outflow of blood.

After the liver was returned to the abdomen, the ends of the suture were brought out through the abdominal wall on each side ca. 3 cm. lateral to the midline at a point just below the costal margin. The mobilized median lobe was then brought through the incised muscle, and the latter was closed with a continuous 000 nylon suture so that the base of the lobe was enclosed closely but was not constricted. The exteriorized lobe was observed briefly.
The effects of in situ procedures were compared with the effects of similar manipulations combined with grafting.

To determine that free exchange of blood occurred. It was then smoothed out over the abdominal musculature, and the skin was closed over it with a continuous 000 nylon suture. The ends of the steel suture were anchored to the skin with metal clips.

Two weeks later the second stage of the operation was completed when the steel ligature was closed by pulling its protruding ends. This separated the subcutaneous transplant from its intra-abdominal base, thereby closing the hepatic artery and vein and the portal vein connecting graft to liver. The ends of the steel suture were clipped at the skin surface and were allowed to retract.

**OTHER OPERATIVE PROCEDURES**

To obtain additional information about conditions resulting in success or failure of grafts, five to twenty animals were subjected to each of the following operations.

1. Seneviratne procedure (19)—i.e., ligation of entire pedicle including lobar bile duct at second stage of operation. (Grafts produced in this manner were compared with those resulting from the modified technic; this procedure tested the effect of biliary obstruction on grafts.)

2. Ligation of pedicle during first 2 days after implantation. (This procedure was used to study the importance of allowing collateral circulation to develop before disrupting the normal blood supply.)

3. Ligation of lobar branches of the portal vein or of the bile duct or of both these structures in situ. (The effects of in situ procedures were compared with the effects of similar manipulations combined with grafting.)

**FUNCTIONAL MEASUREMENTS**

**Bile flow.**—Bile flow from both liver and subcutaneous graft was measured by cannulation of the bile duct. Rats were anesthetized with pentobarbital sodium, and the common bile duct was cannulated with polyethylene tubing (internal diameter, .023 inches); bile flow from the total hepatic mass (liver and graft) was measured at 5-minute intervals for 1 hour. After this period the cannula was wedged into the branch of the duct arising from the median lobe (or graft) and ligated securely in place so that backflow from other lobes could not enter the catheter. Bile flow was again measured for 1 hour at 5-minute intervals. After completion of flow determinations the animals were killed, and the subcutaneous hepatic graft and liver were weighed. Bile flow per gram of parenchyma was computed for both liver and graft. Corrections were made in the gross weight of the graft for the amount of connective tissue estimated histologically.

**Colloid uptake.**—Radioactive chromic phosphate ($\text{Cr}^{3+}\text{PO}_4$) and carbon were used to test the functional competence of Kupffer cells (capacity to phagocytize particulate material) in grafts and to test the exclusion of portal blood from grafts. For the first purpose a mixture of these two substances was injected systemically, and animals were killed 1 hour later. To measure $\text{Cr}^{3+}\text{PO}_4$ uptake, liver and graft were removed and weighed, and the entire specimen, except for thin blocks for histologic sections, was digested in boiling concentrated nitric acid. Radioactivity in an aliquot of digestant was assayed in a deep-well scintillation counter, and results were expressed as counts/min/gm tissue. Corrections were made in the gross weight of grafts for the amount of connective tissue estimated histologically. Carbon was identified in histologic sections, and its localization was determined.

To test whether portal blood was completely excluded from grafts, colloids were injected directly into the portal vein at such a rate that they were cleared from the blood by a single pass through the liver (no colloid could spill over into the systemic circulation). Colloids were detected as previously outlined. Absence of these materials in grafts indicated exclusion of portal blood.

**Autopsy Procedures and Histologic Methods**

Animals were killed at regular intervals beginning 2 days after the first stage and ending 1 year after the second stage of the operation. In all instances grafts and livers were examined carefully, with special emphasis on the condition of portal and hepatic veins, hepatic artery, and bile duct connections to the grafts. The portal and hepatic veins were always probed, and in many instances the portal vein was injected with colloids or with a dye. The condition of the pedicle was examined closely; sections were made of any fibrous tissue connecting each graft and liver, and these were searched for blood vessels. Animals in which any connection between grafts and normal hepatic blood supply could be demonstrated were discarded.

All livers and grafts were weighed after being blotted free of blood. Weights of grafts were corrected for the estimated portion occupied by connective tissue, as determined by the Chalkley method (4) in three or four sections taken from each graft at regular intervals and
stained by the Mallory method. Successful grafts were defined as those that were completely separated from the hepatic circulation and contained more than 0.4 gm. of parenchyma (after correction for connective tissue).

Blocks of tissues for histologic sections were routinely taken from several areas of both graft and liver. Tissue was fixed in an alcohol, acetic acid, and formalin mixture for light microscopy. Stains utilized were hematoxylin and eosin, periodic acid-Schiff (with saliva-digested control sections), Mallory connective tissue, reticulin, Feulgen, and Perle's iron.

RESULTS

OVER-ALL RESULTS

Results of the operation performed on 250 rats are given in Tables 2 and 3. One hundred forty-five animals (58 per cent) survived both phases of the operation and lived from 1 month to 1 year afterward, when they were killed. At the time these animals were killed, ligation of the pedicle was determined to be inadequate in 25 animals (10 per cent), the amount of grafted parenchyma remaining was insignificant in 40 animals (16 per cent) and grafts contained an appreciable amount of parenchyma in 80 animals (32 per cent). Thus, in 32 per cent of the original 250 animals the operative procedure was successful.

Of twenty animals in which the entire pedicle was ligated at the second stage, fifteen were maintained for 1—4 months following complete ligation. All animals subjected to the other operative procedures survived for at least 1 month.

MORPHOLOGIC SEQUENCE RESULTING FROM MODIFIED TECHNIC

The portion of liver remaining within the abdominal cavity was unaltered morphologically during the entire period of observation except for development of focal capsular adhesions. The following observations apply only to grafts.

Gross observations.—During manipulations incident to performing the first stage the median lobe became congested. However, once the procedure was completed, hemostasis was usually relieved, and normal color returned. Fibrous capsular thickening developed during the ensuing weeks. The intramuscular portion of the pedicle atrophied markedly (even before ligation) and ultimately became only a thin fibrovascular band, but the weight of the median lobe exclusive of the pedicle remained relatively constant prior to ligation of the latter. Portal and hepatic veins connecting liver to graft were easily probed. Some grafts were completely or partially surrounded by hematomas which isolated from the subcutaneous tissue the parts of the grafts over which they were located. Presence of hematomas prevented fibrosis of the capsule and development of collateral circulation.

Successful ligation of the pedicle by the second stage of the operation resulted in marked congestion of grafts. During the 1st week following ligation grafts decreased markedly in size until they weighed approximately one-fourth to one-third of their original weight (Chart 2). This decrease in weight was associated with marked thinning. After 3—4 weeks grafts ceased to shrink, and thereafter weight remained relatively constant. Cut surfaces of grafts, although thinned, had the color of normal liver.

By 1 month after successful ligation the muscle defect previously occupied by the pedicle was filled by dense connective tissue, and vascular connections could not be demonstrated by probing or by intraportal injection of dye or colloid.

Following ligation of the pedicle, grafts that had been surrounded by hematomas became completely necrotic and were gradually resorbed.

Microscopic observations.—The first stage was followed by transient centrolobular congestion; after 2 days the structure of the parenchyma returned to normal and remained so in the absence of pedicle ligation. Capsular fibrosis coincided with healing of the subcutaneous wound, and formation of capillary-sized vessels which penetrated the capsule and entered the parenchyma accompanied the fibrosis (Figs. 1—3). Many of these microscopic vascular channels ultimately enlarged and developed muscular walls (Figs. 4, 5). Atrophy of the parenchyma contained in the portion of the pedicle traversing the

| TABLE 2 |
| RESULTS OBTAINED WITH MODIFIED TECHNIC FOR AUTOGRRAFTING PART OF LIVER |

<table>
<thead>
<tr>
<th>Transplantations</th>
<th>No. animals</th>
<th>Per cent of total number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attempted</td>
<td>250</td>
<td>100</td>
</tr>
<tr>
<td>Successful (as defined in the text)</td>
<td>80</td>
<td>32</td>
</tr>
<tr>
<td>Unsuccessful</td>
<td>170</td>
<td>68</td>
</tr>
</tbody>
</table>

| TABLE 3 |
| CAUSES OF UNSUCCESSFUL TRANSPLANTATIONS |

<table>
<thead>
<tr>
<th>Time of discovery of failure</th>
<th>Nature of failure</th>
<th>No. of animals</th>
<th>Per cent of total number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior to establishment of second stage</td>
<td>Death within 2 days after first stage</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Development of hematoma; animals sacrificed</td>
<td>45</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Unsuccessful ligation (ligature breakage, etc.)</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>After second stage but before termination of experiment</td>
<td>Death within 2 days after second stage</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Death from 3 days after second stage up to termination of experiment</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>At termination of experiment</td>
<td>Ligation incomplete</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Ligation complete but insufficient hepatic tissue remaining</td>
<td>45</td>
<td>26</td>
</tr>
</tbody>
</table>
abdominal wall occurred even before ligation of the pedicle. Within 2 weeks after implantation the pedicle usually contained only large lobar branches of portal and hepatic veins, hepatic artery, and bile duct surrounded by connective tissue (Fig. 6).

Successful ligation of the pedicle (second stage) resulted in marked centrolobular congestion. Parenchyma surrounding the centrolobular veins underwent acute degeneration and collapse, the extent of parenchymal degeneration varying from graft to graft (Figs. 7, 8). In successful grafts parenchymal loss involved two-thirds to three-fourths of the area of lobules. Associated with degeneration of parenchyma was accumulation of pigment-laden (iron-positive) macrophages and scattered inflammatory cells (Fig. 9). Connective tissue proliferated in the involved areas, and as it matured it contracted, decreasing the distance between adjacent portal spaces (Figs. 9, 10).

Fibrous tissue in grafts always involved centrolobular areas and was always maximal in these areas. In older grafts fibrosis varied in amount from moderate centrolobular involvement to complete lobular replacement, and all variations between these extremes occurred (Figs. 11–14). In some mature grafts fibrous tissue often connected adjacent central areas, isolating acinar areas of parenchyma around portal spaces (Figs. 12, 13, and 15). In other mature grafts central areas and portal spaces were joined by bands of connective tissue (Figs. 12, 13, and 15). In other mature grafts central areas and portal spaces were joined by bands of connective tissue (Figs. 12, 13, and 15). In other mature grafts central areas and portal spaces were joined by bands of connective tissue (Fig. 14).

Hepatocytes usually occurred in areas immediately surrounding portal spaces, the size of the periportal cuff of parenchyma varying with the extent of centrolobular degeneration and fibrosis (Figs. 11–14). Although hepatic plates in grafts were somewhat irregular compared with uniform plates of single-cell thickness in normal liver (Figs. 17, 18), individual hepatocytes were cytologically normal by light microscopy (Figs. 19, 20).

In areas of extensive fibrosis, single isolated hepatocytes occasionally could be found (Figs. 21, 22). Such incarcerated cells sometimes appeared cytologically normal by light microscopy in grafts several month after implantation, but they often contained a single giant nucleus or up to ten nuclei of normal size. Bile ducts remained normal and manifested little tendency to proliferate (Figs. 9–13, 15, and 18), except for occasional small foci of proliferation at the edges of grafts where bile drainage was probably impaired. This was true even in grafts in which complete parenchymal degeneration and total fibrosis occurred (Figs. 14, 21, and 22). In the latter situation portal spaces could be identified by the presence of bile ducts and vessels.

Sinusoids in grafted parenchyma appeared relatively normal, compared with sinusoids in the liver (Figs. 17–20), except for some coarsening of the reticulin pattern (Figs. 23, 24). Sinusoidal cells were phagocytic as shown by their capacity to sequester carbon particles injected into the systemic circulation (Figs. 25, 26).

Many centrolobular veins underwent fibrous obliteration and in older grafts could rarely be identified; vessels in fibrous tissue in centrolobular areas usually were capillary-sized (Figs. 9, 10, 18). Thrombotic occlusion of some large hepatic veins could be demonstrated soon

---

**Chart 2.** Comparison of weight changes in grafts after ligation of the pedicle with weight changes in median lobes after ligation of the portal vein in situ. Each point is the mean of at least five animals; vertical bars represent two standard deviations above and below means.
after ligation of the pedicle, and in older grafts some of these vessels were completely fibrosed, although partial recanalization occurred (Figs. 27, 28). More frequently in older grafts only marked intimal proliferation of large hepatic veins was noted, a patent reduced lumen remaining (Figs. 29—31). Occasional portal veins were also obliterated by fibrous tissue. However, most portal veins and hepatic arteries were patent in older grafts. Apparently many of these vessels connected with subcutaneous collaterals, since they contained blood even after the pedicle was completely ligated.

**Morphologic Sequence Following Seneviratne Procedure**

Ligation of the entire pedicle including the bile duct resulted in grafts markedly different from those just described. The morphologic sequence occurring in such grafts has been fully described by Seneviratne (19) and will be only reviewed here, since our findings are in essential agreement with his.

Prior to ligation of the pedicle, changes in implanted lobes were identical to those occurring after the modified procedure. Early changes after pedicle ligation were also similar, but by 1 week bile ducts had proliferated conspicuously and by 1 month the majority of cells in grafts were of bile ductal origin (Fig. 32). At 1 month grafts were heavier than those produced by the modified procedure, and they appeared pale and granular. On cut surfaces there were tiny cysts from which clear fluid exuded. At this stage grafts were composed of a mass of ducts (Figs. 33, 34). Coincident with proliferation of ducts, hepatocytes atrophied and rapidly regressed in number until few remained at 1 month (Figs. 33, 34). Surviving hepatocytes were isolated and surrounded by proliferated bile ducts.

**Structural Changes Elicited by Other Operations**

Ligation of the lobar portal vein at the time of implantation or ligation of the pedicle within the first 2 days after implantation resulted in virtually complete loss of parenchymal cells from grafts. Bile ducts often persisted even when all hepatocytes were destroyed.

Ligation of the lobar branch of the portal vein in situ resulted in rapid and marked loss of substance of median lobes (Chart 2). The rate and extent of weight loss were more rapid and more severe than those occurring in grafts, the lobes being reduced from three-fourths to four-fifths within 1—2 weeks and remaining at this low level for the remainder of the time studied. Microscopically, a generalized loss of hepatocytes occurred throughout lobules, and a marked decrease in lobular size corresponded in extent to loss in weight. Centrolobular veins remained visible, and individual hepatocytes appeared only slightly shrunken. Bile ducts remained normal.

After ligation of the lobar bile duct in situ, intralobar bile ducts proliferated considerably. Ligation of both lobar bile duct and lobar portal vein produced more marked ductal proliferation, approaching in extent that occurring in grafts produced by the Seneviratne procedure.

**Functional Observations on Grafts Produced by Modified Technique**

Bile flow from grafts was equivalent on a per gram tissue basis to that from entire livers or to that from median lobes implanted subcutaneously with intact blood supply (Charts 3, 4). Bile flow averaged $1.0 \pm 0.13$ ml/gm/hr from grafts and $0.81 \pm 0.15$ ml/gm/hr from intact livers or median lobes. These values were not statistically different.

When injected into the systemic circulation CrP⁴O₄ and carbon were taken up by both livers and grafts (Table 4 and Figs. 25, 26). On a per gram tissue basis the uptake was less for grafts than for livers. Phagocytic cells lining sinusoids in both livers and grafts contained histologically demonstrable carbon particles (Figs. 25, 26). When injected intraportally neither colloid appeared in grafts if adequate ligation of the pedicle had taken place (Table 4).
TABLE 4
**Cr**32O4 UPTAKE BY LIVER AND GRAFT
(counts/min/gm tissue × 10^4)

<table>
<thead>
<tr>
<th>Route of injection</th>
<th>Condition of pedicle</th>
<th>No. animals</th>
<th>Uptake by liver</th>
<th>Uptake by graft</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraportal</td>
<td>Not ligated</td>
<td>3</td>
<td>20.6</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.0</td>
<td>11.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>19.5</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>Intraportal</td>
<td>Ligated</td>
<td>6</td>
<td>12.3 ± 5.2</td>
<td>0</td>
</tr>
<tr>
<td>Systemic</td>
<td>Not ligated</td>
<td>3</td>
<td>19.2</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.9</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.7</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>Systemic</td>
<td>Ligated</td>
<td>7</td>
<td>15.2 ± 6.4</td>
<td>5.4 ± 3.1</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Early attempts to heterotopically autograft a portion of liver employed free grafts of minute amounts of tissue (Table 1, part A). These grafts were initially separated from the hepatic blood supply and were placed in another area of the body. Survival of hepatic tissue depended upon rapid development of collateral circulation, which usually did not occur. Many such grafts rapidly underwent total necrosis (11, 15, 18, 26), and in most of the other free grafts complete loss of hepatocytes ultimately occurred (2, 3, 7, 11, 12, 17, 26); final grafts in the latter instances consisted of proliferated and dilated bile ducts and pigmented macrophages in a mass of dense connective tissue. Only Knake was successful in preserving some parenchymal cells over long periods (8, 9), and she found it necessary to use extremely thin slices of liver engrafted to the highly vascular mesotestis. Even so, her illustrations showed only small nodules of parenchyma remaining. Others (14) were unsuccessful in grafting liver by Knake's method.

More recent attempts to heterotopically autograft portions of liver depended on the development of collateral circulation to the portion of liver to be grafted before removing it from the hepatic circulation (Table 1, part B). Usually a two-stage technic was used: (a) vascular tissue was anastomosed to the liver, and (b) at a later time, subsequent to the development of collateral blood vessels, a portion of liver attached to the adherent tissue was separated from the normal hepatic circulation. Myren and Vinje (14), who introduced this technic, used the mesotestis in the mouse to vascularize the liver; Thorbjarnarson et al. (25) used the spleen; and Rauber et al. (16) and Sigel et al. (20—23) used portions of the gut in rabbits and in dogs for this purpose. Seneviratne (19) used a slightly different procedure, making a pedicle graft of the median lobe of the rat's liver to the abdominal subcutaneous tissue. Secondarily the pedicle was cut.

These two-stage technics allowed heterotopic autografting of larger amounts of liver, and, in most instances, significant amounts of parenchyma persisted for long periods. However, grafts produced by all these methods suffered from impaired bile drainage. No attempt was made by Myren and Vinje (14), by Seneviratne (19), or by Rauber et al. (16) to preserve bile drainage from grafts. Sigel et al. (23) attempted to do this by enfolding the graft into the lumen of a defunctionalized intestinal loop in the belief that bile might drain from severed ends of intrahepatic ducts. This same group of workers (20) also attempted to graft lobes of liver to which the gall bladder was attached and to achieve bile drainage by choledochoenterostomy or cholecystoenterostomy. All these technics failed because fibrosis prevented continued biliary drainage (22). Grafts produced by all the two-stage methods showed some degree of biliary ductal prolifera-
tion, ranging in extent from the modest proliferation noted in dogs by Sigel et al. (22) to the marked proliferation noted in rats by Seneviratne (19). Furthermore, Sigel et al. (22) considered that steady attrition in weight of the graft was partially the consequence of failure of biliary drainage.

Our study clearly demonstrated the importance of both adequate collateral circulation and adequate biliary drainage for ordered persistence of grafted parenchyma in the rat. Ligation of the pedicle shortly after implantation (rather than 2 weeks later) resulted in total loss of hepatocytes from grafts. Similarly, ligation of the pedicle in animals in which hematomas around grafts had prevented development of collateral circulation resulted in total loss of hepatocytes, although bile ducts occasionally persisted. Grafts resulting from these procedures were cut off from their normal blood supply before they developed collateral circulation and, in this respect, were comparable to free grafts. Failure of hepatic parenchyma to survive these manipulations agreed with results of previous studies of free grafts and demonstrated the necessity of allowing collateral blood supply to develop before destroying the normal supply. Development of significant collateral circulation in successful grafts was demonstrated by the finding that decrease in weight (and in lobular size) was less in grafts after ligation of the pedicle (lobar branches of the portal and hepatic veins and of the hepatic artery) than in median lobes in situ after simple ligation of the portal vein (ligation of both lobar portal vein and lobar hepatic artery in situ resulted in complete necrosis of the lobe [24]).

Adequate biliary drainage was virtually as important as abundant blood supply in allowing persistence of parenchyma in grafts. Total obstruction of the common bile duct in the rat resulted in marked proliferation of biliary ductal cells and degeneration of hepatocytes (24). It was demonstrated recently that concomitant portal vein ligation potentiated the effect of bile duct obstruction in this species (27). These observations were substantiated by our study. Ligation of the entire pedicle resulted in rapid and luxuriant proliferation of bile ducts and in almost total loss of hepatocytes from grafts; these results agreed with Seneviratne's study (19). Preservation of biliary drainage from grafts (by excluding the lobar bile duct from the pedicle ligature) effectively prevented overgrowth by bile ducts. The almost universal occurrence of bile duct proliferation in previous partial hepatic autografts can thus be explained as a consequence of biliary obstruction.

Decrease in the size of grafts after separation from the hepatic circulation was noted following all the two-stage grafting methods (Table 1, Part B). This may be ascribed in part to poorer blood supply to the graft than to the liver (13). However, much of the parenchymal loss occurred immediately after grafts were separated from the normal hepatic blood supply and probably resulted from sudden obstruction of venous outflow with consequent venous congestion and parenchymal atrophy (22). In our study centrolobular congestion was so great that it resulted in acute necrosis of hepatocytes in this region of the lobule. Necrotic areas were ultimately replaced by scar tissue. Sigel suggested that development of collateral venous tracts did not keep pace with development of collateral arterial tracts (22). The marked centrolobular congestion and necrosis that occurred in our grafts was consistent with this idea. Although direct connections of collateral vessels to pre-existing vessels in grafts have been noted (13, 22), such union was doubtless random, and not all vessels acquired such connections.

Impaired capacity of grafts to take up particulate matter injected into the systemic circulation was a finding consistent with deficient blood supply. A lesser blood flow to graft than to liver would result in a comparably lessened amount of colloid presented to Kupffer cells in the graft. Alternative explanations of this decreased uptake in grafts might be the presence of fewer Kupffer cells in grafts than in livers or reduced avidity of these cells in grafts to phagocyte colloid. Histologic localization of phagocytized carbon in grafts disclosed an approximately normal ratio of Kupffer cells to hepatocytes. Our methods did not allow the alternatives of reduced blood flow or reduced avidity to be completely resolved, although evidence from this and previous studies (13) favored the latter.

Subcutaneous location in itself was not inimical to hepatic parenchyma, since simple implantation of the median lobe of the liver under the skin caused no permanent changes other than capsular fibrosis if there was no interference with hepatic blood flow. We found no plasma cell infiltrates in grafts similar to those described by Sigel et al. (20); only focally scattered lymphocytes were noted. Neoplastic changes were not detected in heteropic site.
Figs. 7-10.—Sequence of histologic changes occurring in grafts after ligation of the pedicle. In all figures portal tracts and central areas are shown. Fig. 7 shows a graft 2 days after pedicle ligation. There is marked centrolobular congestion and necrosis of hepatocytes with a rim of viable hepatocytes remaining around portal tracts. Already there is considerable lobular collapse. Fig. 8 represents a graft at 5 days after pedicle ligation. Necrotic hepatocytes have been almost completely removed, and lobular collapse is more extensive than in Fig. 7. Hepatocytes around portal tracts appear normal. Fig. 9 illustrates a graft at 3 weeks after pedicle ligation. Centrolobular areas are completely collapsed and are infiltrated with macrophages, some of which contain iron-positive material. Capillary-sized vessels remain in centrolobular areas. Fig. 10 shows a graft 3 months after pedicle ligation. An original central vein area is marked only by dense connective tissue. A central vein is not visible. Because of lobular collapse portal tracts are much nearer to one another than is normal. (Compare these pictures with Fig. 17, which shows a portion of a lobule from a normal liver at comparable magnification.) H. & E., mag. × 460 in each instance.
FIGS. 11–14.—Sections from different grafts to show variation in extent of hepatocytic necrosis and subsequent fibrosis which occurs after ligation of the pedicle. Fig. 11 is from a 1-year-old graft and shows minimal fibrosis. Fibrosis in this graft is of about the same extent as that illustrated at higher magnification in Fig. 10. Fig. 12 shows a 3-month-old graft with more extensive fibrosis. Rims of parenchyma around portal tracts are preserved. Fig. 13 is from a 6-month-old graft and is even more markedly fibrosed. When nodules of parenchyma persisted in fibrotic grafts (as in this figure and in Fig. 12), a portal tract could usually be found immediately adjacent. Fig. 14 is from a 3-month-old graft and shows almost total fibrosis. Portal tracts remain, but all hepatocytes have been destroyed except for a few trapped in connective tissue (Figs. 20' 21 illustrate similar grafts at higher magnification). Note that in none of these grafts has there been significant bile duct proliferation. H. & E., mag. X 110.

Fig. 15.—Six-month-old graft showing bands of connective tissue surrounding foci of parenchyma. These bands join portal and central areas, although some portal spaces are located at the interior of nodules. H. & E., mag. X 110.

Fig. 16.—One-year-old graft with histologic appearance similar to cirrhotic liver. Connective tissue bands course through the graft. Parenchymal nodules appear to have compressed adjacent fibrous tissue to some extent. In this particular graft almost all portal spaces were located in fibrous bands at edges of parenchymal nodules. Mallory's stain, mag. X 110.
Fig. 17.—Representative portion of a lobule from normal liver showing portal tract (top of picture) and adjacent central vein. Plates of hepatocytes are one cell thick and are regularly arranged. Sinusoids course in an almost direct manner between portal tract and central vein. Compare with Figs. 7-10 and 18. H. & E., mag. X 460.

Fig. 18.—Representative part of 4-month-old graft. Portal tracts are near to each other (four are included in this field). Central areas are difficult to identify. Hepatic plates are irregular and haphazard. Sinusoids are tortuous, and sinusoid lining cells are prominent. H. & E., mag. X 480.

Fig. 19.—Hepatocytes from normal liver showing typical cytologic appearance. H. & E., mag. X 1300.

Fig. 20.—Hepatocytes from same graft as in Fig. 18. These cells appear cytologically normal at this magnification. H. & E., mag. X 1200.

Fig. 21.—An extensively fibrosed graft showing normal portal tracts and a few hepatocytes incarcerated in dense connective tissue. H. & E., mag. X 380.

Fig. 22.—A specimen similar to that illustrated in Fig. 21. Incarcerated hepatocytes are illustrated at higher magnification. Such trapped cells could be found in grafts up to 1 year of age. H. & E., mag. X 900.
FIG. 23.—Delicate pattern of reticulin fibers in normal liver. Reticulin stain, mag. X 390.


FIG. 26.—Phagocytized carbon in Kupffer cells of a graft from the same animal as the liver illustrated in Fig. 25. Kupffer cells in both graft and liver sequestered carbon. H. & E., mag. X 430.

FIGS. 27, 28.—Large hepatic veins from 6-month-old grafts showing occlusion with partial recanalization. Compare with Fig. 29. H. & E., mag. X 120.
Fig. 29.—Normal liver at low magnification to show the thin, delicate walls of large hepatic veins. H. & E., mag. X 100.

Fig. 30.—Two-month-old graft showing marked thickening of the wall of a large hepatic vein. Compare with Fig. 29. H. & E., mag. X 120.

Fig. 31.—A thick-walled, large hepatic vein from a 4-month-old graft. Thickening of the wall is caused by intimal and medial proliferation. H. & E., mag. X 380.

Fig. 32.—One-month-old graft prepared by Seneviratne technic (bile duct occluded). Graft is extremely cellular, and the nature of these cells is shown in Figs. 33, 34. Compare with Figs. 4 and 11-15 which illustrate at comparable magnification grafts prepared by the modified technic. H. & E., mag. X 110.

Fig. 33.—Three-week-old graft prepared by Seneviratne technic. The extreme cellularity is a result of remarkable proliferation of small intrahepatic bile ducts. A few hepatocytes remain. H & E., mag. X 380.

Fig. 34.—One-month-old graft prepared by Seneviratne technic. Most of tissue is made up of proliferated bile ducts. Almost all hepatocytes have been replaced, but a few can still be seen. Compare with Figs. 19 and 20, which show hepatocytes from normal liver and from grafts prepared by the modified technic at comparable magnification. H. & E., mag. X 1200.
autografts produced by the modified technic during 1 year of observation; hepatomas were noted to arise from heterotopically homografted hepatocytes in mice (10).

Bile duct cells did not possess an absolute capacity to grow in grafts to the exclusion of hepatocytes if adequate collateral circulation and adequate bile drainage were effected; however, they did apparently possess a relative growth advantage over hepatocytes as a consequence of the greater sensitivity of the latter to deprivation in blood supply (3). Even in some situations in which hepatocytes were completely destroyed, relatively normal bile ducts remained in an otherwise completely fibrosed graft. Kupffer cells were apparently as labile as hepatocytes to decreased blood supply, since cells that phagocytized carbon were not found in the absence of organized parenchyma.

This study demonstrated that hepatocytes can survive and function in the absence of portal blood. Grafts produced by our method received no portal blood within the limits of our methods of detection; their blood supply was entirely systemic. Colloids injected into the portal vein failed to appear in the graft if the rate of injection allowed the liver to clear the colloid before it spilled into the systemic circulation. Colloid injected into the systemic circulation appeared in both graft and liver. It should be noted that all the two-stage grafts reported previously (except Seneviratne's) were located outside the portal bed. Failure to develop adequate collateral circulation combined with necrosis at the time of ligation of the pedicle may explain the large number of grafts in which all parenchyma was lost. At best, these grafts have a precarious existence between the dangers of poor blood supply, massive congestion, and biliary obstruction.

REFERENCES


Heterotopic Partial Autotransplantation of Rat Liver: Technic and Demonstration of Structure and Function of the Graft

J. W. Grisham, G. F. Leong and B. V. Hole

Cancer Res 1964;24:1474-1495.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/24/8/1474

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.