Experimental Whole-Organ Transplantation of the Liver and of the Spleen *

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Introduction

The rapidly growing experience in experimental homotransplantation is largely based on observations of the skin, kidney, blood and bone marrow.

These studies have been the pioneer ones for reasons that are clear historically: skin for its availability; kidney for its significance as a paired organ with a simple vascular pedicle, the biochemical product of which can easily be measured; blood for its circulatory support; and bone marrow for its use as a replacement tissue after hematopoietic destruction either in cancer or in other transplants.

Experiences with these tissues may give us but a partial view of the total histologic and biologic behavior of the immunogenetic phenomenon. Neither skin nor kidney is itself importantly involved with the production of immune globulins or mononuclear cells of the reticuloocyte-lymphocyte-plasma-cell series. While the bone marrow is involved with both, it is difficult to recover for microscopic study after injection and its study is confined to episodic examinations of peripheral areas where it alights and grows. Even in these areas, its identity is usually established by its peripheral product, rather than its local appearance. Studies of other organs and tissues are therefore of great interest in this field to obtain a broader and more complete picture of the biological response to homotransplantation. Organs which would particularly appeal as a way of broadening our view of the homotransplant phenomena would be organs involved either with a large antigenic mass, or those containing immunologically competent cells. Examples of such are to be found in the liver and in the spleen.

In the present state of our knowledge, clinical homotransplantation must rest on a state of immune tolerance achieved by chimerism, in which donor cells reside in

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the host without exciting an effective immune response. If such can be attained by destruction of the immune system (by irradiation or cytotoxic drugs) followed by hematopoietic restoration (by bone marrow or, in terms of the work herein reported, spleen), then the recipient can accept and hold other tissues from the same donor whose cells are responsible for the hematopoietic restoration.

The Liver

The liver represents the largest single homogeneous antigenic mass of cells that can be readily transplanted in the mammal. Its parenchymatous cells are homogeneous structurally and are importantly involved in metabolic work. Their metabolic efflux can readily be studied and identified in both bile and blood. The liver is concerned with the immune process to the extent that the reticuloendothelial system finds representation in the Kupffer cells lining the hepatic sinusoids.

Technics of transplanting liver tissue to an animal maintaining its own liver have been developed. Studies of the transplantation of small liver slices might hold some promise. In these settings, the acceptance of the transplant is not itself essential for survival of the organism, and one cannot study the effect of the intact transplanted whole organ on the bodily economy. The liver, for its very size and complexity of vascular arrangement, does not lend itself to transplantation to some other portion of the body. It will clearly rest most comfortably and function most normally if placed in its normal site with anatomic restoration of its anatomic relationships, unharmed by vascular deprivation, and free of distortion of its enteric relationships through any kind of vascular shunt. For these reasons, our initial purpose was to transplant the intact liver into its anatomic subdiaphragmatic site.

The short-term hepatectomy suffered by the recipient dog was not anticipated as a problem and did not turn out to be so. The transient anoxia of the transplanted liver could indeed be a major problem had it not been for the striking demonstration that liver, of the various tissues of the body, most outstandingly exhibits the phenomenon of cellular integrity while anoxic, if subjected simultaneously to hypothermia.

Looming larger as a barrier than any of these problems in hepatic transplantation was the circulatory instability of the dog who has no liver in place. Since the liver is removed together with the vena cava this dog suffers from pooling of blood in the kidneys, gut and lower extremities. If simultaneous aortocaval occlusion is instituted for a prolonged period, a severe acidosis of the distal portion of the dog is produced which will result, upon reopening of the occlusive clamps, in a severe hyperkalemic hypoxic acidosis, cardiac arrhythmia, shock and death. This problem was solved by the use of two low-pressure shunt systems from lower caval and portal systems, respectively, to the jugular veins.

The dog was selected for this work because of his size, the lack of any evident inbreeding, and the extensive experience previously gained in these laboratories with kidney transplantation in the dog. Unfortunately, the dog’s tissues harbor anaerobic organisms and for this reason control of infection, both in the liver, in the gastrointestinal tract, and other tissues, is essential for such an operation that may be associated with hypoxia or shock. Antibiotics were used throughout.

1. Operative Procedure in Hepatic Homotransplantation

a. Experimental Group. The steps involved in the development of this operation will not be reviewed in this paper. The procedure currently used, and that employed in its essentials for all of the animals later described, will be briefly recounted.

Two dogs of approximately equal size and of either sex were used. Early in our
experience the dogs were treated preoperatively with antibiotics given by mouth but subsequently these were not commenced until the day of operation.

Anesthesia is induced with intravenous barbiturate and the dog ventilated via a cuffed endotracheal tube. A minimum amount of anesthetic agent (ether) is used. Continuous monitoring of arterial pressure is maintained throughout the operation. High positive pressures are avoided in the airway.*

The recipient animal is operated upon while normothermic. It is possible to do the entire operation without opening the thorax, though many of our earlier experiments involved a short intercostal incision on the right for the upper caval anastomosis.

The vena cava below the renal veins is isolated for the insertion of one of the two temporary shunts. The right adrenal is then dissected off the vena cava above the renal veins, the structures of the porta hepatis are identified, dissected free and all save the hepatic artery and portal vein are divided. Prior to this dissection, 2 per cent procaine is infiltrated around the hepatic artery and in the structures of the porta hepatis, a feature that appears to prevent hepatic outflow obstruction, to which the dog is so prone. It appears to make little difference to the animal's course whether or not the spleen is removed. In the earlier series it was removed, while in all of our recent animals the spleen has been left in place.

Attention is then turned to the suprahepatic area. The vena cava is dissected free to the diaphragm. It is possible to dissect it completely free at the diaphragm and, with care, it is possible to avoid entering the pleura. Isolation and division of the left phrenic vein is the most troublesome detail of this dissection.

The aorta is dissected free above the celiac axis for temporary occlusion during transient portal vein occlusion before its shunt is opened and later during anastomosis. A stainless steel T-tube is then placed in the lower vena cava, and the shunt (1/4 inch internal diameter) from the cava to the right jugular vein is placed. The liver is then ready to be removed.

The portal vein is then divided and the portal blood shunted to the left jugular vein by a flexible plastic ("Tygon") tubing (3/32 inch internal diameter).

During this procedure, a simultaneous operation has been under way on the donor dog. The steps taken are the same as those recounted above save for the fact that no preparation for a caval shunt is needed. When the liver is freed up, the animal is made hypothermic by the application of cold isotonic salt solution to the peritoneal cavity. We have also used immersion and portal perfusion of the liver for cooling after it is removed from the animal. The donor operation should be conducted so as to provide a liver whose edges are sharp, smooth and pink and that does not show any of the dark rounded swelling characteristic of canine hepatic outflow obstruction. When the liver is cooled to the neighborhood of 28°C. or lower, it is taken out with adequate segments of its vessels, and placed in the recipient dog.

The resuture of this liver in the recipient is then carried out anatomically while the two shunts maintain venous return to the right heart. First the inferior vena cava above the liver is sutured end-to-end. Then the portal vein is sutured end-to-end. Upon opening the portal vein anastomosis the clamp above the liver is released so that perfusion of the liver now begins with the recipient's own blood via the portal vein. The liver soon becomes quite normal in appearance if the donor operation has been gently done. The total "dead time" of this

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* We are indebted to Dr. Thomas K. Burnap, currently Assistant Chief Anesthetist at the Mt. Auburn Hospital, Cambridge, Mass., for anesthesia study and care of these animals.
liver is approximately 30–45 minutes up to this point. The cava below the liver is then anastomosed. The hepatic artery is then sutured end-to-end after gentle dilatation of the two ends. As soon as the vena cava is opened, the caval shunt can be removed. The bile ducts can be dealt with in one of several ways. The most satisfactory consists in cholecystoduodenostomy.

The incisions in the neck and the abdomen are then closed. No steps are needed to maintain the liver immobile in its position. It seems to be held very firmly by its anastomotic arrangements and by the pressure of the surrounding viscera.

Transfusions are given as needed, with the use of ion-exchange blood during the anhepatic phase. Acidosis, developing during transplantation, is effectively treated by sodium bicarbonate given intravenously.

No heparin is required for the maintenance of shunt-flow in the anhepatic dog. If the transfusion volume is large the dog will be found to have a coagulation defect at this time that makes hemostasis difficult. When the operation is carried out with more dispatch and with less bleeding, no coagulation defect is identifiable by clotting time, prothrombin time, recalcification time and clot lysis tests.

The plane of anesthesia must be very light. It is desirable that the animal regain consciousness shortly after the operation. Animals that fail to do so often fail to survive the first 24 hours.

b. Control Group. Our control procedures have consisted of two types.

1. Autotransplants. In this procedure, the same operation as that described above for the recipient dog is carried out. The liver is lifted out of the animal, cooled in a bath of isotonic solution and by perfusion, using chilled oxygenated blood and is then replaced in the same animal.

2. Liver dissections, or sham operations. In this procedure, the liver is dissected up, the shunts are placed, and everything is done in the way of dissection, including some of the vascular anastomoses, but the liver is not actually removed from the animal.

2. Results

a. Homotransplants. The homotransplant procedure has been undertaken in 31 animals. There have been 15 survivors over 24 hours. Eight have lived over four days, two have lived five days, and one each have survived 5 1/2, 6, 8 and 12 days. Our observations on clinical course, liver function and histologic change are based on the above animals.

1. Postoperative Course. After operation the animals are maintained on antibiotics; cortisone was used initially but was later not found to be necessary.

In animals surviving the first 24 hours, a characteristic clinical course was pursued. The animal regained consciousness, regained strength and ability to walk about. They recognized their handlers, had adequate urine flow, but in most instances were unable to eat significant amounts of food. A few dogs had biliary diversion through a cholecystostomy, and in these animals the bile output ranged up to 150 ml. per day. A number of animals succumbed during the first four days to anatomical complications of the procedure, including hemorrhage and embolus from the neck incision, and massive hemorrhage from gastroduodenal ulcer. In those that lived beyond this period, clinical and chemical hepatic function continued to within 24 hours of death.

Death was often unassociated with liver failure, was usually unaccompanied by hypotension or shock save terminally, and was presaged in a number of instances only by increasing jaundice, starting about 24 hours before death (see below). The fatal terminus did not resemble a fatality from hepatectomy in most of the animals, and was due to a combination of clinical conditions shown in Table I.
Table 1. Liver Transplantation
Gross Pathological Findings in Dogs Surviving
Two Days or Longer

<table>
<thead>
<tr>
<th>Day of Animal Death</th>
<th>Pathological Findings</th>
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<tbody>
<tr>
<td>X-18 5</td>
<td>Atelectasis; small bowel intussusception; slightly swollen liver.</td>
</tr>
<tr>
<td>X-20 6</td>
<td>Multiple hepatic infarcts; hepatic artery thrombosis; bilateral hemothorax; atelectasis.</td>
</tr>
<tr>
<td>X-24 5</td>
<td>Multiple gastric ulcers; pulmonary congestion and atelectasis.</td>
</tr>
<tr>
<td>X-28 4½</td>
<td>Hepatic infarcts; ? hepatic artery thrombosis; myocardial lesions; partial I.V.C. thrombosis.</td>
</tr>
<tr>
<td>X-35 6½</td>
<td>Hepatic congestion; gastric erosions; jaundice; atelectasis.</td>
</tr>
<tr>
<td>X-36 4</td>
<td>Gastrostomy leak, peritonitis; myocardial lesions; partial hepatic artery thrombosis; focal pancreatic necrosis; renal infarcts.</td>
</tr>
<tr>
<td>X-44 2</td>
<td>Myocardial lesions; focal hepatic infarcts; focal pancreatic necrosis.</td>
</tr>
<tr>
<td>X-50 2</td>
<td>Hepatic artery thrombosis; hepatic necrosis.</td>
</tr>
<tr>
<td>X-58 12</td>
<td>Perforated duodenal ulcers, peritonitis; I.V.C. thrombosis (below reals); green, swollen liver.</td>
</tr>
<tr>
<td>X-69 8½</td>
<td>Small, pale liver; generalized jaundice; renal infarcts.</td>
</tr>
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</table>

2. Chemical course. In many animals the hematocrit was found to be significantly elevated during the first 48 to 72 hours. Three factors played a role here. One was loss of lymph into the peritoneal cavity, although all visible lymphatics were ligated in the porta hepatis. This was treated by infusions of saline or blood. In other instances, the elevated hematocrit was due to inadequately replaced water loss or to overtransfusion of the animal during the operation with subsequent plasma dispersal. If the transplanted liver developed outflow obstruction and became swollen and dark, a very considerable amount of blood was required to maintain the animal in a normotensive state; one to two days later this animal would be found to have a high hematocrit, suggesting that, as hepatic outflow tract obstruction was gradually released and splanchic pooling abated, the animal became hypervolemic, lowering the blood volume towards normal by plasma dispersal and thus showing an elevated hematocrit. An almost unlimited number of chemical and hepatofunctional determinations might be carried out in animals with transplanted livers. The following were selected as being of special interest.

![Liver Function Tests](image)

Fig. 1. Liver function tests. Bilirubin and alkaline phosphatase in the autotransplant are only transiently elevated. In the homotransplant these values rise rapidly prior to death, the rise in alkaline phosphatase preceding that of bilirubin.
The blood urea nitrogen was not significantly elevated in any of these animals save for one in which transient renal failure played a role. Nor was the blood urea nitrogen abnormally low. It remained between 10 and 25 mg.\% in most of the survivors.

The blood sugar was usually normal or elevated. The hyperglycemia was not regarded as a significant finding because of the concomitant infusion of glucose in many instances. Occasionally hypoglycemia was noted during the first 48 hours if the intravenous infusion was stopped.

Alkaline phosphatase shows a progressive increase often antedating the rise in bilirubin concentration (Fig. 1).

The serum bilirubin concentration was generally normal or slightly elevated during the first few postoperative days but rose steadily prior to death. Five out of eight dogs who had this determination carried out 48 hours postoperatively showed values under 2.0 mg.\%. By the fourth postoperative day only one of these eight dogs still had a bilirubin of less than 2.0 mg.\%, and after the fifth day no animal had a serum bilirubin in this range. (Fig. 2).

The plasma albumin and globulin concentration relationships were measured by paper electrophoresis.\* A fall in albumin and a rise in alpha-2 globulin was characteristic.

Coagulation studies (clotting time, recalcification time, prothrombin time and clot lysis) showed abnormalities in those animals early in the series in whom bloodtrapping was unrelieved in limbs or portal circuits, or in whom hemorrhage had demanded large transfusion. As experience grew, no significant abnormalities in these parameters was observed until at or near the time of death when a bleeding tendency associated with a low prothrombin time became clinically evident.

3. Histology (Fig. 3-6). Anatomic findings at death, some of which were responsible for the fatality, are shown in Table 1. Isolated thrombosis in one or another of these vessels was not uncommon but massive thrombosis, either of the portal, caval

\* We are indebted to Mr. John Belko and Mr. William Simpson of the West Roxbury Veterans Administration Hospital for these measurements and study of the clotting indices.
or hepatic systems, was rare. In four animals pulmonary atelectasis was severe. In three animals major gastro-intestinal complications would have been fatal without other disease.

The only organs showing pathognomonic pathologic changes were the liver and the heart. Where bile was diverted from the gastro-intestinal tract, gastroduodenal ulcer was common. The use of an entirely abdominal approach has reduced the incidence of pulmonary complications.

The histologic findings in the liver may be described as an initial infiltration of the portal areas and subhepatic veins with mononuclear cells, lymphocytes and plasma cells, with dilatation of the lymphatics. There is maintenance of the liver architecture with minimum distortion of the hepatic parenchymatous cellular mass and the sinusoids themselves. As the lesion progresses, the portal and centrolobular areas show progressively more cellular infiltration, finally including damage to the epithelium of the bile ducts. In one of the eight-day survivors (X-69) change in the cellular detail was seen in the parenchymatous areas. This was not seen in other dogs. This was unaccompanied by polymorphonuclear leucocyte infiltration or bile stasis and included vacuolization of cells and abnormal mitoses. If due to rejection, this response involved the parenchyma more than in any other dog. Interestingly, our 12-day survivor (X-58) showed less cellular damage than this eight-day survivor; only minimal necrosis was noted in the liver cells near the portal areas.

Early in our experience, lesions were encountered in the hearts of these animals. These were characterized by myocardial necrosis without cellular infiltration or visible thrombosis, going on to small areas of calcification. It was later shown that these can be produced in the dog by long, difficult operations, particularly those producing hypotension and requiring blood transfusion or by hemorrhagic shock. These lesions also occurred in the autotransplants.

b. Controls. Twenty-seven procedures for autotransplantation were instituted. Of these, ten survived 24 hours or more and seven, four days or more: two for four days, three for five days, one for ten days and one for 14 days. Causes of death were analogous to those in the homotransplant group, though the histologic appearance of the liver was quite different.

Of the “sham” dissections, 13 were done.

![Fig. 3](Image)

**Fig. 3.** Liver autotransplantation (X-35). Fifth day. There is dilatation of lymphatics and some interstitial edema. The parenchyma appears normal, and there is no mononuclear cell infiltration (Hematoxylin-Eosin, 100 ×). **Fig. 4.** Liver homotransplantation (X-18). Sixth day. Marked mononuclear cell infiltration is present in the portal areas. There is some lymphatic dilatation. The parenchyma appears virtually normal (Hematoxylin-Eosin, 100 ×). **Fig. 5.** Liver homotransplantation (X-69). Eighth day. The liver shows many morphologic changes in the hepatic cells themselves; necrosis, vacuolization, abnormal mitoses. There is also atrophy of bile duct epithelium and dilatation of the ducts. This animal showed a minimum of extraneous anatomical complications and a clinical picture most closely suggestive of purely hepatic disease (Hematoxylin-Eosin 430 ×). **Fig. 6.** Liver homotransplantation (X-58). Twelfth day. There is mononuclear cell infiltration and lymphatic dilatation in the portal areas, but the parenchymatous cells are well maintained. In this animal death was due to perforated duodenal ulcer (Hematoxylin-Eosin, 100 ×). **Fig. 7.** Spleen homotransplantation (S-12-A). Fourth day. There is a large active lymphoid follicle with many reticulum cells. The appearance is consistent with an immunologic response by the spleen. A few plasma cells are dispersed throughout the follicle (Hematoxylin-Eosin, 100 ×). **Fig. 8.** Spleen homotransplantation (S-12-A). Sixth day. The follicle is packed with plasma cells and lymphocytes. No reticulum cells can be seen. Evidence of follicular activity has disappeared (Hematoxylin-Eosin, 100 ×). **Fig. 9.** Spleen homotransplantation (S-12-A). Ninth day. The lymphoid follicle has undergone involution. No reticulum cells are seen and there is no evidence of follicular activity. Plasma cell infiltration and focal necrosis are now apparent in the adjacent red pulp (Hematoxylin-Eosin, 100 ×). **Fig. 10.** Spleen homotransplantation (S-12-A). Ninth day. The red pulp is virtually replaced by plasma cell infiltration. Focal necrosis can be seen. There is intercellular vacuolization of the splenic trabecula (Hematoxylin-Eosin, 100 ×).
Of these, nine were significant survivors and six lived 14 days or longer, two being sacrificed at six and nine weeks, respectively.

The autotransplants were initially more difficult to accomplish than the homotransplants because there is less vessel-length for anastomosis and it is impossible to achieve parallel hypothermic arrangements for the anoxic liver without cooling the whole animal, a step that is definitely undesirable in this procedure. In those autotransplants and sham dissections that had prolonged survival, the most striking difference in contrast to the transplanted dogs were to be seen in their better clinical course, going on to normal dietary intake, and the finding of a liver which did not show the lymphocytic and plasma cell infiltration, degeneration of bile duct epithelium or hepatocellular damage. In common with the homotransplants, the autotransplants showed marked dilatation of the lymphatics in the portal areas and in the wall of the sublobular veins.

The Spleen

The nature of the histologic and clinical picture involved in homotransplantation of the spleen is a matter of concern because the spleen is an organ containing a large mass of immunologically competent cells. If grafted tissues react immunologically against the host such should be seen maximally in a structure such as lymph node or spleen. Beyond this lies the possibility that after whole body irradiation, hematopoietic restoration might be achieved by homotransplantation of the spleen from an unirradiated dog. The mass of whole spleen is many hundred-fold larger than any dose of cellular suspension or marrow aspirate that might be injected for such a purpose. The concept of hematopoietic restoration by intact spleen in whole body irradiation finds its basis in the work of Jacobson,5 in which shielding of the spleen from whole body irradiation resulted in hematopoietic survival and clinical resuscitation of laboratory rodents after massive whole body irradiation. In Jacobson's experiments, the shielded spleen needed to be left in the animal but a short time to achieve its hematologic effect. In adults of other species (dog, man), the spleen does not normally harbor mature, active, hematopoietic tissue. It appears to be convertible to a multipotential hematopoietic organ as suggested by changes observed in such disease as agnogenic myeloid metaplasia or certain myelophthisic anemias in which the bone marrow becomes aplastic or is destroyed and the spleen becomes an active site of hematopoiesis.

The present experiments were undertaken to perfect a method for splenic transplantation, and to view the histologic and clinical results in the untreated normal dog.

1. Operative Procedure in Splenic Transplantation

Both dogs were operated upon normothermic and no effort at producing hypothermia in the transplanted spleen was made in most of the experiments. Experiments demonstrated that cooling by immersion or perfusion results in a rapid drop of spleen temperature, should such be shown to be necessary.

Before the spleen is removed from the donor, its vascular pedicle is isolated in such a way as to isolate the splenic artery near its origin as a point at which anastomosis can be done. When this artery bifurcates near the spleen the anastomotic procedure is facilitated. If its bifurcation is close to its origin, the operation is more difficult. In most of these procedures, the spleens were traded between two dogs. They were sutured in place by end-to-end Anastomosis of artery and vein after gentle dilatation of the arterial ends. The dog's spleen is a large organ, occupying a larger fraction of the body weight and abdominal cavity than is the case in man. When transplanted to its normal site, it occupies a normal position free of torsion or distortion.
Control Autotransplants. In control animals, the spleen was removed and sutured back in place in the same dog.

2. Results

Splenic homotransplants were carried out in 29 dogs. In many of these animals (several of them early in our experience) the spleen did not become properly vascularized, and splenic hemorrhage or infarction caused the death of the dog. There were ten animals in whom good splenic vascularization was established as shown by gross and microscopic appearance at biopsy and by fresh arterial bleeding after the second postoperative day. Many animals underwent subsequent splenectomy if the condition of the spleen threatened their survival. The longest survival of spleen was in one animal at 65 days (S-12-B), established by biopsy and showing good vascular maintenance.

a. Clinical Course. If splenic vascularization was satisfactory, these dogs had no characteristic clinical disturbance after their operation. They were not carried on antibiotics and did not require intravenous therapy after the first day. If the spleen became necrotic due to distortion of its blood supply, the animal became ill. In those animals in which venous obstruction occurred with engorgement of the spleen, the animal might succumb to splenic rupture or hemorrhage, particularly after biopsy.

Biopsies were taken at various intervals after the operation in both homotransplants and autotransplants.

b. Hematology. No characteristic hematologic changes (hematocrit, white count, smear) were identified in the peripheral blood of these animals. Leucocytosis was usually present, especially when the rejection response in the spleen seemed to be at its height.

c. Histology (Fig. 7–10). There was a striking difference in the histologic appearance of the homotransplanted and auto-transplanted spleen. In those spleens that failed to achieve surgical viability early diffuse necrosis occurred, a picture contrasting with that of rejection.

The rate of histologic change is variable. As an illustration, the histologic sequence (slower than most) in homotransplanted spleen of dog S-12-A included the following:

1. (Days 2–4.) Reticulum cell hyperplasia in large prominent active lymphoid follicles, compatible with a brief “graft vs. host” response.

2. (Well established on day 6.) Plasma cell infiltration, first in the follicles, then the red pulp and subcapsular areas.

3. (Well-established by day 9.) Disappearance of reticulum cells and involution of follicles with intercellular vacuolization in trabeculae. Diffuse massive plasma cell infiltration, particularly in the red pulp, with focal areas of necrosis.

4. Thrombosis of small vessels and complete infarction.

In all the other animals showing rejection, necrosis was established at the end of one week.

The course of one long-surviving splenic homograft is difficult to interpret (S-12-B). On day 36 there were follicles with active centers, present also on days 51 and 65. The cellularity of the red pulp was reduced in comparison with earlier time intervals. There is the possibility that chimerism may have been established. There is also the possibility that the cells seen in the follicles and red pulp represent a repopulation of donor spleen with cells of the new host.

Discussion

One-stage whole organ transplantation both of the liver and of the spleen may be accomplished in the laboratory. The transplanted liver survives and functions well for a time. Although we have had several survivors over 48 hours, we have had but a few animals whose surgical and clinical
course was so smooth that one could feel confident that the clinical course was that of liver rejection. In such animals, the rejection process seemed initially to be focused largely on the portal areas leaving the parenchymatous liver cell quite free. This was reminiscent of kidney in which early rejection is directed at the tubular mass, leaving the glomeruli free of attack.

Future work on liver transplantation in the animal may take a number of different directions. The most important is a further extension of these experiments to view the unmodified rejection response more clearly. A second form of study will depend upon treatment of the recipient with radiation, radiomimetic drugs, with or without bone marrow or splenic transplant, to achieve a state of tolerance in which prolonged hepatic survival might be achieved. A third form of experiment would consist of transplantation into an animal in liver failure.

Looking to the clinical problem, it should be noted that the liver of a recently deceased animal appears to maintain viability so long as either the entire body, the liver perfusate, or the liver itself is rapidly cooled and maintained in the cool state. Techniques for the postmortem preservation of tissues will rest on the temperature coefficients for metabolic activity in each organ and the mechanical details of speed of cooling. The treatment of cirrhosis or other liver disease by hepatic transplantation offers promise clinically only where there is a comparative lack of conjoint pathology in other organs. The treatment of metastatic carcinoma by liver transplantation would offer salvage only in those rare patients where the liver was the only site of metastasis.

The splenic experiments demonstrate the histologic nature of splenic rejection and the fact that the rejection process seems to involve but a transient phase of graft reaction against the host.

Looking to the future of splenic transplantation in the laboratory, it is essential that experiments be carried out to discover methods by which the spleen may be converted into a more active hematopoietic organ. Experiments of this type will involve whole body irradiation or preliminary treatment with radiomimetic drugs (both with splenic protection against the damaging agent). If the spleen can be converted to a more active hematopoietic organ, one may reasonably expect that after whole body irradiation or the use of radiomimetic drugs in the recipient animal, one may achieve splenic acceptance as has been the case with the skin grafts and bone marrow. Whether or not the destructive therapy required as a preliminary must be so severe as to endanger the life of the recipient dog can only be discerned by further study. In an animal harboring and accepting a spleen from a donor animals, one might hope to achieve an ideal situation for subsequent acceptance of other organs because such donor would then be available for transplantation studies of skin, kidney, liver, or other organs. In this regard, if splenic hematopoiesis can be achieved, one has a more fitting donor-recipient relationship than is the case with the bone marrow transplantation. It is difficult to secure enough marrow from a single donor (particularly in man) to repopulate the destroyed marrow of a potential recipient. Multiple-donor experiments are therefore of great interest.

For any of the these future possibilities to become a reality, the first two steps required are those of the development of a method for surgical transplantation of these two viscera, and the description of the normal course of transplantation without other treatment. Such has been the objective of the work herein described.

Conclusions

Experimental whole-organ homotransplantation of the liver and of the spleen has been carried out experimentally in the dog, transplanting these organs to their normal anatomical position and blood supply.
Control experiments have tended to demonstrate that autotransplantation of the organs, returning them to their normal sites.

In both cases a satisfactory surgical technique has been achieved which produces a viable organ. Hepatic hypothermia is an essential feature of the liver transplant. Rejection of these viscera is a gradual cellular process, sharply to be differentiated from avascular necrosis or surgical failure.

In the case of the liver, a significant sparing of the parenchymatous liver cell mass is noticed early in the rejection process, the latter being directed initially entirely towards the portal areas and centrolobular veins.

In the case of the spleen, the rejection process is diffuse, results in atrophy of follicles (after an initial follicular stimulation), loss of follicular activity, and infiltration of the entire spleen with plasma cells that appear to arise from the recipient since there are no nests of active plasma-cell-producing areas in these spleens.

The implications of this work with respect to therapeutic transplantation of the two organs, and future directions for research in this field, are briefly discussed.

Bibliography

Discussion

Dr. Stephen E. Hedberg: Dr. Cole, members of the Association and Guests, I should first like to congratulate Dr. Moore on the presentation of this interesting and excellent paper.

At Walter Reed we also have been interested in the transplantation of canine spleens and our work with transplantation of normal spleens to normal dogs has tended to confirm what Dr. Moore has shown you here today.

One of our aims in spleen transplantation has been to explore the possibility of protecting the recipient animals against high doses of ionizing radiation by means of a spleen transplant.

Very early in the game we satisfied ourselves that the normal spleen will not protect an irradiated recipient. Our protocol was therefore modified, and Dr. Moore thought that you might be interested to see what we are doing now and in hearing about some of our early results.

The first slide is a diagram of our experiment. Our donor dogs are females of D-negative blood type, immunized against typhoid and diphtheria and prepared according to a method outlined by