# Regulation of Spleen Growth in Hepatic Dysfunction

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IN PATIENTS WITH HEPATIC OR BILIARY CIRRHOSIS, spleno-
megaly is generally considered to be the result of
extrahepatic portal congestion, a concept which under
lies the term "congesive splenomegaly."[19,20,31] Informa-
tion from experiments in animals and from recent clini-
cal observations in surgical patients suggests that this
simple mechanical explanation may be inadequate. In-
terruption of splenic venous drainage for example is not
followed by sustained splenomegaly in experimental ani-
mals.[32] In addition, chemically induced injury to the
liver results in splenomegaly even when the spleen is
transplanted out of the portal system.[7] In patients with
hepatic cirrhosis and bleeding esophageal varices, suc-
cessful portacaval shunt frequently fails to function as a
"physiological splenectomy" even though portal pressure
falls.[10] Moreover, preshunt levels of portal pressure may
be higher in patients without splenomegaly.[10] Consid-
ered together these findings provide little support for the
concept of impaired splenic venous outflow as the domi-
nant factor in the pathogenesis of splenomegaly.

Extrahepatic portal congestion develops in animals
with chronic biliary obstruction[27] and it is generally as-
sumed that this circulatory abnormality underlies the
development of splenomegaly in such animals.[12,19,21,25]
Based on these considerations, an attempt was made to
determine whether the effects of surgically induced bi-
liary obstruction on growth of the spleen are actually due
to the extrahepatic portal congestion which develops
concomitantly. As free autotransplants of splenic tissue
regenerate and function when placed in the systemic
circulation[6,15,22] regeneration rate and sequestering func-

tion of such grafts were compared with transplants in
the portal system following surgical ligation and division
of the common bile duct.

## Method

### 1. Preparation of Animals

All studies were performed on Bartonella bacilliformis-
free Caesarian delivered strain of Sprague-Dawley rats
(Charles River c.d.).* As transplanted splenic tissue
grows in a more predictable manner in young animals,[23]
weanling rats were used weighing 50–75g. Under ether
anesthesia and using aseptic precautions, the spleen was
completely removed and a small (2–3 mm) weighed
transverse sections was autotransplanted into either a
subcutaneous pocket in the anterior abdominal wall or,
in a second group, into the peritoneal cavity within an
omental envelope. Three weeks later the common bile
duct was double ligated and divided. A third and fourth
group of animals underwent splenectomy and autotrans-
plantation in the same two locations but without biliary
obstruction and served as controls. A total of 44 animals
were studied, divided equally into four groups. In order
to avoid gram negative sepsis and endotoxinemia, a de-
velopment which could in itself increase growth of the
spleen, oxytetracycline was administered to all animals
in their drinking water.

## 2. Measurement of Growth and Sequestering Function of Transplants

At the end of 7 weeks after transplantation animals in each group were injected by tail vein with 1 ml. of a 50% washed suspension of isologous red cells labeled with 5 u.c. of $Na_2Cr^{51}O_4$.[*] These cells were altered by exposure of a 50% washed cell suspension to an equivalent volume of N-ethylmaleimide[†] (12 m M in isotonic saline) a concentration that provided for preferential splenic uptake of the altered cells.[16] Three hours after injection animals were sacrificed and cardiac blood, liver, kidney, lung, femur and splenic transplant were removed, weighed and assayed for radioactivity in a well-type scintillation counter as previously described.[15] Weight of the transplant on removal was compared with original weight at the time of operation. Sections of livers and splenic autotransplants were then placed in formalin and prepared for histologic study. Serial sections were stained by the following technics: Hematoxylin and eosin, Giemsa and toluidine blue for estimation of cell type and distribution; reticulum and trichrome for determination of connective tissue, periodic acid Schiff (PAS) and phosphotungstic acid-hematolylin (PTAH) to determine presence of "deposit" materials. All sections were examined without reference to a particular experimental group.

## Results

### A. Growth and Function of Spleen Autotransplants: Control Animals

The transplant failed to grow and function in two of the animals undergoing subcutaneous transplantation.
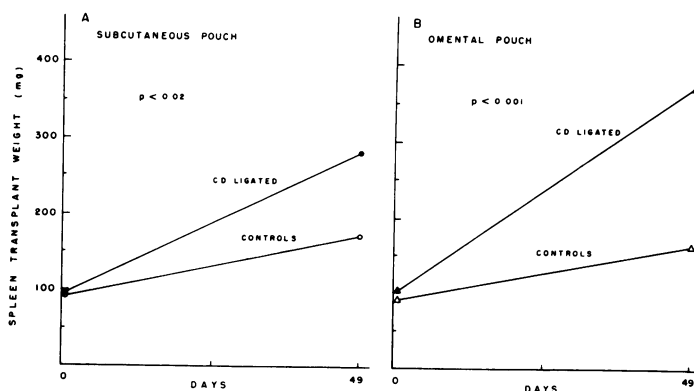


FIG. 1. Effect of CD ligation on gain in weight of spleen autotransplants in subcutaneous (A) and omental (B) pouches. Note that in CD ligated animals growth rates of splenc transplants are significantly greater than in control animals whether or not the transplants are in the portal circulation. Each of the points represents the mean valve from 6–11 animals.

* Obtained from Abbott Laboratories, Chicago, Illinois.
† Obtained from Schwarz Bio Research Inc., Orangeburg, N.Y.

In the remaining animals in both groups gross and histologic findings conformed to those reported previously by others.[15] At the end of the 7th week after transplantation subcutaneous transplants had gained an average of 82 mg compared to 73 mg for grafts in the omentum, an increase of about .75 times the weight of the original transplant (Fig. 1a).

Sequestering function was well established and approximately equal in both groups by the 7th week. Total uptake of $Cr^{51}$ labeled cells averaged 73 ul in subcutaneous and 115 ul in omental grafts.

### B. Common Duct Ligated Animals

Transplants failed to grow and function in two of the animals undergoing subcutaneous transplantation and in five of those undergoing transplantation into the omentum. At the end of 7 weeks after transplantation transplants in the remaining animals had gained an average of 183 mg in subcutaneous locations and 267 mg in the omentum. For subcutaneous grafts this increase in weight was 1.5 times that observed in control animals and for omental grafts twice that of controls. (Fig. 1b) Thus in CD ligated animals growth rates of splenic transplants were significantly greater than in control animals whether or not the transplants were in the portal circulation. Weight of regenerated spleen in some animals undergoing CD ligation exceeded that of the normal spleen in situ in animals of similar size and age. By extrapolation it can be estimated that such spleens would weigh about 600 grams in man.

At 7 weeks well developed vascular channels to and from each functioning transplant were visible grossly. In the case of omental transplants venous connections to nearby omental veins were regularly identified and were particularly prominent in CD ligated animals.

On a total organ basis, splenic sequestration was similar in all control and common duct ligated animals. Nevertheless, in CD ligated animals as in controls, grafts in the omentum tended to sequester slightly more of the injected cells 87 ul vs 54 ul, the differences not being significant.

Data regarding growth of splenic transplants and measurements of sequestering function are summarized in Table 1.

### C. Changes in Liver Size and Sequestering Function

In control animals position of the splenic transplant did not affect either liver size or sequestering function. In animals undergoing CD ligation the liver weighed twice as much as that of control animals in those with transplants in the omentum and about one and a half times more than controls in animals with subcutaneous transplants (Fig. 2). On a gram for gram basis sequestering function decreased slightly in both CD ligated groups

TABLE 1. *Weight Gain and R. B. C. Uptake of Splenic Autotransplants*

| Animal | Controls Weight Gain (mg) | A) Subcutaneous Transplants R. B. C. Uptake (ul) | Animal | CD Ligated Weight Gain (mg) | R. B. C. Uptake (ul) |
|---|---|---|---|---|---|
| 1 | 66.3 | 48 | 10 | 212.8 | 40 |
| 2 | 155.6 | 94 | 11 | 365.5 | 52 |
| 3 | 8.9 | 31 | 12 | 48.9 | 65 |
| 4 | 132.3 | 117 | 13 | 180.3 | 41 |
| 5 | 46.7 | 64 | 14 | 120.6 | 67 |
| 6 | 156.5 | 87 | 15 | 97.5 | 66 |
| 7 | 139.0 | 87 | 16 | 401.9 | 66 |
| 8 | 22.9 | 82 | 17 | 146.7 | 49 |
| 8 | 42.6 | 50 | 18 | 88.3 | 41 |
| | | B) Omental Transplants | | | |
| 19 | 44.6 | 56 | 30 | 200.1 | 74 |
| 20 | 20.6 | 56 | 31 | 357.0 | 104 |
| 21 | 188.7 | 125 | 32 | 175.0 | 77 |
| 22 | 139.7 | 107 | 33 | 331.3 | 132 |
| 23 | 21.9 | 70 | 34 | 218.0 | 76 |
| 24 | 31.0 | 59 | 35 | 325.3 | 62 |
| 25 | 73.2 | 122 | | | |
| 26 | 66.6 | 78 | | | |
| 27 | 59.3 | 109 | | | |
| 28 | 108.2 | 150 | | | |
| 29 | 50.8 | 294 | | | |

compared with corresponding controls but this change was not significant.

### D. Histologic Findings

Livers of control rats were normal. In animals undergoing CD ligation intrahepatic bile duct proliferation was striking[8] and accompanied at times by occasional necrotic foci: fibrosis was absent.

All regenerating spleens displayed a thickened capsule and vascular adhesions. Apart from the capsule no increased deposition of fibrous tissue was noted. Neither PTAH nor PAS positive material was found and in control animals transplants closely resembled normal spleen.

Two striking alterations characterized transplants in animals undergoing CD ligation. (Figs. 3 and 4) First, the lymphocyte cell mass or white pulp was markedly increased. Lymphocytes were often so widely distributed and numerous that identification of a distinct red pulp was difficult. At times, and particularly in omental transplants, collections of excess lymphocytes were found outside the capsule. In addition, aggregations of small lymphocytes formed Malphigian corpucles which were two to four times larger than in controls. Second, germinal activity was markedly reduced and often absent in such transplants. Small accumulations of germinal cells were seen occasionally or less often, small numbers of such cells were scattered throughout the white pulp. Histologic appearance of transplants in control animals conformed to that reported previously.[15]

### E. Portal Pressure

To confirm the effect of biliary obstruction on portal pressure,[27] the common bile duct was doubly ligated and divided in a separate group of six rats. Three weeks later the animals were re-examined and portal pressure measured with a saline monometer attached to a cannula in the portal vein. At this time pressures ranged from 15–19 cm $H_2O$ compared to 5–10 cm in a control group of six rats.

### Discussion

In these experiments, weight of subcutaneous transplants in CD ligated animals was 1.5 times more than control values but was less than that of transplants in the portal circulation. The results recall earlier studies of Cameron and DeSaram who observed increased growth of splenic transplants placed in the systemic circulation of animals exposed to carbon tetrachloride.[7] The mechanism underlying this increased growth of the spleen during hepatic dysfunction is obscure but clearly does not depend upon "congestive splenomegaly" a term almost always used to describe the process.[17,19,21,30] Introduced almost 40 years ago by Larabee[20] to imply that portal congestion alone underlies enlargement of the spleen in patients or in animals with hepatic dysfunction, "congestive splenomegaly" has become a convenient clinical expression but it implies the presence of information which is in fact lacking; such hemodynamic effect would be unusual and is incapable of experimental dupli-
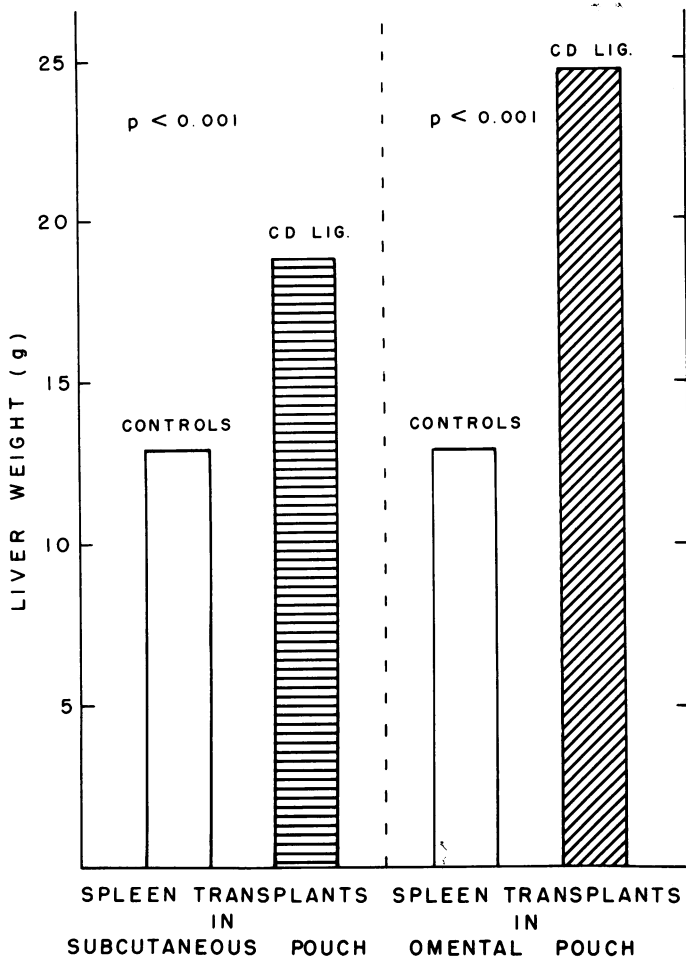
Fig. 2. Effect of CD ligation on liver weight. In CD ligated animals liver weights are signigficantly greater than in controls regardless of the location of the spleen transplants. Increase in liver weight is more marked however with spleen autotransplants in the portal circulation. Each bar represents the mean valve from 6–11 animals.

cation. In CD ligated animals the observation that transplants in omentum grew larger on the average than those placed subcutaneously suggests however that portal congestion may influence spleen growth under certain circumstances. Thus venous congestion appears to be a supplemental factor and not essential for increased spleen growth. The effect of this factor could be related to an increase in splenic lymph formation, an abnormality regularly associated with splenic congestion.[11]

Histologic examination of autopsy specimens of liver and spleen obtained from patients with hepatic disorders led McMichael to suggest a dominant role for reticuloendothelial hyperactivity in initiating increased growth of the spleen.[24] Recently the relationship between splenic growth and RE function has been clarified and it is recognized that: 1) decreased blood flow through hepatic sinusoids is a general feature of most kinds of liver injury,[3] a derangement which is accompanied by reduction

in hepatic sequestering activity,[3,18] 2) a reciprocal relationship exists between hepatic and splenic sequestering function,[15] and 3) increased requirement for splenic sequestering function stimulates growth of splenic transplants.[15] In the light of this knowledge, earlier observations by Whipple,[31] Moschowitz,[25] Rousselot and Thompson[26] and others which seemed to demonstrate that splenomegaly results from portal hypertension could reflect instead the inhibitory effect of decreased transsinusoidal blood flow on hepatic RE activity with compensatory enhancement of splenic RE activity. While support for this view is provided by findings of increased spleen growth following partial hepatectomy or ligation of a branch of the portal vein,[2,28] it is curious that in published reports detailing the effects of an Eck fistula in experimental animals, references to development of splenomegaly are completely lacking. It is tempting to speculate that under these conditions an abnormally rapid transplenic blood flow[33] may allow excess splenic RE activity but prevent splenomegaly.

In animals undergoing CD ligation the finding that splenic transplants in the congested portal circulation sequestered approximately the same number of red blood cells as transplants in the systemic circulation conforms to clinical observations reported recently by Holzbach, Shipley, Clark and Chudzik.[14] In patients with cirrhosis they were unable to correlate increased splenic red cell sequestration with raised portal pressure and suggested instead that splenomegaly in itself may promote increased sequestration. In the experiments described here however sequestering function of splenic transplants measured after 3 weeks of complete biliary obstruction was the same on the average as in control animals, an unexpected observation considering their increased size. Results seem to vary in published reports regarding the effect of biliary obstruction on RE sequestering function. Helpern, Biozzi, Nicol and Bilbey, for example measuring uptake of carbon particles found RE function essentially unchanged in liver and increased in spleen on a unit basis.[12] Increased weight of both liver and spleen resulted in a net increase in sequestering activity. Aronsen, Nylander and Ohlsson measuring uptake of colloidal gold found hepatic sequestering activity lower than in controls, due to decrease in effective transhepatic portal flow.[1] In the experiments described here sequestration of altered red blood cells in liver also decreased slightly per gram of liver tissue, a change balanced by increase in liver mass so that net hepatic sequestering function was restored to normal. The fact that this increase in liver mass was consistently greater in animals with transplants in the portal system is unexplained but suggests that the well known regulatory influence of portal venous blood on hepatic regeneration may derive in part from the spleen. Based on these considerations two factors could

FIG. 3. Microphotograph of a section of regenerating spleen in a control animal 49 days after transplantation. Intense germinal activity is evident in the center of a normal corpuscle. Densely packed small lymphocytes surround this germinal center. H & E × 250.
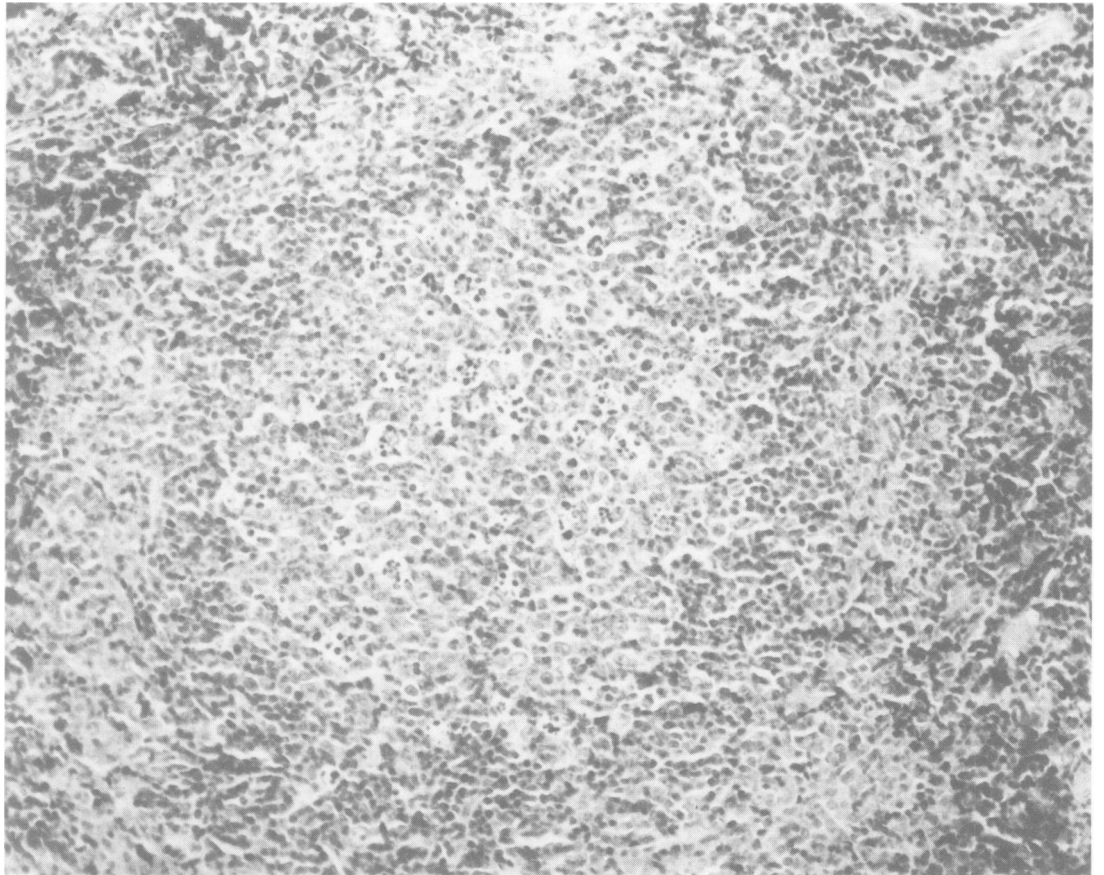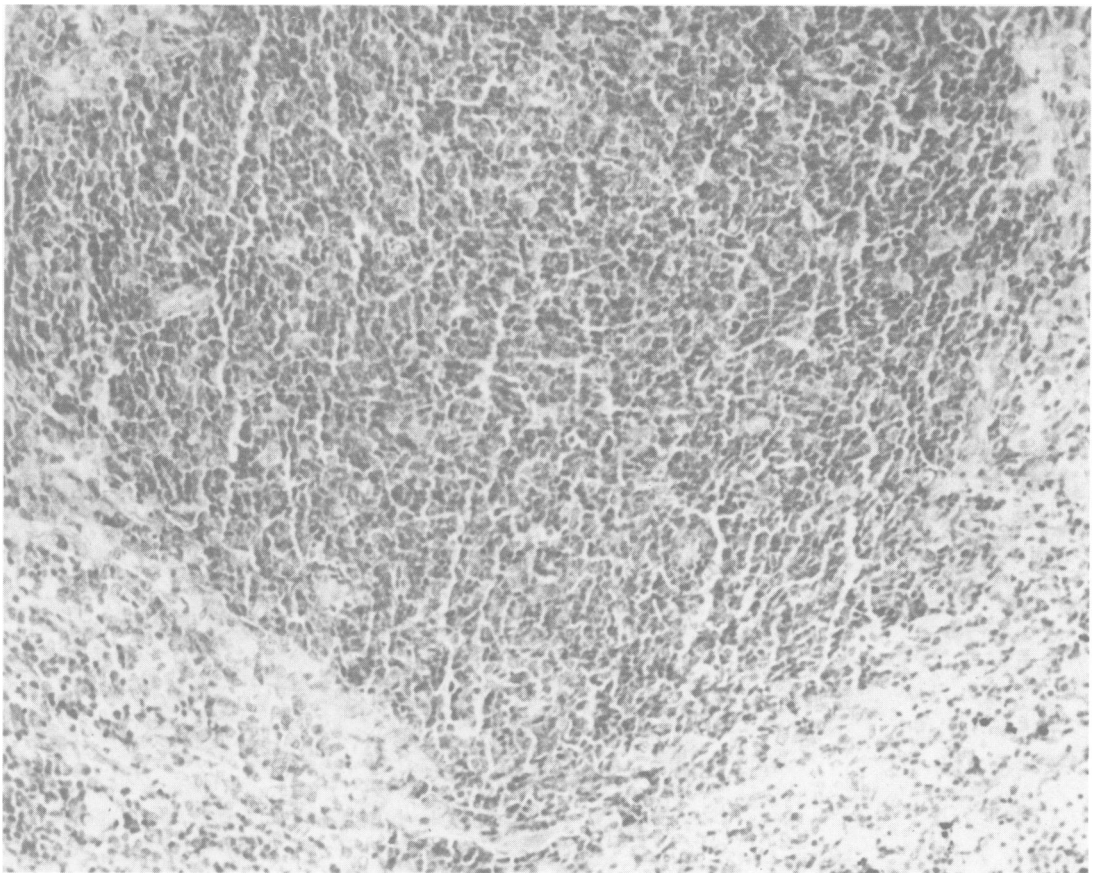


FIG. 4. Microphotograph of a section of regenerating spleen in a CD ligated animal 49 days after transplantation. Approximately one half of an abnormally large, elliptically shaped corpuscle can be seen at the same magnification as in Fig. 3. Germinal activity is absent. H & E × 250.

explain failure of the larger transplants in CD ligated animals to sequester more of the labeled cells. First, decrease in total hepatic RE function was transient, persisting only until gain in liver weight restored net hepatic sequestering function to normal. Second, available evidence indicates that reticulum cells, active elements in initiating splenic growth, are the components which respond to unusual functional demands on the RE system. As a large portion of such cells in regenerating transplants differentiate by 2–3 weeks into lymphocytes and other cell types with concomitant loss of sequestering ability, it is likely that the residual pool of cells with sequestering ability progressively decreases as growth and differentiation proceed.[8] A continuous stimulus such as one which results from a marked degree of persistent excess hemolysis is probably required for continued proliferation of cells with sequestering function. In this regard, Jacob, MacDonald and Jandl found that growth rate as well as sequestering function of subcutaneous transplants were significantly greater when splenectomy was combined with sustained phenylhydrazine induced hemolysis.[15]

In CD ligated animals hyperplasia of the white pulp, the immunologically competent component of spleen, constituted the predominant histologic alteration in transplants in the portal as well as those in the systemic circulation. Cameron and DeSaram found that the same change predominated in whole spleen transplanted out of the portal bed in carbon tetrachloride treated animals.[7] Hyperplasia of splenic white pulp without germinal center activity also regularly follows strong or sustained antigenic stimulation[13] and recent studies suggest that in hepatic dysfunction this stimulation could result from altered disposition of circulating antigens absorbed from the gastrointestinal tract. While the strategic interposition of liver between the gastrointestinal tract and the general circulation normally insures against sensitization to such antigen, derangements in transsinusoidal portal flow or spontaneous porta caval shunts or both may so alter the disposition of antigen that effectiveness of the liver in this regard is lost, antigen continues to circulate and sustained immunologic stimulation of the spleen results.[4,9,29] Whether or not splenic white pulp hyperplasia develops in normal animals following creation of an Eck fistula is unknown, but in patients with liver disease abnormal serum levels of immunologically active protein and abnormal sensitivity to antigens absorbed from the alimentary tract are much more prominent in those who have undergone a porta caval shunt.[4,5]

## Summary

In hepatic or biliary cirrhosis splenomegaly is generally considered to be the result of extrahepatic portal congestion, a concept which underlies the term "congestive splenomegaly." In these experiments an attempt was made to determine whether the portal congestion assoiated with experimental ligation of the common duct is actually responsible for the increased growth of the spleen which also follows this maneuver. Based on the fact that free, 2–3 mm slices of splenic tissue regenerate and function in subcutaneous as well as in omental pouches, regeneration rate, sequestering function (uptake of $Cr^{51}$ tagged rbc) and histology of 7-week-old splenic autotransplants in the systemic circulation were compared with similar autotransplants in the portal bed in 44 splenectomized rats following 3 weeks of CD obstruction and in controls.

Weights of subcutaneous splenic transplants in CD ligated animals averaged more than 1½ times those of corresponsing transplants in non-CD ligated controls. Transplants in the omentum of CD ligated animals weighed almost 2 times more than corresponding transplants in controls. Uptake of $Cr^{51}$ tagged cells by transplants was similar in all controls and CD ligated animals. Hyperplasia of white pulp and absence of germinal activity characterized both subcutaneous and omental splenic transplants in CD ligated animals.

Portal congestion is not a requirement for increased growth of spleen in animals with hepatic dysfunction. Underlying mechanisms are still obscure but may depend on transient stimulation of splenic RE activity secondary to decrease in hepatic sinusoidal blood flow.

## Acknowledgments

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