EXPERIMENTAL HEPATIC ISCHEMIA*

COLLATERAL CIRCULATION OF THE LIVER

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RECENT SURGICAL PROCEDURES in the treatment of portal hypertension have emphasized the problem of hepatic ischemia, for they involve curtailing the blood supply of the liver. This problem often arises in the radical treatment of carcinoma of upper abdominal organs, and may also occur through accidental damage to the hepatic artery during cholecystectomy.

Our knowledge of hepatic ischemia has been limited to the effects of diversion of the portal flow, since interference with the arterial blood supply of the liver had a fatal outcome. Following the discovery in Toronto that dogs would survive ligation of the hepatic artery when treated with antibiotics,[8, 21] further experimental investigation of hepatic ischemia became possible.[32]

Such investigations were carried out on a series of 29 dogs with a three-stage operation, and biochemical and histological studies were done after each stage. The following methods were used:

METHODS

Adult dogs of either sex, weighing 10 to 15 Kg. were used. All were fed "Purina Dog Chow" *ad libitum*. After operation, this diet was supplemented by a meat-free diet consisting of 300 Gm. milk, 200 Gm. bread and 30 Gm. corn syrup.

Operative Technic

The first-stage procedure consisted of ligation of the common hepatic artery and forming a partial Eck fistula, *i.e.*, a portacaval anastomosis and ligation of the portal stem caudal to its pancreatico-duodenal tributary. The latter continued to empty its blood into the liver.

Under Nembutal anesthesia, 35 mg./Kg. body weight, an upper abdominal midline incision was made. The duodenum was packed off to the left, gathering the intestinal loops in its mesenteric fold. A linen thread was looped around the common hepatic artery and another was placed around the portal vein, caudal to its pancreatico-duodenal tributary (Fig. 1). The portal vein was freed of its overlying tissue for one and one-half inches peripherally. A side-to-side anastomosis of the portal vein with the inferior vena cava was carried out with the cutting-thread technic described by Fischler and Schröeder as modified by Fishback.[6] After completion of the anastomosis the thread around the portal stem was tied. The linen thread around the common hepatic artery was then tied and the abdomen was closed in three layers.

The second-stage procedure was carried out under intratracheal ether insufflation, six to eight weeks after the first operation. It consisted of ligating all branches of the hepatic artery. Through a midline incision the hepato-duodenal ligament was exposed and freed of adhesions. The hepatic artery proper and all its branches were exposed by blunt dissection. By means of "climbing ligatures" the arterial branches were dissected upward and ligated with linen thread close

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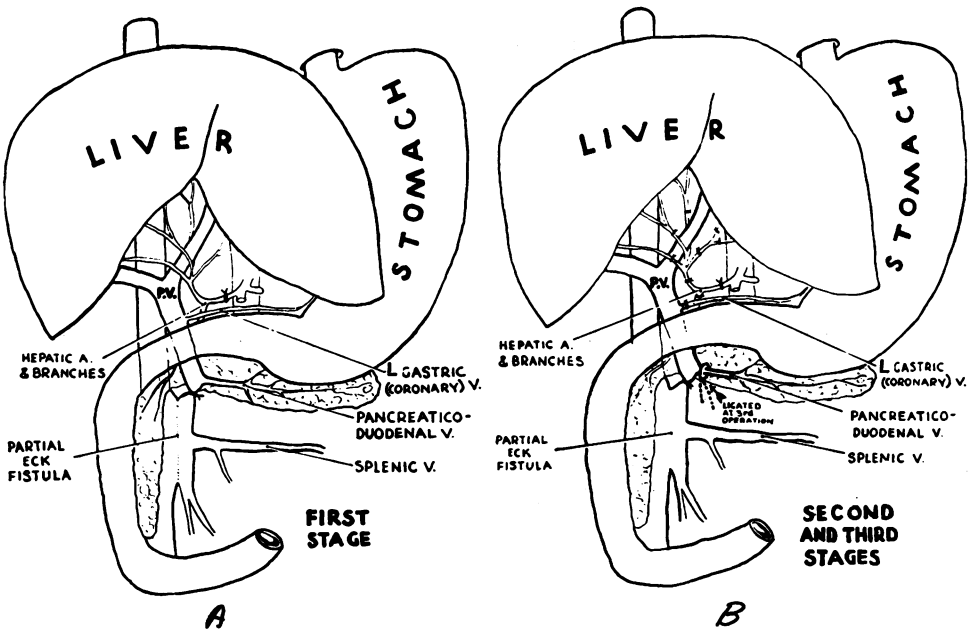

FIG. 1. (A) First stage. The stem of the common hepatic artery is ligated proximal to the gastroduodenal artery. Portacaval anastomosis and ligation of the portal vein caudal to the pancreatico-duodenal vein (partial Eck fistula). The small left gastric vein flows into the portal stem. (B) Second stage. All branches of the hepatic artery and the left (coronary) gastric vein are ligated. The liver is supplied with blood (besides that from its arterial collaterals) mainly by the pancreatico-duodenal vein. Third stage. The pancreatico-duodenal vein is ligated. The hepatic artery and the portal vein are completely tied off.

to the portal fissure (Fig. 1A). The gastroduodenal artery was tied off. The technic was the same as that used in our joint work with Dr. D. Fraser.[8] The portal vein was again inspected for tributaries (other than the pancreatico-duodenal) that might have increased in size and become visible. When present, they were tied off. The abdomen was closed as usual. Penicillin, 300,000 units, was given daily for eight days postoperatively.

The third-stage operation consisted of ligating the pancreatico-duodenal vein, the last tributary of the portal vein. Under ether anesthesia, six to eight weeks after the second-stage procedure, a midline incision was made. The hepato-duodenal area was freed of the many adhesions which had formed around the linen ligatures in the hepatoduodenal ligament. The duodenal loop, usually adherent to liver and gallbladder, was freed by sharp dissection and packed off to the left. The ligature obstructing the portal vein caudal to the pancreatico-duodenal vein served as a good landmark for entrance of the latter into the portal vein. The thin-walled pancreatico-duodenal vein, usually increased in size because of its temporary collateral function, was prepared with sharp dissection and ligated with two 000 silk ligatures (Fig. 1A). The closure of the abdomen sometimes required the preparation of the anterior rectus sheath.

Pathoanatomical Studies

Liver biopsies. Slices of liver tissue were removed during the different stages of operation. Bleeding was controlled by mattress sutures of plain catgut No. 1. Where the biopsy was taken 48 hours after the first-stage procedure, there was no bleeding whatsoever.

Autopsy material. Portions of liver and other organs were obtained from animals that died or were sacrificed, and were usually fixed in formal-calcium or Bouin's solu-

tion. The following staining methods were used: Hematoxylin-eosin, Sudan IV or Oil Red O–light green (Wilson's stain) for the demonstration of fatty changes, modified Mallory's or Bielschowski's stains for reticulin fibers.

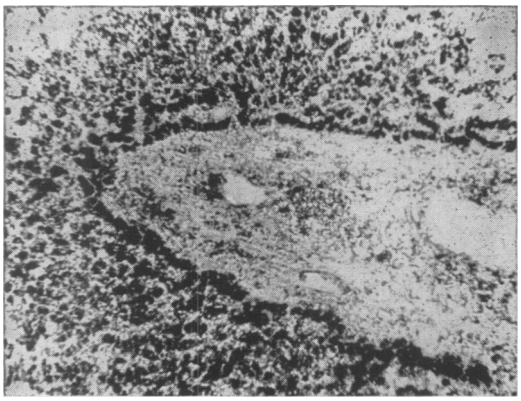

FIG. 2. Around the portal canal there is a plate of fatty, degenerated non-necrotic parenchymal cells. Frozen section, Wilson's stain. Oil Red O, hematoxylin, light green (x 76).

Biochemical Studies

Liver function tests were carried out after the second and third stages of operation.

Thymol turbidity test method of Maclagan,[18] using the standard curve of Shank and Hoagland.[43]

Serum bilirubin electrophotometric method of Malloy and Evelyn[20] as modified by Schiff.[42]

Bromsulphalein retention, Rosenthal and White's[40] method, as modified by Gornall and Bardawill,[9] using a dose of 10 mg. bromsulphalein/Kg. body weight, taking samples after 15 and 30 minutes.

Electrophoresis of the plasma proteins. Heparinized blood samples were taken in the morning to avoid any postprandial increase of lipoid material. Three milliliters of heparinized plasma were diluted to 12 ml. with barbiturate buffer of pH 8.6 and ionic strength 0.1. The diluted plasma was dialyzed against 1 liter of buffer for 18 to 20 hours at 0° C. The standard analytical cell of the Aminco-Stern electrophoresis apparatus was used for all determinations.

Glucose tolerance test. Glucose tolerance was investigated in normal dogs and in dogs after each of the three operative stages. The tests were carried out after a 24-hour fast, usually during the first or second postoperative week and again after two to three months. Some dogs were tested six months after the third-stage operation. Glucose, 1 Gm. per Kg. body weight, prepared as a 50 per cent sterile solution, was injected intravenously. Blood sugar was determined according to the method of Miller and van Slyke.[27] The normal values obtained from 18 normal dogs and postoperative findings were plotted for comparison.

Studies on Collateral Hepatic Vessels

Latex injections were carried out to assess the effectiveness of our technic for ligating the hepatic vessels and to study the collateral vescular channels that had developed after all the named vessels of the liver were tied off. The animals were heparinized and sacrificed under Nembutal anesthesia by exsanguination. Following thoracotomy, a large cannula was introduced into the thoracic aorta and its tip fixed with a ligature above the diaphragm. The abdominal aorta was ligated below the renal arteries. Another angled glass cannula was introduced into one of the main branches of the portal vein at the portal fissure. The tip of this cannula was directed away from the liver. One liter of warm physiological saline was flushed through the aortic cannula and all blood washed out of the liver. Red latex was introduced through the aortic cannula under a pressure of 100 cm. water. Blue latex was forced retrogradely into the portal vein. The cannulas were left in place and clamped off for 12 hours. The preparations were dissected and the vascular pattern was sketched.

Radiological Studies

Radiograms of the hepatic vessels were taken after guided catheterization of either

the hepatic veins[33] or the celiac axis.[34] For the hepatic veins, this was carried out through the femoral vein; for the celiac axis, via the femoral artery. A transflex catheter (French 7 to 10) with a special metal leader inside was introduced into the exposed femoral vein or artery of the anesthetized and heparinized animal. Catheter and leader were moved upwards into the great abdominal vessels until the diaphragm was reached. Here the end of the metal leader was curved by turning the control knob on the handle and moved with the catheter caudally. As soon as the tip of the curved catheter met the opening of a side-vessel it hooked into it. The catheter was then slid over the curved leader, which was held in position, and allowed to penetrate as deeply as the width of the catheterized branch of the large vessel permitted. The leader was removed while its curve was gently released. A radiopaque substance was injected into the catheter and roentgenograms of the explored vessel were taken.

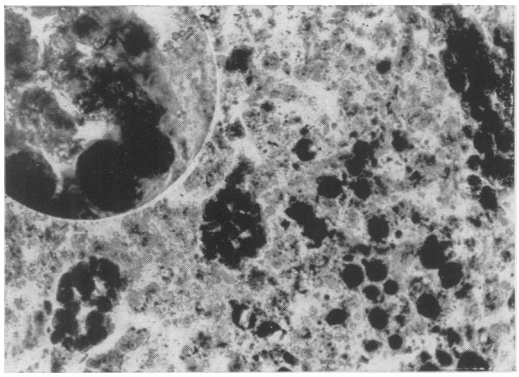

FIG. 3. Liver biopsy eight months after second stage operation. The collections of sudanophilic material have formed from altered red blood cells. These sudanophilic iron-containing masses represent the stigmata of areas of hemorrhagic necrosis. Frozen section, Wilson's stain. Oil Red O, hematoxylin, light green (x 71. Inset x 320).

Estimated Hepatic Blood Flow

The blood flow through the liver was estimated by the method of Bradley, Ingelfinger and their associates.[3] The arterial samples were obtained from the exposed femoral artery. The blood samples from a left or right hepatic vein were procured by guided catheterization. The animals were anesthetized with Nembutal and heparinized. Continuous bromsulphalein infusion was accomplished with a small electric-powered pump. The rates of infusion were 2 ml./min., the saline solution infused containing 0.5 mg. dye per milliliter. Packed cell volumes were determined, using copper sulphate solutions and a nomogram. Plasma B.S.P. concentrations were determined colorimetrically. The calculations were made as outlined by Ingelfinger and his associates. For these the total blood volume was estimated as 10.8 per cent of the body weight.

RESULTS

First Stage

Twenty-nine dogs underwent ligation of the common hepatic artery and formation of a partial Eck fistula in a one-stage procedure. One dog succumbed after acute eventration. Another seven died within 24 to 96 hours; three of them showed signs of hepatic coma lasting from 31 to 96 hours. Twenty-one dogs recovered following several days of illness. One of these displayed characteristic hepatic coma for seven days. The other dogs were able to undergo the second-stage operation six to eight weeks later.

Pathological changes in the biopsy and autopsy material. Both the external and the cut surfaces of the livers were mottled by areas of ischemic necrosis and congestion. The latter was sometimes generalized, producing subserous suffusions and petechiae on all abdominal organs. The congestion of the kidneys was remarkable. Microscopically, as a rule, in the liver there was widespread centrolobular necrosis of hemorrhagic and lysing nature, sparing from destruction only small islands of periportal parenchyma. The non-necrotic periportal parenchyma varied in amount, and exhibited varying degrees of fatty change.

TABLE I. *Liver Function Tests in Dogs.*

	After second stage operation				
	Serum Bilirubin		Bromsulphalein Retention		Thymol Turbidity
	Direct	Indirect	15 min.	30 min.	
	mg%	mg%	%	%	units
7–10 days					
A35	0.045	0.25	—	3.5	1.0
A55	0.14	0.06	24.0	15.6	0.6
A58	0.075	0.0	23.5	17.5	1.4
A46	0.09	0.24	11.8	4.4	1.4
2 months					
A35	0.06	0.02	—	3.1	1.1
A46	0.24	0.33	14.7	7.2	1.2
A47	0.015	0.33	9.3	5.6	0.8
A48	0.12	0.6	20.8	9.8	0.9
4 months					
A55	0.090	0.150	9.8	—	0.8
A58	0.075	0.210	8.3	6.2	1.2
A23	0.015	0.270	11.8	5.2	1.5
Mean values for normal dogs	0.12	0.27	6.3	3.0	1.8

Around some of the portal canals, single layers of fatty parenchymal cells (Fig. 2) were present. Some of the cells immediately adjacent to the necrotic areas were dissociated, swollen, and contained sudanophilic material. Early accumulation of iron pigment in macrophages and in parenchymal cells was observed in the same areas.

Liver function tests, radiological studies, and estimations of hepatic blood flow, were not carried out after the first stage operation, which was considered as merely a preliminary step to a further reduction in blood flow.

Glucose tolerance test. The dogs did not show a lowered glucose tolerance after they had recovered from the immediate postoperative sequelae. When tested two to three months later, the values were still normal.

Second Stage

Nineteen dogs underwent the second-stage operation which consisted of ligating *all* the branches of the hepatic artery. Thereafter the liver received only blood from the pancreaticoduodenal vein. Four dogs died. Three of them showed characteristic signs of bile peritonitis due, in two cases, to perforation of the gallbladder, and in one instance to injury of the bile duct. One animal wasted away five and one-half months after operation. The 15 surviving dogs regained strength and health quickly. Four of them were sacrificed for histopathological studies. Nine underwent the third-stage operation and two were still alive and in good condition after two years.

Pathoanatomy. There were no striking gross lesions. Some adhesions around the gallbladder were present. The livers were normal in size, shape, color and consistency. Microscopically there was regeneration and repair of the pericentral ischemic necrotic lesions. The liver cords, however, appeared somewhat small and embryonic-like. Some parenchymal cells were dusted with fine powderings of sudanophilic droplets. In addition there were scattered throughout, and in both central and portal areas, collections of sudanophilic masses (Fig. 3) which appeared to consist of altered red blood cells in long-standing areas of focal hepatic necrosis. A yellowish-brown pigment, intermixed with these masses, gave a positive Prussian blue stain, revealing it as hemosiderin. These masses apparently were stigmata of areas of hemorrhagic necrosis. Frank necrosis and subsequent fibrosis were completely absent except in the periportal areas of the liver of one dog which had received an injection of toxic radiopaque material (Thorothrast) for radiological studies.

Liver function tests. To assess the more lasting disturbances of hepatic function produced by the operation described above, the dogs were subjected to a series of liver function tests commencing at least seven to ten days after surgical intervention. The results were compared with the mean values given at the end of each table, which were determined by Dr. Fraser[7] for a group of 20 apparently normal dogs. There was bromsulphalein retention in all dogs in the immediate postoperative period and even four

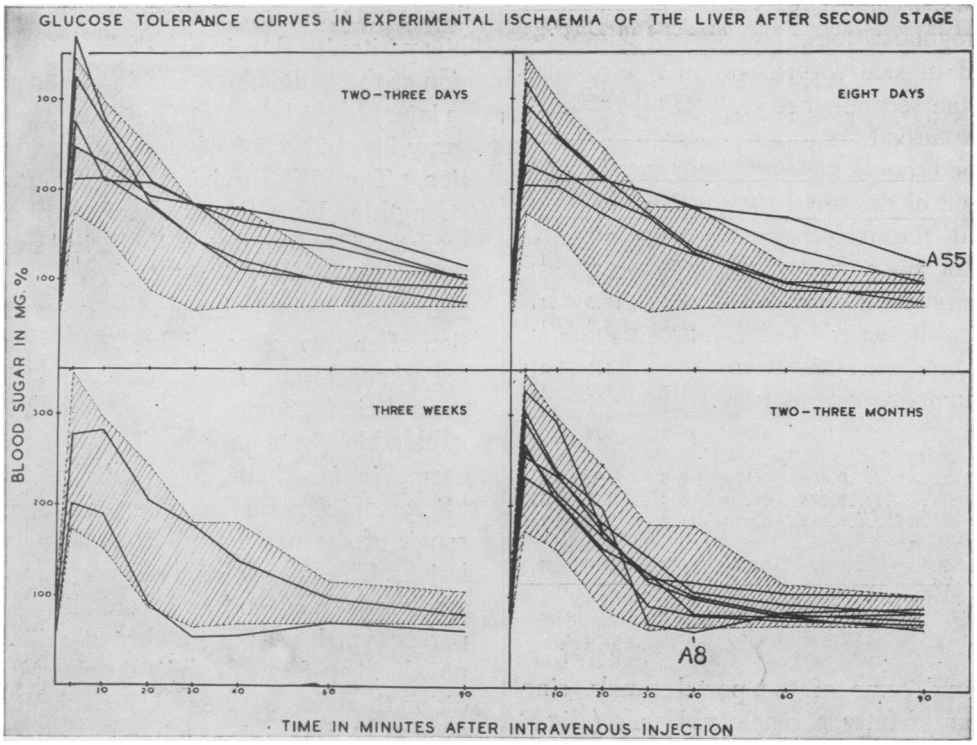

FIG. 4. The glucose tolerance is slightly lowered during the first few days after operation and becomes almost normal by the eighth postoperative day. Later on, the curves stay within the range of normal (shaded areas).

months later (Table I). Dog A-55, which had a stormy recovery from generalized peritonitis due to the perforation of the gallbladder following ligation of the hepatic artery, showed, in addition to the B.S.P. retention, an abnormally high serum bilirubin level.

We wonder if this constantly delayed excretion of B.S.P. is related to the alteration in hepatic blood flow rather than to the slight parenchymal damage.

Electrophoretic study. Two to three weeks after the second operation the plasma of these animals showed a slight decrease in the albumin fraction and increases in the beta and gamma globulins and in fibrinogen. The A/G ratio was lower than normal.[35]

Glucose tolerance. The glucose tolerance was tested two to three days, eight days, three weeks and two to three months after the second-stage operation (Fig. 4). In all animals a lowered glucose tolerance was observed during the first few days after operation. It was probably due to the reduced food intake at this time. Except for the plateau-like curve of dog A-55 (in self-starvation because of its bile-peritonitis) the results were normal by the eighth postoperative day. The tolerance curves, three weeks and two to three months after operation, are practically normal. Noteworthy are the one for dog A-25 which delimits a smaller area than normal, and that for A-8 with a slightly hypoglycemic phase. In both these animals the pancreatico-duodenal artery had been ligated during operation to eliminate the hepatic blood supply through this collateral route.

Studies on collateral hepatic vessels. An increase of collateral arterial and portal blood supply to the liver was always observed during the second- and third-stage

operations. The pancreatico-duodenal vein was regularly found to have doubled or tripled in size within one to two months after the second-stage operation. Although the portacaval shunt was patent and the portal blood should have preferred the pressure gradient of this angioanastomosis, it flowed instead through collateral routes to the ischemic liver. In two cases, however, the anastomosis had sealed and the subsequent ligation of the markedly enlarged pancreatico-duodenal vein at the third operation was immediately followed by complete arrest of the mesenteric venous flow. One animal died three hours after operation, while in the other the portacaval anastomosis was re-established during operation.

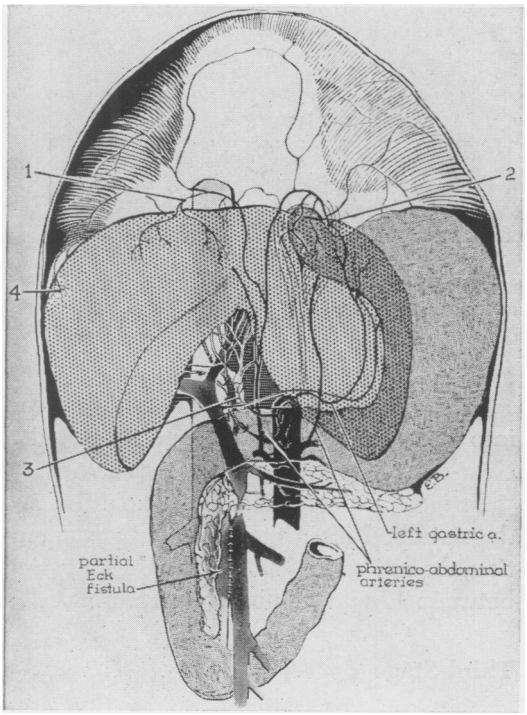

FIG. 5. Collateral arterial circulation of the dog's liver with partial Eck fistula after complete ligation of the hepatic artery. (1) A crown of collateral vessels around the vena cava coming up from the phrenico-abdominal arteries and sending fair-sized twigs to the right and central liver lobes. (2) Collateral arterial twigs to the left liver lobe arising from the left gastric artery and its anastomosis with branches of the phrenico-abdominal arteries. (3) A plexus of collateral vessels spun around the bile duct and situated in the subserosa of the hepatoduodenal ligament sends twigs into the liver. This plexus is formed by the anastomosis of branches from the left gastric with ones from the phrenico-abdominal arteries. (4) Collateral arterial twigs coming up from the right phrenico-abdominal artery and coursing through the right coronary ligament into the liver.

Another affluent of the portal vein to which little attention has been paid in the experimental literature is the coronary vein of the stomach. It sometimes joins the pancreatico-duodenal vein but, more regularly, it empties as a separate branch directly into the portal vein. During the second-stage operation this vessel was often found to have remarkably increased in size in spite of complete patency of the portacaval anastomosis. Thus, to achieve a total diversion of venous blood from the liver, all small veins emptying into the portal vein cranial to the site of ligation in a classical Eck-fistula (*i.e.*, above the pancreatico-duodenal vein), had to be ligated separately. The gastro-duodenal artery was sometimes doubled in size, and its anastomoses with the inferior pancreatico-duodenal artery were conspicuous. Strong pulsations along the tortuous course of these vessels revealed the role they had played in conveying blood as collaterals to the hepatic artery proper after the common hepatic artery had been ligated.

Injections with red latex through the thoracic aorta were carried out to reveal the collateral hepatic arterial circulation. Retrograde injection with blue latex through the left branch of the portal stem was done to discover any additional or untied venous vessel emptying into the portal vein. The pathways of collateral arterial hepatic circulation that developed are illustrated in the composite drawing, sketched from dissected autopsy specimens (Fig. 5).

A crown of collateral vessels (1) around the subdiaphragmatic part of the inferior vena cava was observed to arise from the phrenicoabdominal arteries and to send fair-sized branches to the right and central liver lobes. Collateral arterial twigs to the left liver lobe (2) arose from the left gastric artery and its anastomosis with branches of the phrenicoabdominal arteries. A plexus of

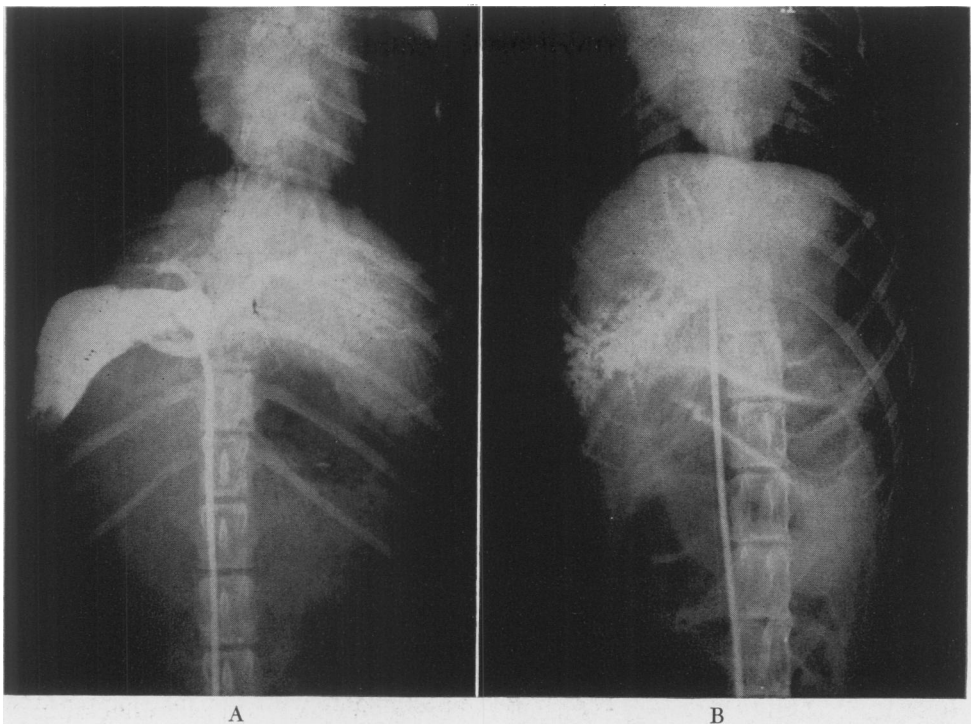

FIG. 6. Hepatic venography. (A) Vascular pattern of the right and left hepatic veins in a normal dog's liver. Branches of the fifth order can be distinguished. (B) Sacculation of the hepatic vein branches of the third order in an atrophic liver after second stage operation. The border of the left liver lobe is sharply delineated.

collateral vessels woven around the common bile duct (3) was situated in the subserosa of the hepato-duodenal ligament and sent its twigs into the liver. This plexus was formed from branches of the left gastric artery and those of the phrenicoabdominal arteries. Collateral arterial twigs also rose from the right phrenicoabdominal artery and coursed through the right coronary ligament (4) into the liver.

Hepatic Venography showed sacculation of the venous branches (Fig. 6B) instead of the finer arborizations of the vascular tree as seen in the normal liver (Fig. 6A), where the entire hepatic venous system is visible. The hepatic veins in these atrophic livers are apparently deprived of their fine root-like tributaries, which help to interconnect different vascular areas. The rapid injection of diodrast into these veins might have produced a temporary forceful dilatation of their third-order branches. Nevertheless, sufficient radiopaque substance has spread into the rest of the liver and produced a clear-cut delineation of the organ.

Estimated hepatic blood flow. In the two dogs studied after the second-stage operation, the estimated hepatic blood flow varied between 10 and 21 per cent of normal.

Third Stage

Nine of the dogs that survived the second-stage operation and two other dogs that had previously undergone a portacaval anastomosis and complete ligation of the hepatic artery, were subjected to the third-stage operation. It consists of ligating the pancreatico-duodenal vein, thus completing the shunting of all portal blood away from the liver into the systemic circulation. One animal died immediately after operation from acute intestinal venous stasis following ligation of the pancreatico-duodenal vein be-

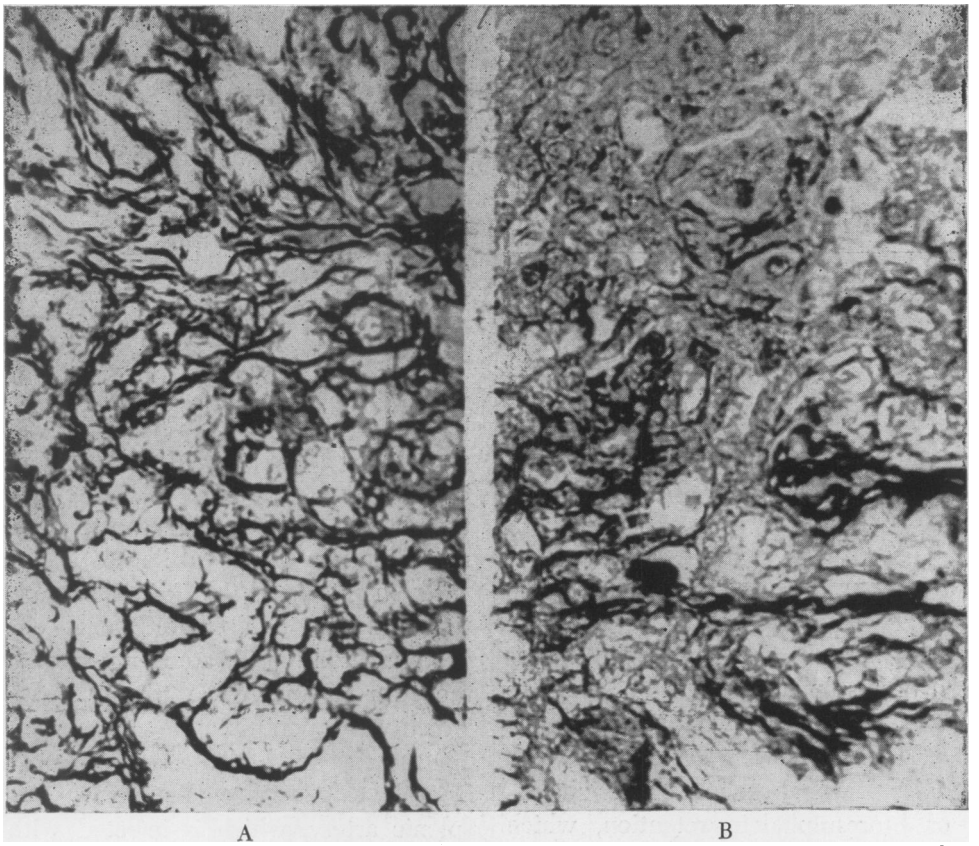

FIG. 7. Reticular and connective tissues three months after third stage operation. The atrophic parenchymal cells are disorganized. The stromal framework is more conspicuous due to increased reticulin fibers and early fibrosis. Right half: Paraffin section, connective tissue stain (x 475). Left half: Paraffin section, Bielschowsky silver stain (x 475).

cause the portacaval anastomosis had become occluded. The other ten dogs survived, but remained in a precarious state of health. Although meat was rigorously withheld from all their diets, four soon developed severe bouts of hepatic lethargy (the so-called "meat intoxication"), and one died of this complication. The surviving nine dogs were sacrificed for pathoanatomical studies at different intervals after operation, four of them as long as 16 months later.

Pathoanatomy. The livers showed parenchymal atrophy ranging from reduction in size to complete disappearance of liver lobes. There was involution of liver cords which became small, especially around the central vein where the cells were dedifferentiated, disorganized and the stroma was much increased (Fig. 7). Comparing the histological findings with those seen in biopsies after the second stage, it was apparent that the liver cells had suffered after the last of the portal blood was shunted away from them. The microscopic examination in some of the animals (Fig. 8), however, did not reveal marked abnormalities. At closer inspection a certain degree of atrophy of the hepatic cells and hemosiderin in the v. Kupffer cells could be observed. Regenerative and hypertrophic processes were marked in the periportal cells of only one dog.

Liver function tests were carried out in four animals seven to ten days postoperatively (Table II). Except for one dog, which soon lapsed into a state of "meat intoxication" from which it did not recover, the values for bilirubin and thymol turbidity were

TABLE II. *Liver Function Tests in Dogs.*

After third stage operation

	Serum Bilirubin		Bromsulphalein Retention		Thymol Turbidity
	Direct	Indirect	15 min.	30 min.	
	mg%	mg%	%	%	units
7–10 days					
A35	0.045	0	18.9	11.5	1.3
A46	0.12	.36	15.6	5.3	1.0
A48	0.045	0	23.1	13.9	1.5
A30	0.18	.12	25.0	13.5	2.2
3–4 months					
A48	0.135	.150	21.0	13.0	.2
A50	0.12	.060	11.5	5.1	1.9
A35	0.075	.150	9.8	5.0	1.5
A46	0.060	.240	12.5	7.8	1.2
D20	0.075	.180	21.8	9.7	0.8
A8	0.045	.060	18.7	9.9	0.6
Mean values for normal dogs	0.12	.27	6.3	3.0	1.8

within the normal range. However, all dogs showed bromsulphalein retention. Three to four months later the remaining three, as well as three others, were again tested. The results were normal except for a slight degree of bromsulphalein retention, which would indicate subclinical liver impairment.[23, 31] These findings correlated well with the autopsy and biopsy findings.

Glucose tolerance was determined in eight dogs three to eight days, two to three and six months after the third-stage operation (Fig. 9). Except during the immediate postoperative period, the results were within the normal range.

Electrophoretic study of the plasma proteins two to three weeks after the third stage showed abnormal patterns. The total albumin was lower than after the second-stage operation, and much lower than normal. Conversely, the beta and gamma globulins and fibrinogen were increased in comparison with those of the animals after the second-stage operation. The electrophoretic patterns five to six months after the third stage demonstrated that, in some dogs, plasma protein production had become almost normal. It might be due to a well-developed collateral blood supply which helped the parenchymal cells to recover. However, in other dogs with prolonged convalescences and poor food intakes, the albumin level remained low, indicating that the case history of each experimental animal is as important as the time factor in interpreting abnormal electrophoretic patterns.[35]

Studies in Collateral Blood Flow

Latex injections through the thoracic aorta showed the same collateral arterial pattern as described after the second-stage operation (Fig. 5). Retrograde injection of the ligated portal vessels with blue latex via the main left branch of the portal stem did not reveal any collateral flow.

Radiological studies. Guided catheterization and arteriography of the celiac axis demonstrated the collateral arterial flow to the liver *in vivo*. The transflex catheter which was guided into the celiac axis (Fig. 10) is suspended in the aorta like a cane from the celiac orifice. It points towards the splenic artery, which is injected with diodrast and is markedly enlarged. Its branches and finer ramifications enter the splenic pulp. The larger branches run towards the upper pole of the spleen and the stomach. The lienogastric artery, continuing into the left gastroepiploic artery, is of larger caliber than the left gastric artery. A dense network of fair-sized arterial branches marks the site of the gastrosplenic ligament. One must interpret this arterial lacework as the rearguard of an advanced group of collaterals that penetrated the liver substance in a semicircle, above and to the right of the left gastric artery. The emptiness of the right hypochondrium contrasts markedly with the numerous vessels present on the left. However, there is a Y-shaped channel approaching from below the liver and dividing before it penetrates the organ. Its contour is rather jagged. Upon closer inspection the irregularities are recognized as small, looping branches of a dense vascular plexus spun around the common bile duct.

This plexus, situated in the subserosa of the hepato-duodenal ligament, was also observed in our latex preparations. The vessels, normally nourishing the adjacent structures (*e.g.*, the wall of the common bile duct), have now become an additional route

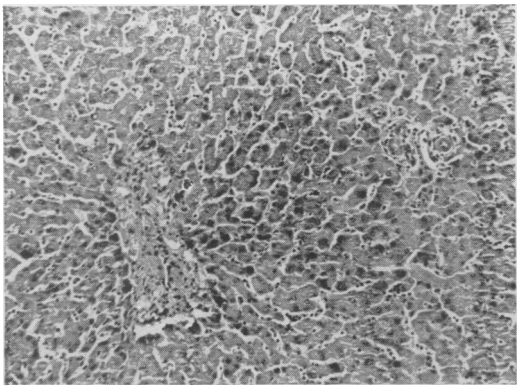

FIG. 8. Liver of an animal sacrificed six weeks after third stage operation. There are no marked abnormalities. Slight atrophy of the parenchymal cells and an increase in stroma can be observed around the central vein. Many neutrophils are present in the portal triad (right upper) H & E stain (x 48).

of collateral blood supply to the liver. The stump of the ligated hepatic artery, from which the pancreatico-duodenal artery curves downward, can be seen above and to the right of the catheter. A metal cap at the dome of the right diaphragm represents the tip of another catheter guided into the lumen of a right hepatic vein for estimation of hepatic blood flow in this animal.

Estimated hepatic blood flow. In the three dogs studied, the estimated hepatic blood flow values were reduced to from 18 to 48 per cent of normal.

DISCUSSION

The experiments that we have presented were originally designed to ascertain how much the hepatic circulation can be reduced without being fatal to the animal. Diversion of portal blood was accomplished by anastomosing the portal and caval systems. It was found that the classical Eck- fistula does not always shunt all of the portal blood. Small vessels emptying into the portal stem proximal to the ligature (above the pancreatico-duodenal vein) can increase in diameter to the size of the portal vein itself, and deliver blood accordingly. This might explain why the reported biochemical changes following Eck-fistula formation are sometimes so contradictory. Rigorous technic is a fundamental condition in experiments curtailing the blood supply.

The partial Eck-fistula method which we introduced permits simultaneous reduction of the portal and hepatic arterial flows. By allowing the pancreatico-duodenal vein to empty its blood into the liver, the circulatory interrelationship between liver and pancreas can be studied. Indeed, the liver of an animal that has undergone the second-stage operation is supplied with venous blood by the pancreatico-duodenal vein alone, and this blood first passes through the vascular area of the pancreas. An analogous situation arises in human cases when the superior mesenteric vein has been anastomosed end to side with the inferior vena cava. Then much of the portal blood coming into the liver by the pancreatico-duodenal and splenic vein passes the pancreatic area first. However, we could not agree with Meythaler and associates[25, 26] that such a circulatory arrangement, in which blood from the pancreas flows into the liver, is essential for the glucose tolerance of the animal. After our second-stage operations, almost all venous blood flowing to the liver first passed through the pancreatic area. After the third-stage operation the pancreatic hormone could reach the liver only by arterial collaterals. Nevertheless, at either stage, the results of the glucose tolerance tests on our animals were normal. In general, this test reflected little of the extensive hepatic damage evidenced in the biopsy material.

The liver function tests we used were intended to reveal any hepatic impairment due to parenchymal damage. Serum bilirubin levels[44] and the thymol turbidity test[13] may reveal diffuse parenchymal dam-

age. The disturbances observed in our animals, however, were transient. B.S.P. retention after 30 minutes was constantly increased even ten months after operation, at a time when all the other tests were normal. Might this delayed excretion of the dye be evidence of reduced circulation in the livers? In 1947 Ingelfinger[12] drew attention to the relation between bromsulphalein excretion and hepatic blood flow.

The changes in the plasma proteins we observed may have some clinical significance. The consistent decrease in the albumin and the increase in the beta and gamma globulins and fibrinogen, all of which were normal preoperatively, suggest that even greater changes might occur in cirrhotics with portal hypertension after surgical procedures curtailing the arterial or venous blood supply to their livers have been carried out. Such methods, both clinical and experimental, have been repeatedly communicated since our first reports on preventing death after ligation of the hepatic artery were published.[8,21]

Antibiotic therapy is well justified in dogs because their livers usually harbor anaerobes. The data on the bacterial content of the human liver are inconclusive as yet.[22, 39, 41] Thus ligation of the hepatic artery proper, even when followed by antibiotic therapy, may be a hazardous procedure. In our experiments on dogs, ligation of the hepatic artery combined with reduction of the portal circulation was followed by a high mortality (eight out of 29 dogs). Some of the animals developed hepatic coma due to a widespread aseptic hepatic necrosis. Ligation of arterial vessels supplying the livers of cirrhotics can be considered analogous to our operative procedures, and in some instances the results are similar.[36]

The phenomena of recurrent stupor often seen in Eck-fistula dogs fed only meat has its clinical counterpart in the recently described observations on patients with various types of anastomoses between the portal and caval systems. The increase in blood ammonia is considered responsible for the bouts of hepatic stupor.[24, 38] A similar syndrome could be induced in our animals after the third-stage operation by several feedings with meat. Some of them, however, showed attacks of hepatic lethargy even though meat was rigorously withheld from their diets. These attacks usually heralded a downhill course.

Circulatory factors in hepatic repair are of greater significance than usually thought. The experimental study we have presented demonstrates anew the enormous regenerative capacity of the liver. This faculty is commonly attributed to the hepatic parenchymal cell. However, parenchymal lesions due to occlusion of blood vessels can be repaired only after new vascular channels have been opened. Repair of hepatic necrosis due to toxic substances also requires increased blood supply. The role of collateral blood flow for the survival of an ischemic liver has been demonstrated by many investigators.[1, 8, 10, 11, 29] From our experiments in which a huge vascular organ, the liver, was totally deprived of its regular blood supply and survived and functioned on collateral circulation alone, we have learned that gradual and stepwise obstruction of vessels favors the increase of flow from collateral vascular areas. This conclusion is not valid for the portal channels. A total Eck fistula (*i.e.*, a portacaval anastomosis with ligation of the portal stem close to the *porta hepatis*, with careful search for and tying off of the smallest venous twigs that could empty into the portal stem beyond its ligature), definitely precludes any portal blood from flowing into the liver. Hepatic arterial collaterals, however, are quite numerous. Indeed, on closer consideration we find that the liver is not being supplied by an end artery, but is rather intercalated into the large vascular area of the celiac axis. The branches of this artery are anastomosed with each other by vessels arranged in modified vascular arcades. These represent the architectural principle of vas-

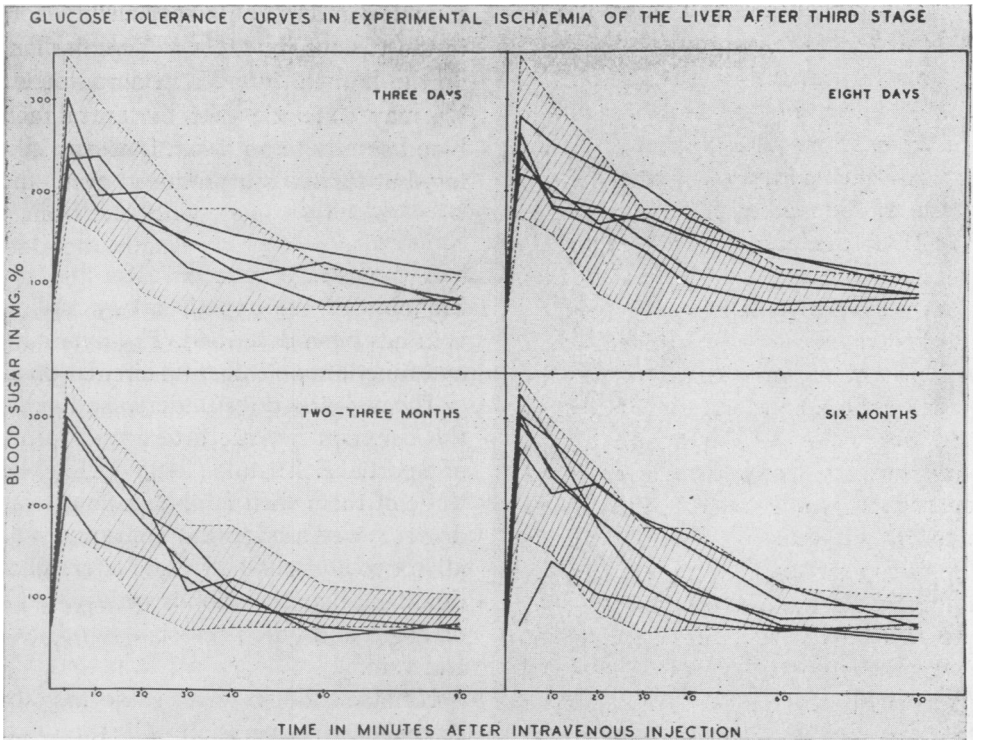

FIG. 9. Except during the immediate postoperative period the curves are within the normal range (shaded area).

cular supply in the digestive system. Thus in our dogs hepatic repair occurred surprisingly quickly after each stage of the operation. Even after all branches of the hepatic artery have been ligated and all the portal blood has been shunted away, the livers still showed fair weight. However, hepatic function can be disturbed even when the histological changes are minimal.[17]

The collateral flow may be brought about by enlargement of pre-existing small vessels connecting the hepatic circulation to other vascular areas (Fig. 5), or by angiogenesis.[28]

The fascinating problem of the hemodynamics of collateral circulation requires a brief discussion. What makes the collateral vessels open, enlarge, and increase in number? Initially the opening and enlargement of the collateral vessels was considered to be caused solely by the higher blood pressure in the collateral channels as compared with the occluded vessel.[30, 37] The retrograde blood flow corresponded to this pressure gradient. Bier[2] investigated the "reactive hyperemia" following vascular occlusion. He attributed this reaction to an "avidity of the ischemic tissue for blood." Later, the accumulation of CO_2, lactic acid, and an H-like substance were considered as responsible for the dilatation of the capillaries which draw blood into the ischemic area. The French school, headed by Leriche,[15, 16] emphasized the role of the vasomotor nerves in amelioration of the blood supply to an ischemic organ. Sympathectomy is now most commonly used to improve the circulation to an ischemic limb. As for the liver, improvement of 14 patients with impending hepatic coma by sympathetic block between D11 and D12 can be explained similarly.[4, 5] The amelioration of chronic hepatitis in 17 cases after periarterial neurectomy on the common hepatic artery was described by Mallet-Guy[19] as due to im-

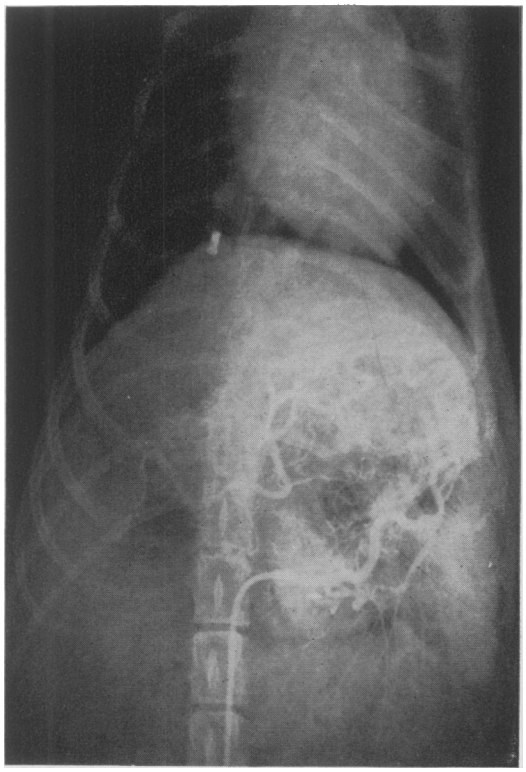

FIG. 10. Arteriogram of collateral vessels to the liver, obtained by guided catheterization of the celiac axis (description in text).

proved blood flow to the liver. Ligation of the vessels *en bloc* in our dogs might have damaged the perivascular sympathetic fibers and thus facilitated the dilatation of the smaller branches drawing blood from adjoining collateral vascular areas into the ischemic organ. According to Sir James Learmonth,[14] chances for repair would be greatest if the flow through a main branch of an occluded vessel could be re-established. Unfortunately, vascular surgery is a poor risk in an organism with a failing liver.

The liver, the venous antechamber of the splanchnic area, interposed between celiac axis and mesenteric vessels, is subjected to the many variations of blood flow of this region. Although the organ is adaptable to these circulatory changes, its cells are very sensitive to anoxia. More attention should therefore be given to hepatic ischemia, its sequelae and eventual permanent damage in the various practices of vascular surgery on the human liver. From our experiments we may conclude that the curtailment of blood supply to an organ is *always* damaging, but *least* when produced gradually.

SUMMARY

A three-stage procedure for ligating *all* branches of the hepatic artery and portal vein has been described. The liver survives and functions on collateral circulation alone.

Twenty-nine dogs underwent ligation of the common hepatic artery and formation of a partial Eck fistula (Stage I). Eight died; three of them with fatal hepatic coma. One dog survived a seven-day coma and was still alive two and one-half years later. The predominant hepatic lesion is widespread hemorrhagic and lytic necrosis around the central vein.

Nineteen dogs were subjected to the second-stage operation (ligation of all branches of the hepatic artery), six to eight weeks after Stage I. Three died immediately postoperatively of bile peritonitis. Another wasted away five and one-half months later. Adhesions were noted around the slightly atrophic liver lobes. Microscopically, there was dedifferentiation of parenchymal cells around the hepatic veins and vestiges of hemorrhagic necrosis in the form of fatty and iron-laden cells.

In 11 dogs the pancreatico-duodenal vein was tied off (Stage III), thereby completing ligation of the portal vein six to eight weeks after ligating all branches of the hepatic artery. One dog died immediately because the previously formed portacaval anastomosis was not patent. Four others suffered episodes of hepatic lethargy, the so-called "meat intoxication," although fed no meat. The patho-anatomical findings in the animals sacrificed up to 16 months later revealed atrophy of the liver, ranging from some reduction in size to complete disappearance of liver lobes. Microscopically, the cells around the hepatic veins were involuted.

Some parenchymal and Kupffer cells showed sudanophilic and iron-containing masses.

The function of the liver was tested after the operative stages. Slight changes in serum bilirubin and the thymol turbidity test were noted in the dogs immediately following the second and third stages. Bromsulphalein retention persisted even ten months after operation. Electrophoretic analyses showed decreases in the total plasma proteins, in the albumin and A/G ratios accompanied by increases in alpha and gamma globulin, and in the Phi fraction. Intravenous glucose tolerance tests performed on the dogs after each stage of the operation showed delayed returns to fasting levels only during the immediate postoperative days, and were probably due to poor food intake.

Estimation of the hepatic blood flow by the bromsulphalein clearance method, with guided catheterization of the hepatic veins, showed a reduction of 10 to 20 per cent after the second-stage, and of 18 to 48 per cent after the third-stage operation. Guided catheterization of the celiac axis and arteriograms of this vessel demonstrated the collateral arterial circulation *in vivo*. Hepatic venography evidenced changes in the smaller branches of the hepatic veins. Collateral arterial supply to the liver was demonstrated by latex injection through the thoracic aorta at autopsy.

The mechanism of development of collateral circulation and its significance in the ischemic liver has been briefly discussed.

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