LIVER REGENERATION FOLLOWING PORTACAVAL TRANSPOSITION IN DOGS*

CHARLES G. CHILD, III, M.D., DAVID BARR, M.D.,
GEORGE R. HOLSWADE, M.D., AND CHARLES S. HARRISON, M.D.

NEW YORK, NEW YORK

FROM THE DEPARTMENT OF SURGICAL RESEARCH OF THE DEPARTMENT OF SURGERY OF THE NEW YORK HOSPITAL-CORNELL MEDICAL CENTER

INTRODUCTION

THE LIVER IN NORMAL DOGS regenerates rapidly and completely following partial removal.[1] If, however, its portal blood supply is compromised either by partial ligation of the portal vein[7] or side to side portacaval anastomosis,[5] or diverted through an Eck fistula,[6] liver regeneration is then inhibited or prevented. It is not clear whether this is due to lack of portal blood itself, or to simple reduction in afferent hepatic blood flow. As yet, no substance necessary for liver regeneration has been found in the portal blood. The existence of a portal factor remains hypothetical.

Attempts have been made to demonstrate the relationship of blood flow to hepatic regeneration. Higgins, Mann and Priestley[4] combined partial hepatectomy with ligation of the postcaval vein in fowl. Fowl were chosen because there exists in them a natural anastomosis between their postcaval and hepatic portal venous systems via the inferior mesenteric vein. Therefore, ligation of the postcaval vein in effect produced a reverse Eck fistula. There followed greater hepatic regeneration than anticipated. This was interpreted as being due to an excessive volume of venous blood coursing through the liver. This was not confirmed by Grindlay and Bollman[2] in a similar experiment in dogs. They found that hepatic regeneration after partial hepatectomy in dogs with reverse Eck fistulae was no greater than in normal dogs. They also found that liver regeneration after partial hepatectomy was enhanced by constriction of the inferior vena cava above the diaphragm in dogs with classic Eck fistulae, or with their portal veins partly occluded. This effect was presumed to be due to a secondary increase in hepatic venous filling. A similar effect was observed in these dogs if the inferior vena cava was constricted just below the liver. The explanation for this was less clear, unless one accepted the author's suggestion that rhythmic stasis, or even alternating reversal of blood flow, occurred in the inferior vena cava secondary to respiration distending hepatic veins.

Mann[5] performed partial hepatectomy in dogs with side to side portacaval anastomosis. He found that where the anastomoses were patent, liver regeneration was decreased in comparison to normal dogs. He concluded that the reduction in hepatic regeneration in the dogs with portacaval fistulae was due to a fall in portal pressure and blood flow, and not to lack of a hypothetical portal factor.

It is felt, however, that insufficient experimental distinction has been made in explaining the failure of hepatic regeneration in dogs with Eck fistulae between the requirement by the liver for a factor peculiar to portal blood and its requirement for an adequate blood supply.

* Read before the American Surgical Association, Los Angeles, California, April 3, 1953. Supported by U. S. Public Health Service Grant-in-Aid H-167 (C), (C2), (C3).

It is the purpose of this paper to report experiments in dogs designed to determine whether portal blood is essential for liver regeneration. An operation was devised in dogs that accomplished complete diversion of the portal stream and at the same time provided the liver with a profuse supply of systemic venous blood. In this preparation, the response of the liver to partial hepatectomy was studied.

METHOD

Healthy adult mongrel dogs of both sexes and ranging in weight from 10 to 22 Kg. were used. The dogs were divided into three groups.

Group I. Portacaval Transposition Followed by Partial Hepatectomy. Portacaval transposition was carried out through a long right subcostal incision (Fig. 1). The inferior vena cava was dissected free of peritoneum and adventitia from the renal veins to the liver and around its entire circumference. The right adrenal vein and lumbar veins were divided and ligated. The entire portal vein was mobilized from its mesenteric and splenic tributaries to its bifurcation at the liver. All tributaries between these two points were divided between ligatures. A bulldog clamp was placed on the root of the mesentery, so as to temporarily occlude the superior mesenteric artery. The inferior vena cava was divided between Blalock clamps about 1.5 cm. below the liver. The portal vein was divided between Blalock clamps approximately 1.5 cm. above its principal tributaries. The distal portal vein was sutured to the proximal vena cava end to end, using three continuous everting mattress sutures. Upon completion of this anastomosis, the bulldog clamp on the superior mesenteric artery was removed. The distal inferior vena cava was then similarly sutured to the proximal portal vein. All splanchnic blood was thus diverted from the liver directly into the inferior vena cava. The intrahepatic portal bed was filled with systemic venous blood.

Approximately one month later, partial hepatectomy was performed. The left lobe, the gallbladder lobe, and most of the papillary lobe were removed.

Hepatic venograms, obtained by injecting 75 per cent neo-iopax directly into the inferior vena cava, were made prior to partial hepatectomy to prove patency of the caval-hepatic anastomosis and to visualize the intrahepatic portal bed (Fig. 2A). Venography was repeated at intervals afterward to demonstrate regeneration *in vivo*. At these times, inferior vena cava pressures were recorded. The areas of the shadows cast on the venograms by the right lobes before and after partial hepatectomy were measured with a planimeter and compared. The increase in area is expressed simply in planimeter units.

TABLE I. *Area of Shadow Cast by Right Lobes of Liver on Consecutive Venograms Before and After Partial Hepatectomy in Dogs with Portacaval Transposition.*

Dog Number	Areas in Planimeter Units			
	Before Partial Hepatectomy	After Partial Hepatectomy		
		1 Week	4 Weeks	6 Weeks
738.....	265	369	516	570
737.....	213	310	372	397
741.....	202	456	474	508
746.....	217	214	333	
756.....	381	795	692	
760.....	335	598	664	

After periods of observation ranging from 35 to 60 days, the dogs were sacrificed by exsanguination under nembutal anesthesia. Their livers were removed and weighed, and the degree of regeneration calculated. The weight of the liver removed at operation was multiplied by 3/7 to estimate the weight of the hepatic remnant. This, subtracted from the weight of the liver removed at autopsy, gave an estimate of the gain in liver weight during the experimental

period. The gain divided by the weight of liver removed at operation was multiplied by 100 to express regeneration in percent. Thus, if gain equalled the weight of liver removed at operation, then regeneration would be 100 per cent.

in normal dogs by the same method. After periods of observation ranging from 30 to 57 days, these dogs were sacrificed by exsanguination under nembutal anesthesia. Their livers were removed and weighed, and similar calculations made.

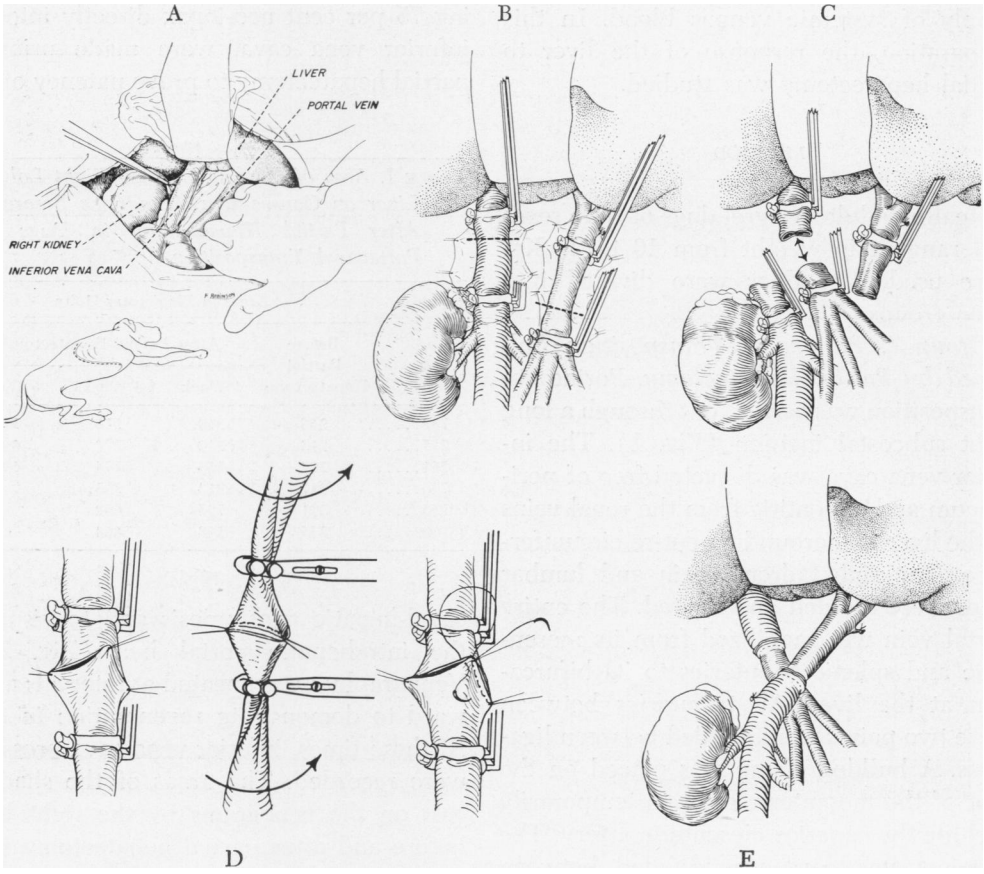

FIG. 1. Exposure for portacaval transposition. (B) Placement of Blalock clamps and proposed sites of division of portal vein and inferior vena cava. (C) Distal portal vein is to be anastomosed to proximal inferior vena cava. (D) Three continuous everting mattress sutures are used for the anastomoses. (E) Completed portacaval transposition.

Throughout the experiment, the dogs were maintained on standard kennel rations. Intravenous fluids and unmatched whole blood transfusions were given as indicated during and immediately after operations. After partial hepatectomy, the dogs received intramuscular penicillin and dihydrostreptomycin for one week.

Group II. Partial Hepatectomy in Normal Dogs. Partial hepatectomy was carried out

Group III. Eck Fistula Followed by Partial Hepatectomy. Eck fistulae were created by end to side portacaval anastomosis. Partial hepatectomy was performed at the same time in some animals, and at a later date in others. Liver regeneration was calculated by the method described.

Determinations of bromsulfalein retention and serum concentrations of albumin, globulin, alkaline phosphatase, sodium and

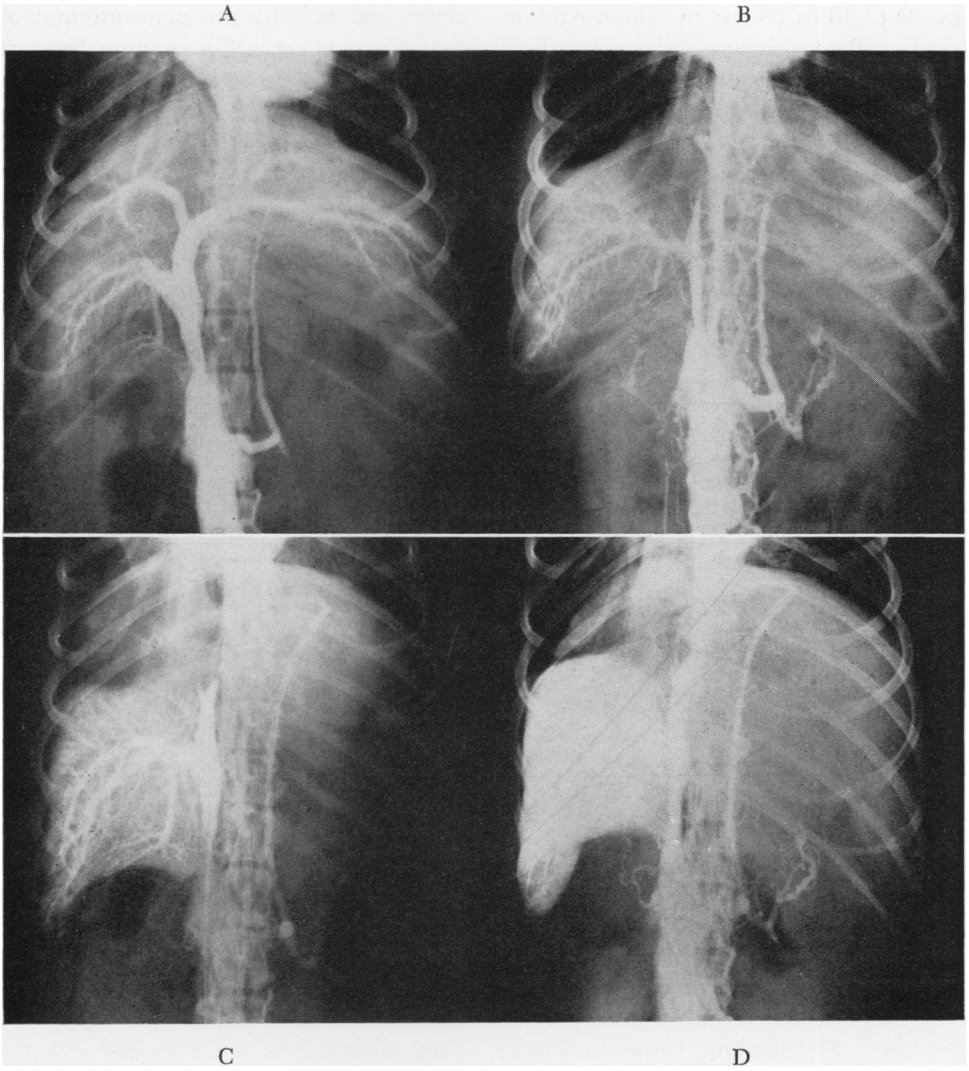

FIG. 2. *Hepatic Venograms*: (A) Portacaval transposition. (B) One week, (C) Four weeks, and (D) Six weeks after 70 per cent hepatectomy.

potassium were made during the experiment in most animals. All liver specimens were examined histologically.

RESULTS

Group I. Portacaval transposition was well tolerated. Initially, three dogs died at operation, but thereafter 14 survived without an operative death. Two dogs died approximately three weeks after operation. These lost weight progressively and were in a cachectic state at death. At autopsy their anastomoses were patent, and the cause of death was not determined in either animal. Liver function tests in the surviving dogs showed little deviation from normal. Hepatic venograms in all dogs but one demonstrated patency of the cavalhepatic anastomosis. Inferior vena cava pressures were consistently elevated at this time in

the range of 15 to 26 cm. of saline. Abdominal wall collaterals were enlarged only in the dog with the thrombosed caval-hepatic anastomosis.

Partial hepatectomy was carried out in the 12 survivors of the initial operation. The only death occurred on the second postoperative day, of bleeding from an inadequately transfixed hepatic vein. One dog became deeply jaundiced after operation, and at exploratory celiotomy four weeks after partial hepatectomy, a ligature was found obstructing the right hepatic duct. An attempt was made to decompress the biliary tract, but he died after operation. His liver showed moderately advanced biliary cirrhosis. One dog who appeared to be doing well died suddenly 35 days after partial hepatectomy. The cause of death was not established at autopsy. The dog with the thrombosed caval-hepatic anastomosis survived partial hepatectomy, but lost weight progressively during the ensuing 32 days, and at the time of sacrifice, was extremely cachectic. These dogs, because of the complications described, have been excluded from consideration of the group as a whole.

The other eight dogs had uncomplicated courses and appeared to do well, although they all lost 1 to 3 Kg. of body weight. In all, the regeneration observed in the venograms was striking. When the films taken prior to partial hepatectomy were compared with those taken at intervals afterward, it was clear that there was a great increase in the area and density of the liver filled with contrast material (Fig. 2). The main branches of the portal vein were enlarged; the actual measurements in planimeter units are shown in Table I.

In all animals, the right lobes of the liver appeared to have increased in size in all dimensions (Fig. 3). The edges of the lobes were blunted, and the surface of the liver was smooth. Their color was a light red-

TABLE II. *Hepatic Regeneration Following Partial Hepatectomy in Dogs with:*
A. *Portacaval Transposition*
B. *Normal Hepatic Circulation*
C. *Eck Fistula*

Dog Number	Weight of Dog at Part. hepatect. Kg.	Weight of Liver Removed Gm.	Estim. Weight of Hep Remnant Gm.	Weight of dog at Autopsy Kg.	Weight of Liver at Autopsy Gm.	Estim. Gain in Liver Weight Gm.	Interval Between Op. Autopsy Days	Liver Regeneration per cent
A. Portacaval Transposition								
729	15.5	273	117	13.6	302	185	38	68
736	16.1	302	130	15.2	371	241	36	80
738	13.6	335	143	12.3	230	87	60	25
737	10.8	251	108	9.3	159	51	50	20
741	11.1	201	81	9.6	177	96	50	49
746	10.2	159	68	9.3	161	93	45	59
756	21.5	322	138	18.2	320	182	45	57
760	15.4	272	117	14.3	230	113	35	42
Average Regeneration: 50 per cent ±S. D. 21								
B. Normal Hepatic Circulation								
769	11.4	279	120	10.4	285	165	42	59
770	12.3	348	147	12.9	322	185	57	53
797	13.9	208	89	15.0	337	238	30	114
829	19.3	255	109	17.3	318	209	43	82
830	22.7	325	140	21.8	451	311	43	96
831	17.2	329	141	15.9	288	147	42	45
Average Regeneration: 75 per cent ±S. D. 27								
C. Eck Fistula								
772	16.1	421	181	10.7	174	−7	29	0

brown. Histologically, four of the dogs' livers were normal. Four others, however, showed loss of hepatic cells in the central areas (Fig. 4). These changes may have accounted for the slight elevation in serum alkaline phosphatase and minimal bromsulfalein retention that persisted in some of the dogs.

generation ranged from 45 to 114 per cent and averaged 75 per cent (± S.D. 27—Table II).

Group III. End to side portacaval anastomosis was performed as the initial procedure in four dogs. Only one of these dogs thrived. One unquestionably had distemper and had to be destroyed 35 days

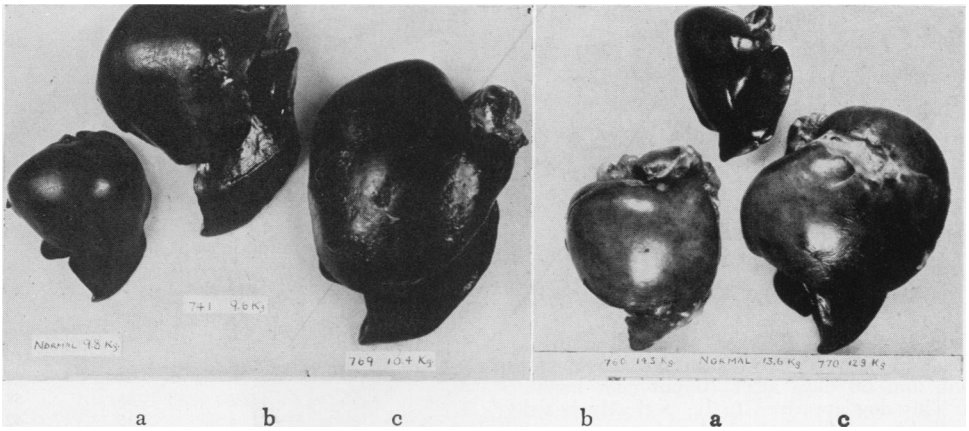

FIG. 3. (A) Postmortem specimens of dogs' livers after 70 per cent hepatectomy. Weights shown are body weights in kilograms at the time of autopsy. (a) Right lobes of liver of normal dog of body weight comparable to the others sacrificed for purposes of comparison. (b) Right lobes of liver 85 days after portacaval transposition and 50 days after 70 per cent hepatectomy. (c) Right lobes of liver 42 days after 70 per cent hepatectomy alone.

(B) Postmortem specimens of dogs' livers after 70 per cent hepatectomy. Weights shown are body weights in kilograms at the time of autopsy. (a) Right lobes of liver of normal dog sacrificed for the purpose of comparison. (b) Right lobes of liver 70 days after portacaval transposition and 35 days after 70 per cent hepatectomy. (c) Right lobes of liver 57 days after 70 per cent hepatectomy alone.

The enlargement of the liver after partial hepatectomy in the dogs with portacaval transposition is obvious, although not as great as after the same hepatectomy in normal dogs.

Regeneration, as calculated by the method described, ranged from 20 to 80 per cent, and averaged 50 per cent (± S.D. 21—Table II).

Inferior vena cava pressures averaged 21 cm. prior to partial hepatectomy, and 17 cm. of saline four weeks or more after partial hepatectomy.

Group II. Partial hepatectomy was performed in six normal dogs; all survived and did well. Two gained ½ to 1 Kg. and four lost 1 to 2 Kg. of body weight during the experimental period. At autopsy their livers grossly were enlarged with smooth, light, red-brown surfaces and blunted edges (Fig. 3), and histologically appeared normal. Re-

after operation. One died of unknown cause 30 days after operation. One did not do well, but was subjected to partial hepatectomy anyway 25 days after shunting, and he died 3 days later with massive pulmonary consolidation and hemothorax. The dog that thrived was subjected to partial hepatectomy 16 days after portacaval shunt. Following operation, he became jaundiced and intermittently lapsed into a stuporous state, during which periods he was maintained on infusions of 10 per cent glucose and vitamins. He died 29 days after partial hepatectomy. His right hepatic duct was compromised, but not totally obstructed. There was bile in the distal com-

mon bile duct. His liver showed mild biliary cirrhosis and did not measurably regenerate.

An attempt was made in three dogs to perform the end to side portacaval shunt and partial hepatectomy at one operation.

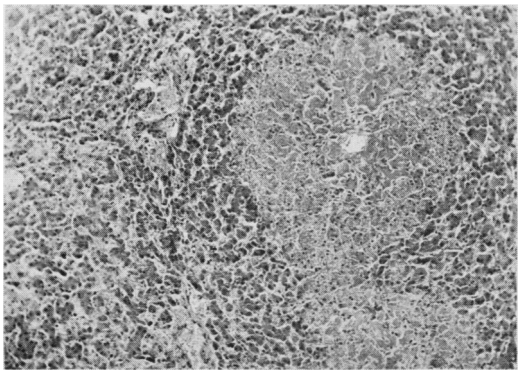

FIG. 4. Section of liver removed from dog #741 at autopsy 85 days after portacaval transposition and 50 days after 70 per cent hepatectomy. This dog appeared to be perfectly healthy, although his serum alkaline phosphatase was 19.8 at the time of sacrifice. Central necrosis was found in 4 dogs; persistently elevated alkaline phosphatase in only 2. The reason for the central necrosis is purely speculative. It may be secondary to anoxemia in the central regions of the sinusoids.

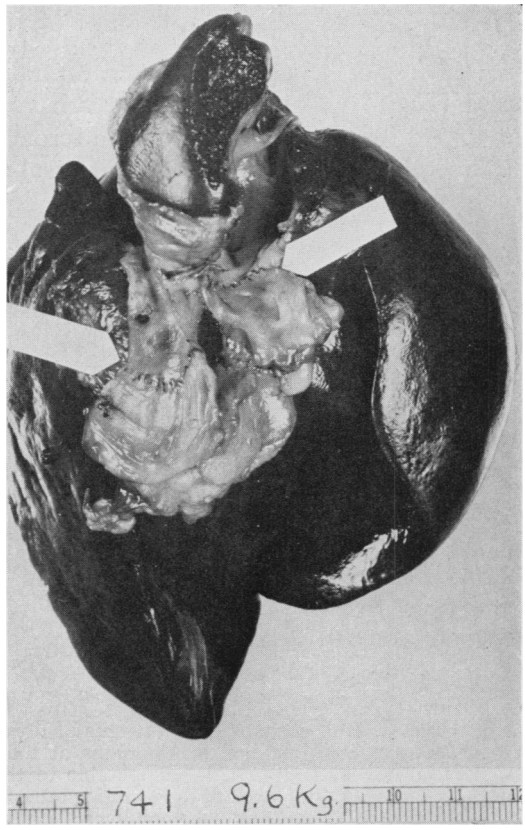

FIG. 5. Postmortem specimen of liver of dog 85 days after portacaval transposition and 50 days after 70 per cent hepatectomy viewed from behind, showing the portacaval anastomoses. Arrow on left points toward the distal caval-proximal portal vein anastomosis. Arrow on right points toward the distal portal vein-proximal caval anastomosis.

All of these dogs died. Eleven days was the longest survival period.

Thus, of this group of dogs, only one survived long enough to be considered comparable to those in Group I.

DISCUSSION

In the dogs with portacaval transposition, liver regeneration after partial hepatectomy was measured by two independent methods, venography and calculations of percent regeneration based upon liver weight. Of the two methods, venography was the better controlled, since each set of venograms provided a control film for each animal. The calculations, on the other hand, relied upon comparisons of liver weights in two dissimilar groups of dogs.

The venograms in every instance showed an increase in the area and density of the shadow cast by the hepatic remnant during the experimental period. This in itself is interpreted as indicative of hepatic regeneration.

The calculations of percent regeneration were less easily evaluated. The normal dogs, in whom liver regeneration after partial hepatectomy is presumed to be complete in six weeks by this method of calculation, showed a mean regeneration of only 75 per cent. This apparent discrepancy may be accounted for in part by the error inherent in the method, and in part by factors of individual variation. The estimate of gain in liver weight during the

post-hepatectomy period was based upon the measured weights of liver removed at operation and at autopsy, and the assumption that a 70 per cent hepatectomy had been performed. In support of this assumption it was found in dissections of normal livers (23 dogs) that the portion of liver removed amounted to 71 per cent of the total weight of the liver ($\pm$ S.D. 4). The liver removed at operation, however, contained an unknown quantity of blood, whereas the liver removed at autopsy after exsanguination contained very little. The estimate, then, of the weight of the liver left at operation must have been unduly high and the weight of the postmortem specimen low by comparison. These considerations fix the error in the calculations of gain and percent regeneration on the low side. Since all dogs were similarly treated, the error should be fairly constant. There is no way of assessing the relative importance of the error of the method and individual variation in accounting for this discrepancy, but the error must be significant.

The increase in liver weight after partial hepatectomy was great in the dogs with portacaval transposition as compared with that found after partial hepatectomy in dogs with Eck fistulae. Since only one of the latter in this experiment survived, it was necessary to draw comparisons from the work of others, which has shown that liver regeneration does not occur after partial hepatectomy in dogs with Eck fistulae. If the premise that liver regeneration in these dogs is zero is accepted, then it can be proved statistically that liver regeneration occurred in the dogs with portacaval transposition. For example, according to the t test, it is necessary for t to equal or exceed 2.18 for the calculated liver regeneration in the dogs with portacaval transposition to be significant at the 5 per cent level as compared with the dogs with Eck fistulae where none occurs. T is found to be 6.02. There is no question that a significant difference exists between these two groups.

How much liver regeneration occurred in the dogs with portacaval transposition cannot be stated with equal certainty. A rough idea can be gained by comparison with the normal dogs. There was a mean of 50 per cent regeneration in the dogs with portacaval transposition, and a mean of 75 per cent in the normal. Again, according to the t test for there to be a significant difference in the regeneration observed between the two groups at the 5 per cent level, t must equal or exceed 2.18. In this situation, t equals 1.98. It therefore cannot be stated that a statistically significant difference exists between these two groups of dogs. However, t is close to the critical value, and the difference between the two means is sufficiently great to suggest that there really may be a difference between the groups in question. Other statistical tests such as Smirnov's and Wilcoxon's which, in contrast to the t test, do not assume that the results conform to the normal distribution curve, give results similar to the t test. The inference, then, is that somewhat less regeneration of liver took place after partial hepatectomy in the dogs with portacaval transposition than in the normal dogs.

The physiological significance of these data lies in the fact that systemic venous blood has been shown to be capable of supporting liver regeneration. The likelihood of the existence of a hypothetical portal factor is thereby decreased, but since systemic venous blood did not seem to be quite as effective as portal blood, the possibility of the existence of a portal factor of secondary importance cannot be unequivocally excluded. The conclusion, however, seems justified that portal blood is by no means essential for hepatic regeneration and that its failure after partial hepatectomy in dogs with Eck fistulae is

largely due to diminished hepatic blood flow. These conclusions are consistent with the observation that the portal vein supplies the liver in dogs with at least 70 per cent of its total blood supply.[3]

SUMMARY

1. Partial hepatectomy has been performed in dogs with portacaval transposition.

2. Hepatic venograms showed striking regeneration of the liver in these animals.

3. Hepatic regeneration averaged 50 per cent (± S.D. 21) in the dogs with portacaval transposition as compared to 75 per cent (± S.D. 27) in normal dogs subjected to a similar partial hepatectomy.

4. It is concluded from these data that portal blood itself is not essential for liver regeneration, and that the failure of liver regeneration in dogs with Eck fistulae is due to diminished hepatic blood flow, rather than to lack of a factor present only in portal blood.

BIBLIOGRAPHY

1 Fishback, F. C.: A Morphologic Study of Regeneration of the Liver after Partial Removal. Arch. Path., 7: 855, 1929.

2 Grindlay, J. H., and J. L. Bollman: Regeneration of the Liver in the Dog after Partial Hepatectomy. Surg., Gynec. & Obst., 94: 491, 1952.

3 Grindlay, J. H., J. F. Herrick and F. C. Mann: Measurement of the Blood Flow of the Liver. Am. J. Physiol., 132: 489, 1941.

4 Higgins, G. M., F. C. Mann and J. T. Priestley: Experimental Pathology of the Liver. X. Restoration of the Liver of Domestic Fowl. Arch. Path., 14: 491, 1932.

5 Mann, F. C.: The Portal Circulation and Restoration of the Liver after Partial Removal. Surgery, 8: 225, 1940.

6 Mann, F. C., F. C. Fishback, J. G. Gray and G. F. Greem: Experimental Pathology of the Liver. III. Effect of Diverting Portal Blood on the Restoration of the Liver after Partial Removal. Arch. Path., 12: 787, 1931.

7 Stephenson, G. W.: Experimental Pathology of the Liver. IX. Restoration of the Liver after Partial Hepatectomy and Partial Ligation of the Portal Vein. Arch. Path., 14: 484, 1932.

DISCUSSION.—DR. JOHN H. MULHOLLAND (New York University College of Medicine, 477 First Avenue, New York 16, N. Y.): I would like to discuss Dr. Stewart's very nice biochemical evidence of cellular activity within the liver. This is a difficult area to explore and is, for that reason, obscure.

He has measured elevation of potassium in the hepatic vein blood but makes little attempt to explain the mechanism. It remains for his listeners to speculate and, perhaps, conclude that selective permeability of cell membrane is the function he is estimating.

He referred to potassium increase in venous blood as loss from the liver. I am sure he used the word "loss" as the best word to describe the phenomenon, because it may very well be displacement. A certain element of this potassium rise is normal homeostatic adjustment by the liver cell. Another element may be, as he mentioned, death in the cell.

This difference might be defined in the charts he showed, which had two increments of potassium increase in hepatic vein blood under varying conditions of the experiment.

The facts are evidence that the liver is the site of effective homeostatic adjustments, and may very well be the site of failure of those adjustments in certain circumstances.

It is well known, for instance, that bleeding a dog from its hepatic artery brings about failure of adjustment to loss of blood volume more quickly than does bleeding a similar amount from a peripheral artery.

There is an analogy in the human being, whose hepatic artery has a greater function than in the dog, in the clinical fact that bleeding from branches of the hepatic artery in the posterior wall of the duodenum (thus tapping the pressure head of hepatic artery blood) is much more injurious to homeostatic mechanisms than peripheral bleeding.

I enjoyed the paper very much, and congratulate Dr. Stewart.