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THE ORIGIN, HORMONAL NATURE, AND ACTION OF HEPATOTROPHIC SUBSTANCES IN PORTAL VENOUS BLOOD

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LONG AGO, Rous and Larimore (39) were intrigued with the possibility that portal venous blood contained hepatotrophic factors and that the extrahepatic diversion of these factors by portacaval shunt was responsible for the poor health of dogs with Eck fistula. However, the observations of Mann (27) did not support the hepatotrophic hypothesis, and the work in 1953 of Child and his associates (11) using portacaval transposition was generally interpreted as crucial evidence against it. By replacing the diverted splanchnic venous blood with an inflow to the portal vein from the inferior vena cava, Child avoided most of the adverse effects of Eck fistula. The concept became rooted from these studies and those of Fisher and his associates (15-17) and a number of subsequent authors that the quality of portal venous inflow was not a prime determinant of good hepatic structure, function, or capacity for regeneration. Instead, it became accepted that the quantity of total hepatic

blood flow was the main consideration. In spite of the demonstration that canine livers submitted to transposition underwent major deglycogenation and were, thus, not actually normal (43, 52), the flow oriented view held sway until it was definitively challenged by investigations that had their origin in studies of experimental liver transplantation as has been thoroughly reviewed in a recent monograph (50).

First, it was noted that auxiliary hepatic homografts underwent remarkable atrophy if these extra livers were revascularized in an ectopic location with a double systemic blood supply analogous to that with the Child preparation (51). One possible explanation that was advanced was that the organ which was perfused first by splanchnic venous blood extracted a disproportionate share of unspecified substances and that the other organ atrophied because of its disadvantaged competitive situation. The hypothesis was supported by Marchioro and his associates (29) who showed that the transplant atrophy could be prevented by diverting the nonhepatic splanchnic venous blood away from the host liver and through the graft. By so doing, the atrophy now afflicted the native organ. Confirmatory observations were reported by Thomford (56), Halgrimson (20), and Tretbar (57) and their

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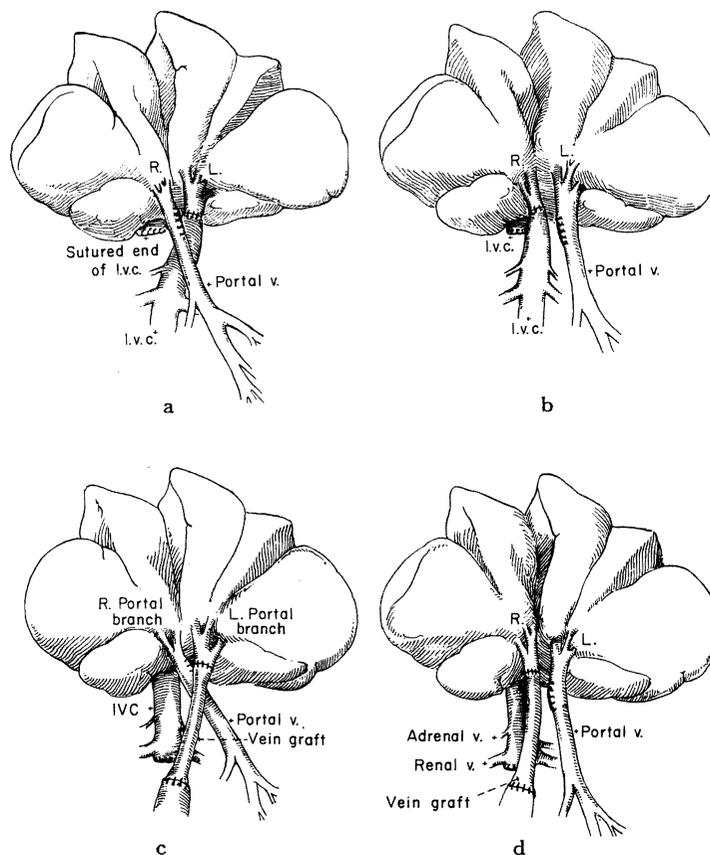


Fig. 1. Partial portacaval transposition. a and b, Entire vena caval flow is directed into either the left or the right portal venous branch. c and d, This differs from a and b in that the vena caval flow excludes the adrenal and renal blood. A venous graft is always required to bridge the gap.

associates. Thomford (56) showed that the atrophy in Welch auxiliary homografts could be prevented in recipients which had undergone immunosuppression if the host livers were removed within a few days after transplantation, and Tretbar (57) and Halgrimson (20) and their colleagues demonstrated that the shrinkage could be reduced by diversion of portal blood away from the host liver even though it was not directly rechanneled through the transplant. Observations by Sigel and his associates (47, 49) with hepatic autografts implanted to intestinal pedicles or directly revascularized in the neck could be interpreted in the same general way.

The transplant preparations which made apparent the foregoing physiologic effects had two serious flaws which prevented definitive conclusions about the pathogenesis of the atrophy. First, the total flows delivered to the two coexisting livers were often different. Second, there was by definition an additional inherent inequality of the two

organs since the homograft was usually under immunologic attack despite host immunosuppression whereas the animal's own liver was not. Consequently, other experiments were undertaken which were designed to circumvent one or both deficiencies.

One preparation not involving transplantation was used by Marchioro and his associates (28, 30) and termed a split transposition. Splanchnic venous blood was provided for one portal vein branch of the liver whereas the other portal branch was supplied with blood from the inferior vena cava. Later, Price (36), Lee (25), and Chandler (9) and their associates performed analogous experiments, with either canine partial hepatic autografts or isografts of inbred rat livers. All these experiments showed hypertrophy in the hepatic tissue which was perfused with splanchnic blood and atrophy of the other hepatic fragments. In addition to hypertrophy, Marchioro and his associates (28) showed that the advantaged hepatic portion had binucleate

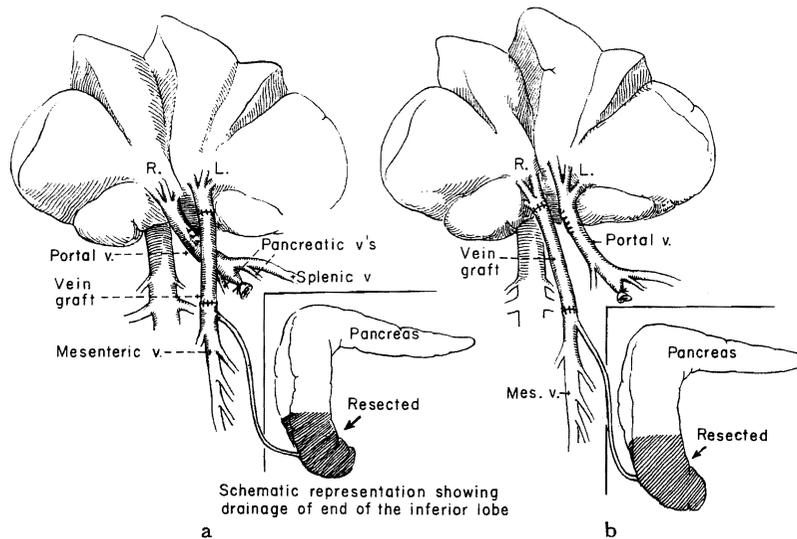


FIG. 2. Technique of division of splanchnic venous flow into a pancreatico-gastroduodenal-splenic compartment and an intestinal compartment. Blood from these respective sources is directed into the right or left lobes. The tail of the inferior lobe of the pancreas was resected since it drains separately into the mesenteric vein.

and trinucleate hepatic cells, mitoses, and proliferating bile ducts, all indications of hyperplasia. With quantitative studies of deoxyribonucleic acid synthesis, Lee (25), Chandler (9) and Fisher (18) and their associates proved that the hepatotrophic effects of splanchnic blood upon the liver include hyperplasia as well as hypertrophy. It has become increasingly accepted that the portal hepatotrophic factors are probably not just artifacts of transplantation and other experimental maneuvers but are prime determinants of the initiation and control of liver hypertrophy and hyperplasia in many circumstances.

This article was undertaken to report anatomic and biochemical studies of the source and mechanism of the hepatotrophic factors in splanchnic venous blood. The results have indicated that these originate from the pancreas and are hormonal in nature.

METHODS

Portal Diversion Procedures

One hundred and one mongrel dogs, weighing 12.4 to 24.1 kilograms, were used. Six normal dogs were sacrificed to obtain tissues for control studies and liver lobe weights, and the remaining 95 dogs had one of the following operative procedures.

Group 1, partial portacaval transposition. a, Split transposition.—In 15 dogs, the left (Fig. 1a) and in 18 dogs the right (Fig. 1b) portal vein was detached from the main portal trunk and revascularized by

an end-to-end anastomosis to the supra-adrenal inferior vena cava by the method of Marchioro and his associates (28). The procedure divides the liver into two compartments which are dissimilar in that one receives portal blood from the total splanchnic venous bed and the other obtains its portal supply from systemic sources, including the effluent from the kidneys, adrenals, and hindquarters.

b, Split transposition minus adrenal and renal inflow.—The procedure was identical to that just described except that the systemic venous blood was derived from the infrarenal inferior vena cava thereby excluding the renal and adrenal effluent. This blood was transmitted to the appropriate branch of the portal vein by way of an internal jugular vein graft. In six dogs, the systemic venous input was to the left portal branch (Fig. 1c) and in two dogs, to the right portal vein (Fig. 1d).

Group 2, splanchnic flow division. The two portal branches were isolated. One was left undisturbed; the other was detached and anastomosed by means of an iliac vein graft to the common mesenteric venous input. Proximally, the mesenteric trunk was ligated just below the splenic vein (Fig. 2). Thus, one side of the liver received portal blood of an intestinal source, and the other side received venous blood returning from the pancreatic, splenic, and gastroduodenal beds. Twenty experiments each were performed to the right and the left sides.

In the dog, the pancreas has two distinct lobes.

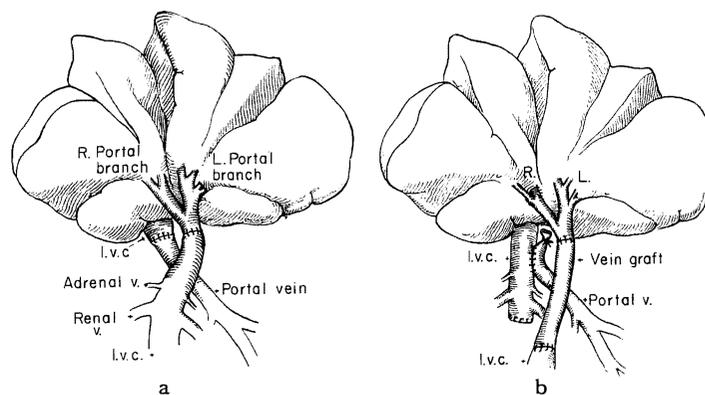


FIG. 3. Techniques of transposition for the dogs in group 3. a, Standard portacaval transposition of Child. b, Modified transposition which eliminates adrenal and renal venous blood from the portal blood samples. A venous graft is always required.

Early in the series, it was discovered that the tail of the inferior pancreatic lobe almost invariably drains into the mesenteric venous circulation (Fig. 2, insets). Thereafter, this portion of the pancreas was always resected at the time of the splanchnic division procedure. Three dogs operated upon before this observation was made had delayed partial pancreatectomy at the time of the first biopsy one month after the original operation.

Group 3, total portacaval transpositions. a, Standard transposition.—Nine dogs underwent portacaval transposition by means of Child's method (11) and as shown in Figure 3a. An open liver biopsy was performed before the transposition.

b, Total transposition minus adrenal and renal inflow.—Five dogs had a modified portacaval transposition (Fig. 3b) with revascularization of the portal vein by means of a venous graft from the infrarenal inferior vena cava.

Postoperative Studies

The dogs were maintained on a standard kennel diet. Bilirubin, alkaline phosphatase, and serum glutamic oxalacetic transaminase values were checked twice a week. These values were never abnormal.

Vessel patency. Prior to liver biopsy, the patency of the anastomoses was determined either by angiography or at exploratory laparotomy. At angiography of the dogs in groups 1 and 2, an effort was made to see if dye injected in one venous pool spilled into the other bed. Complete gross separation was always demonstrated.

Liver biopsies. In all dogs found to have patency of the two portal branches, biopsy specimens were taken from both sides. To minimize any effects due to the anesthetic agents, the specimens were re-

moved under normothermia as soon as possible after induction of anesthesia with phencyclidine hydrochloride (Sernylan®), atropine, and pentobarbital sodium. The liver was first carefully examined for gross evidence of hypertrophy or atrophy. Blood flow to the area of the biopsy was not interfered with in any way until the specimen had been removed. In all experiments except those of group 3, both sides of the liver were biopsied and the specimens processed separately. Two grams of liver tissue were excised. One and one-half grams of tissue were snap frozen within five seconds and stored in liquid nitrogen at -158 degrees C. until the biochemical studies were performed. The rest of the biopsy specimen was used for pathologic studies. A portion was fixed in formalin, and the remaining tissue was frozen with dry ice.

Autopsy procedures. Most of the dogs were sacrificed after the last biopsy. In the dogs in groups 1 and 2, the liver was excised and extraneous tissue, including the gallbladder, trimmed from it. After weighing the entire liver, the two portal branches were carefully dissected out and a decision made as to the exact portal venous distribution to each of the lobes and sublobes of the liver. When the entire liver had been thus subdivided into the right and left components, it was cut along that axis and the right and left portions weighed separately. The normal weight ratio for the right lobes versus the left lobes had been shown by Child (11), Marchioro (28), and Pouyet (34) and their associates to be about 30:70. Of the six normal control dogs which were sacrificed, these same proportions were verified as will be noted later.

In dogs dying prior to biopsy, the same morphologic evaluation for atrophy and hypertrophy was performed, and specimens were taken for histo-

logic studies, but no arrangements were made for biochemical determinations.

Criteria for hypertrophy. On histopathologic study, relative atrophy or hypertrophy of the different liver portions was usually evident, and differences in lobule size, fat content, reticulin pattern, and glycogen content could be detected with the appropriate stains.

To obtain a quantitative estimation of the hepatocyte size, a tracing device was attached to the light microscope, and large numbers of hepatocytes in each experiment were drawn on a standard thickness paper. Forty representative traced hepatocytes were then cut out, and the pieces of paper they occupied were weighed (Fig. 4). The weight in grams was used to denote size units. We have shown this to be an accurate method for comparing cell sizes by confirmatory planimetry and by studies of unicellular organisms, the size of which could be directly determined.

Criteria for hyperplasia. The following hallmarks of hyperplasia were looked for: increased numbers of mitoses, the presence of binucleate and trinucleate hepatocytes, increased numbers of bile ductules, and increased thickness of the hepatic cell plates.

Biochemical Determinations

Hepatic glycogen. The method of Bloom and his associates (4) was used to separate the trichloroacetic acid soluble glycogen fraction from the insoluble one. Both fractions were quantitated with the anthrone method of Seifter and his colleagues (42), and the results were expressed in milligrams of glycogen per gram wet weight of liver.

Active and total phosphorylase. The active form of hepatic phosphorylase, Enzyme Commission Number 2.4.11., was measured by the method of Shimazu and Fukada (45), wherein additional activation during the assay of phosphorylase, Enzyme Commission Number 2.7.1.38., is prevented by inhibiting dephosphophosphorylase kinase and phosphorylase phosphatase, Enzyme Commission Number 3.1.3.17., with ethylenediaminetetraacetate and sodium fluoride, respectively. The assay for the phosphorylase determination contained 50 millimoles of glucose-1-phosphate and 1 per cent glycogen, to which a glycerol extract of liver was added. Incubation was for ten minutes at 37 degrees C., following which the inorganic phosphate released was measured by the method of Takahashi (55). The activity of phosphorylase was expressed as millimicromoles of phosphate liberated in one minute by 1 milligram of protein of liver extract.

Total phosphorylase activity was assayed in the

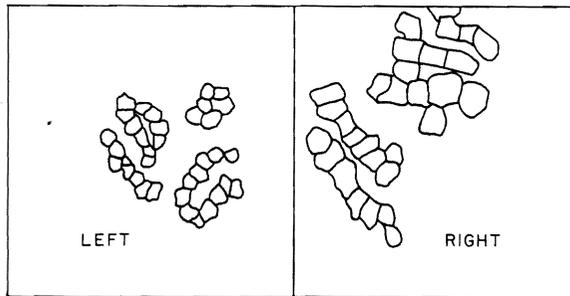


FIG. 4. Hepatocyte shadows traced during histopathologic examination. These were later cut out on standard paper and weighed as an index of hepatocyte size. The specimens depicted were from the experimental group 2 (see Fig. 2a). The right lobes with the large hepatic cells received venous blood from the pancreas, stomach, duodenum, and spleen. The relatively shrunken left lobes with the small hepatocytes received intestinal blood.

same way except that inactive phosphorylase was first rendered active with adenosine triphosphate according to the method described by Shimazu and Amakawa (44).

Hepatic cyclic 3', 5'-adenosine monophosphate (cyclic AMP). The method for extracting the liver sequentially with trichloroacetic acid and cold water-saturated diethyl ether has been described by Wastila and his associates (60). The extract was directly assayed for cyclic 3', 5'-adenosine monophosphate by the protein binding method of Gilman (19). The values obtained represent the means of four determinations of each sample and are expressed as picomoles per gram wet weight of liver.

Protein concentration. The protein of a weighed liver specimen was extracted with 10 per cent trichloroacetic acid and digested with 3 per cent desoxycholic acid in sodium hydroxide. The protein concentration was then measured with the biuret method of Henry and his colleagues (21).

Protein synthesis. Twenty-four hours before biopsy, the dogs were given an intravenous injection of 60 millicuries of ^{14}C -leucine which had a specific activity of 28.1 millicuries per millimole. A 70 to 100 milligram portion of the biopsy specimen was processed by the method of Schneider and Hogeboom (41) as modified by Siekevitz (46), and the resulting protein powder was collected on a millipore filter. The filters were introduced into counting vials containing 20 milliliters of Albano and Francavilla's standard scintillation solution (1) and counted on a Picker Liquimat[®]. The results were expressed as counts per minute per gram wet weight of liver.

Total lipids and triglycerides. Total lipids were measured by a modification of the method of MacKenzie and his colleagues (26), by which a tissue

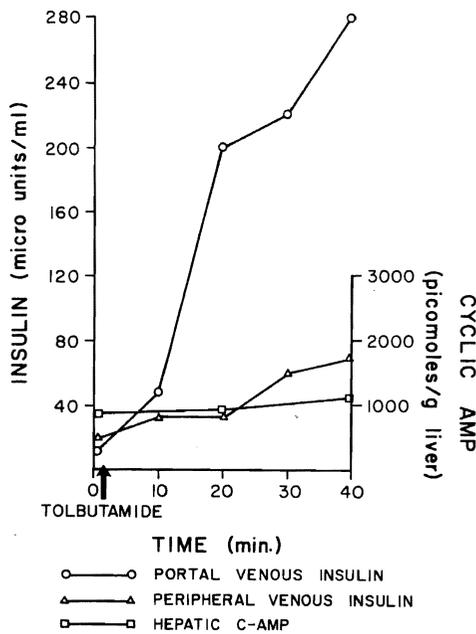


FIG. 5. Changes in peripheral and portal venous insulin and hepatic cyclic 3', 5'-adenosine monophosphate, *cyclic AMP*, occurring in a normal dog infused with tolbutamide. Note that the peak insulin response in the portal blood occurred 25 to 40 minutes after infusion and that no significant alterations in hepatic cyclic 3', 5'-adenosine monophosphate were caused acutely by the tolbutamide itself.

sample is homogenized in and extracted with a cold 1:1 ethyl alcohol:ethyl ether solution, washed, dried, and weighed.

The triglyceride fraction was extracted and weighed by a modification of MacKenzie's micro-method as derived from Barron and Hanahan (2), using silicic acid column chromatography to separate the triglyceride from the other lipid components. The column was first washed with 5 grams of 85:15 hexane:benzene. Then, the first two fractions of 4 grams each were eluted with a mixture of 97:3 hexane:ethyl ether and collected for triglyceride determinations.

The total lipids and triglyceride fractions were expressed as milligrams per gram wet weight of liver.

Glucokinase. Glucose phosphorylation by glucokinase, Enzyme Commission Number 2.7.1.2., was assayed by the glucose-6-phosphate dehydrogenase spectrophotometric method of Salas and his colleagues (40). The results were expressed as micromoles of glucose phosphorylated per minute per gram wet weight of liver at room temperature.

Kinetic Studies of Cyclic 3', 5'-adenosine Monophosphate (Cyclic AMP)

Aminophylline test. To determine the rate of formation of liver cyclic 3', 5'-adenosine monophos-

phate, 13 dogs of groups 1 or 2 were submitted to an aminophylline infusion test two months after their operation. As described by Robison and his group (38), the methylxanthines in appropriate dosages are essentially complete inhibitors of cyclic 3', 5'-adenosine monophosphate phosphodiesterase. Since Butcher and Sutherland (8) and Cheung (10) have shown this phosphodiesterase to be the principal enzyme involved in the catabolism of cyclic 3', 5'-adenosine monophosphate, inhibition of the enzyme with aminophylline allowed the quantitation of cyclic 3', 5'-adenosine monophosphate formation. A large dosage of aminophylline was used. This was given rapidly to permit prompt completion of the studies before the onset of delayed physiologic effects, such as aminophylline-induced changes in insulin levels which might by themselves affect the metabolism of cyclic 3', 5'-adenosine monophosphate.

The dogs were anesthetized, and open biopsy specimens of the right and left lobes were taken for base line levels. Then, 3 grams of aminophylline in 300 milliliters of 5 per cent dextrose in normal saline solution were infused by way of a peripheral vein at a constant rate during a six minute interval. Biopsy specimens of 100 to 200 milligrams in weight were removed from both sides of the liver at two, four, and six minutes after the infusion was begun, taking care to minimize blood loss. These were immediately frozen in liquid nitrogen and assayed for cyclic 3', 5'-adenosine monophosphate as described previously.

Tolbutamide-glucagon test. As reviewed by Exton and Park (13), the level of hepatic cyclic 3', 5'-adenosine monophosphate is, at least in part, under the control of endogenous insulin. To determine whether or not the livers of dogs remained responsive to changes in insulin level after a portal diversion procedure, a test was developed in which the cyclic 3', 5'-adenosine monophosphate response to a measured dosage of endogenous insulin was measured.

To generate a standard endogenous insulin response, a 40 milligram per kilogram dose of tolbutamide was given to normal dogs as a one minute infusion by way of a peripheral vein. With the insulin antibody method of Morgan and Lazarow (32), it was found that the peak of insulin increase in the portal blood occurs 25 to 40 minutes after the infusion of tolbutamide (Fig. 5).

The tolbutamide itself did not cause major changes in hepatic cyclic 3', 5'-adenosine monophosphate (Fig. 5). Nevertheless, the levels of portal blood insulin generated by this test are known from other studies of Robison and his colleagues (38) to

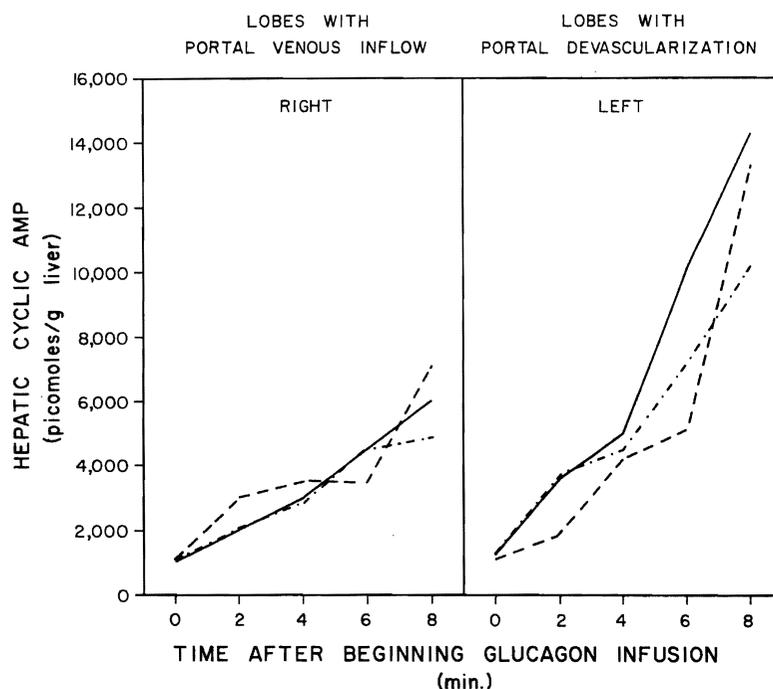


FIG. 6. Results with the tolbutamide-glucagon test in three dogs from groups 1a or 1c in which there was clotting of the venous grafts. Consequently, the left branch of the portal vein transmitted no blood at all while the right lobes had a full splanchnic inflow. Note that the hepatic cyclic 3', 5'-adenosine monophosphate response to exogenous glucagon was considerably less in the lobes having a portal inflow compared with the lobes suffering from portal devascularization. The relatively lower cyclic 3', 5'-adenosine monophosphate presumably represented a restraining insulin effect.

produce a reduction in the hepatic cyclic 3', 5'-adenosine monophosphate, even though these are so slight that they are difficult to measure by direct techniques. However, the effect of the insulin can be demonstrated by giving glucagon simultaneously, as was demonstrated in three control experiments (Fig. 6). Glucagon normally produces a manyfold increase in liver cyclic 3', 5'-adenosine monophosphate when given by systemic vein, but when the liver received both endogenous insulin and exogenous glucagon simultaneously, the net effect was a more modest rise in cyclic 3', 5'-adenosine monophosphate (Fig. 6). In these control dogs, the right lobes of the liver had a normal blood supply whereas the left portal branch was occluded so that the left lobes did not directly receive any venous effluent from the pancreas.

The conditions of the tests in the 13 definitive dogs of groups 1 or 2 were as follows: the anesthetized dogs were maintained on lactated Ringer's solution while biopsy specimens of each side of the liver were taken. Tolbutamide, 40 milligrams per kilogram body weight, was given intravenously for one minute, following which an infusion of 5 grams

per kilogram of dextrose was given over 25 minutes. Then, 1.40 gammas per kilogram body weight of glucagon were given in 300 milliliters of 5 per cent dextrose during eight minutes. Biopsy specimens were taken at two, four, six, and eight minutes after the glucagon infusion was begun, frozen in liquid nitrogen, and assayed for cyclic 3', 5'-adenosine monophosphate, as described previously.

RESULTS

Partial Portacaval Transposition

Morphologic findings. Eighteen of the 33 experiments with unmodified split transposition were carried to completion with proof of the patency of the venous channels after approximately two months. In 11 of these successful experiments, the inferior vena cava was anastomosed to the left portal branch (Fig. 1a), and in the other seven, the vena caval anastomosis was to the right branch (Fig. 1b).

The results which are partially summarized in Figure 7 confirmed and extended the observations previously reported from our laboratories (28, 30). The liver lobes receiving splanchnic venous inflow

TABLE I.—THE EFFECT IN SPLIT TRANSPOSITION EXPERIMENTS OF GROUP 1 OF PORTAL VENOUS INFLOW FROM THE SPLANCHNIC VERSUS THE INFERIOR VENA CAVAL BEDS

	<i>Splanchnic flow to right lobes.</i>								<i>Splanchnic flow to left lobes.</i>							
	<i>One month</i>				<i>Two months</i>				<i>One month</i>				<i>Two months</i>			
	No.	Right	Left	p	No.	Right	Left	p	No.	Right	Left	p	No.	Right	Left	p
Glycogen, mgm./gm. of liver																
Total.....	7	5.15 ±1.00	3.79 ±0.43	<0.02	11	3.74 ±1.16	2.26 ±1.08	<0.001	3	2.92 ±1.63	3.74 ±2.69	NS	7	2.45 ±0.70	4.66 ±0.76	<0.001
Trichloroacetic acid soluble.....	7	4.65 ±1.11	2.81 ±0.81	<0.02	11	3.00 ±1.03	1.59 ±0.88	<0.001					7	1.89 ±0.83	3.55 ±0.58	<0.01
Cyclic adenosine monophosphate, picomoles/gm. of liver..	7	1,050 ±159	1,638 ±284	<0.001	11	1,162 ±86	1,695 ±256	<0.001	3	1,276 ±185	906 ±211	<0.1	6	1,718 ±96	1,363 ±267	<0.05
Phosphorylase, millimicromoles/min./mgm. of liver protein																
Total.....	7	111.5 ±11.3	*		11	123.1 ±17.7	*		3	* ±17.0	123.5 ±17.0	NS	4	* ±10.1	110.7 ±10.1	
Active.....	7	51.9 ±14.0	72.7 ±18.2	<0.1	11	54.8 ±11.8	76.0 ±13.2	<0.001	3	66.6 ±18.4	46.0 ±12.3	NS	4	72.3 ±4.3	44.8 ±6.1	<0.02
Glucokinase, micromoles/gm./min....					9	3.04 ±1.14	1.73 ±0.67	<0.001					7	1.59 ±0.97	2.33 ±0.81	<0.01
Protein concentration, mgm./gm. of liver.	7	197.0 ±14.0	216.3 ±21.0	<0.05	11	205.4 ±21.4	211.7 ±20.9	NS	3	195.8 ±7.8	175.5 ±63.9	NS	7	188.5 ±17.6	184.2 ±15.6	NS
Protein synthesis, counts/min./gm. of liver.....	1	2,175	2,558		6	1,758 ±112	1,866 ±246	NS					2	2,016 ±378	2,021 ±243	NS
Triglycerides, mgm./gm. of liver.....					3	16.8 ±3.0	11.6 ±1.6	<0.1								

Means, numbers of determinations, and standard deviations are given.

All p values compare the two liver sides.

*Within the limits of the analytic method used, the measurements of total phosphorylase activity were constant in a given experiment, no matter what the site of the liver biopsy. For this reason, these determinations are given only for the side of the liver receiving splanchnic blood.

gained weight after 2 ± 0.5 (standard deviation) months and had striking hypertrophy of the individual hepatocytes, whereas the lobes being perfused with vena caval blood shrank with a diminution of the liver cell size. Good histopathologic studies were obtained from 17 of the 18 livers. The liver lobules were larger on the splanchnic side in all of the 17 experiments, and lipid was less prominent in 12 of 17 and about equal in the other five. Glycogen, as judged with periodic acid-Schiff stained sections, often seemed more prominent in the lobes supplied with splanchnic venous blood although this finding was inconstant. Consequently, heavy reliance was placed on the quantitative glycogen assays which will be described.

There was also evidence of hyperplasia on the side receiving splanchnic blood. This consisted of the presence of hepatic cell plates two or more cells thick, binucleate and trinucleate hepatocytes, increased numbers of bile ductules, and a slightly increased mitosis count.

Of the eight attempts at split transposition with exclusion of the adrenal and renal blood, only one experiment was completely successful, that with

vena caval flow to the right lobes (Fig. 1d). The morphologic effects of this procedure at nine weeks (Fig. 7) were comparable but slightly less extreme than with the regular split transposition.

Biochemical concentrations. Beginning within four weeks and continuing until two months there were marked biochemical differences between the lobes according to the source of the portal venous inflow (Table I). Hepatic tissue receiving splanchnic blood, whether this be on the right or left side, had significantly higher concentrations of glycogen and glucokinase relative to the lobes supplied by vena caval blood. In contrast, the lobes supplied by the vena caval blood had significantly higher cyclic 3', 5'-adenosine monophosphate and active phosphorylase concentrations. The hepatic triglyceride concentration seemed to be increased by splanchnic venous inflow, but the observations were too few to permit statistical significance. The comparisons of protein concentration and protein synthesis were not strikingly different (Table I).

In the one successful experiment of group 1b (Fig. 1d) in which adrenal and renal venous blood was excluded from the vena caval supply to the

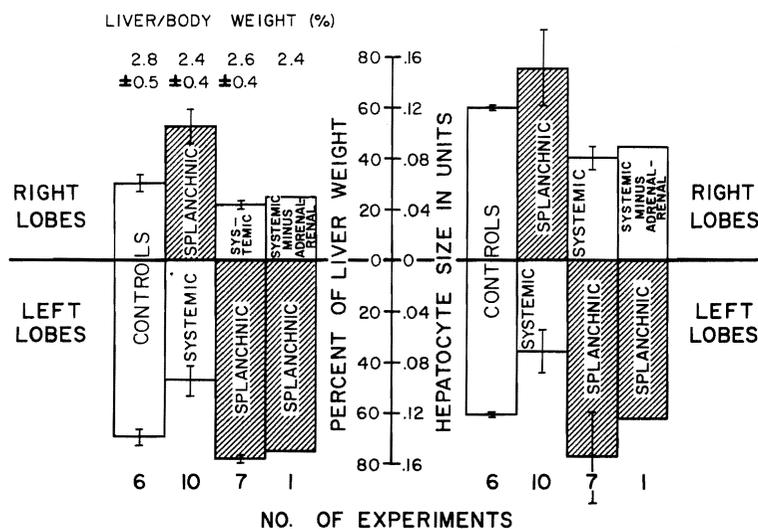


FIG. 7. The morphologic consequences, after 2 ± 0.5 (S. D.) months in the dogs of group 1, of supplying parts of the liver with nonhepatic splanchnic venous blood, shaded areas, as compared with vena caval blood. Results are also given for six normal dogs. Note the gross weight gain and hepatocyte hypertrophy on the side perfused with splanchnic blood. Although the relative sizes of the lobes were altered according to the source of portal venous inflow, note that the ratio of the liver weight to total body weight was relatively unchanged. One standard deviation is depicted in the bar graphs and written out for the weight percentages.

right lobes, the chemical pattern was altered after nine weeks in a different way than in the dogs of group 1a. In the right and left lobes, respectively, glycogen was 5.7 and 5.25 milligrams, glucokinase was 1.0 and 1.5 micromoles, active phosphorylase was 47.4 and 45.3 millimicromoles, and cyclic 3', 5'-adenosine monophosphate was 974 and 1,225 picomoles.

Splanchnic Flow Division

Forty experiments were attempted, and 12 were carried to completion. In six successful experiments, the pancreatic, splenic, and gastroduodenal blood passed to the right lobes (Fig. 2a), and in the other six this blood passed to the left lobes (Fig. 2b).

Morphologic findings. The effects of these alterations on the liver tissues after one to five and one-half months, 73 ± 53 (S. D.) days, are summarized in Figure 8 and compared with the liver tissue from six normal dogs. The lobes which received the pancreatic, gastroduodenal, and splenic blood retained or increased their expected weight compared with the other lobes supplied by intestinal venous blood, and the individual hepatocytes in the favored lobes increased in size (Fig. 8). The latter hepatocyte and lobe size increases occurred whether the pancreatic, gastroduodenal, and splenic flow was to the left or right lobes. In addition to the hepatocyte size, complete histopathologic analysis was performed on all

12 livers. In nine of these 12 experiments, the liver lobules were obviously larger on the side supplied by the pancreatic-gastroduodenal-splenic venous effluent (Fig. 9). The degree of lipid deposition was less on this side in one experiment but about the same in the other 11.

There was also evidence of hyperplasia in the liver tissue supplied by blood from the pancreas, stomach, duodenum, and spleen (Fig. 9). This consisted of hepatic cell plates two or more cells thick, binucleate and trinucleate hepatocytes, and increased numbers of bile ductules. A slightly raised mitosis count was also found in those livers biopsied less than three months after splanchnic flow division.

Biochemical concentration. After 10.4 ± 7.6 (S. D.) weeks, range one to five and one-half months, the liver tissue provided with pancreatic-gastroduodenal-splenic blood contained higher concentrations of glycogen in spite of the fact that alimentary glucose was passing primarily to the contralateral hepatic lobes. These findings were statistically significant in the experiments of group 2b in which the pancreatic-gastroduodenal-splenic blood went to the left lobes (Table II). When this blood was directed to the right side in the dogs in group 2a, the right lobes behaved similarly, but the changes were short of statistical significance. Under both conditions, the glucokinase was elevated in parallel

TABLE II.—THE EFFECT IN SPLANCHNIC DIVISION EXPERIMENTS OF GROUP 2 OF PERFUSING THE LIVER WITH PANCREATIC DUODENAL SPLENIC VERSUS INTESTINAL BLOOD

	Splanchnic flow to right lobes						Splanchnic flow to left lobes									
	One month			Two months			One month			Two months						
	No.	Right	Left	p	No.	Right	Left	p	No.	Right	Left	p				
Glycogen, mgm./gm. of liver																
Total.....	5	5.28 ±1.26	5.26 ±1.47	NS	4	3.64 ±1.63	3.13 ±1.67	NS	4	3.31 ±1.90	4.51 ±1.91	<0.02	6	2.83 ±1.87	4.07 ±1.64	<0.01
Trichloroacetic acid soluble.....	5	4.11 ±1.05	4.03 ±1.05	NS	4	3.35 ±0.40	2.35 ±1.55	NS	4	2.93 ±1.90	3.70 ±2.05	<0.1	6	2.00 ±1.31	3.23 ±1.41	<0.001
Cyclic adenosine monophosphate picomoles/gm. of liver.....	5	1,276 ±102	1,258 ±76	NS	4	1,215 ±191	1,123 ±175	NS	4	725 ±63	1,052 ±394	NS	6	1,077 ±237	1,164 ±218	<0.1
Phosphorylase, millimicromoles/min./mgm. of liver protein																
Total.....	5	115.1 ±21.3	*		4	104.2 ±6.4	*		4	*	88.8 ±23.5		6	*	113.2 ±11.6	
Active.....	4	46.5 ±10.0	48.1 ±7.3	NS	4	52.0 ±6.3	50.4 ±6.3	NS	4	43.2 ±4.8	47.3 ±15.0	NS	6	49.2 ±7.9	48.5 ±7.3	NS
Glucokinase, micro-moles/gm./min....					4	2.16 ±1.27	1.62 ±1.06	<0.1					4	2.07 ±1.13	2.67 ±0.88	NS
Protein concentration, mgm./gm. of liver.	5	188.3 ±35.9	190.7 ±29.9	NS	4	205.6 ±2.5	202.4 ±6.2	NS	4	179.0 ±7.0	210.0 ±19.8	<0.02	6	192.7 ±33.1	198.2 ±33.9	NS
Protein synthesis, counts/min./gm. of liver.....					4	2,696 ±394	2,753 ±358	NS	4	2,971 ±1,044	3,021 ±1,043	NS	3	2,340 ±970	2,509 ±341	NS

Means, number of determinations, and standard deviations are given.

All p values compare the two sides.

*Within the limits of the analytic method used, the measurements of total phosphorylase activity were constant in a given experiment, no matter what the site of the liver biopsy. For this reason, these determinations are given only for the side of the liver receiving splanchnic blood.

TABLE III.—THE EFFECT OF PORTACAVAL TRANSPOSITION (LEFT) AND PORTACAVAL TRANSPOSITION MINUS THE RENAL AND ADRENAL BLOOD (RIGHT) UPON GLYCOGEN CONCENTRATION AND CYCLIC 3', 5'-ADENOSINE MONOPHOSPHATE

	Transposition				Transposition minus renal, adrenal			
	No.	Preoperative	Postoperative	p	No.	Preoperative	Postoperative	p
Glycogen, mgm./gm. of liver								
Total.....	5	3.82±0.53	2.10±1.04	<0.02	2	4.08±1.12	2.27±0.40	NS
Trichloroacetic acid soluble.....	5	3.13±0.42	1.70±0.83	<0.001	2	2.99±0.55	1.89±0.31	NS
Cyclic adenosine monophosphate, picomoles/gm. of liver...	5	(1107)*	1,729±215	<0.01	2	(1107)*	1,701±46	<0.05

The experiments are group 3a and 3b.

All p values compare the preoperative and postoperative determinations.

*This "normal" for cyclic 3', 5'-adenosine monophosphate was derived by taking the mean of a series of cyclic 3', 5'-adenosine monophosphate determinations in biopsy specimens taken from canine livers having a normal portal blood supply.

with the glycogen but not to a statistically significant degree (Table II).

Cyclic 3', 5'-adenosine monophosphate, active phosphorylase, protein concentration, and protein synthesis did not conform to a consistent pattern.

Total Portacaval Transposition

The five dogs with successful conventional transposition (Fig. 3a) underwent follow-up study for 10.2 ± 2.0 (S. D.) weeks, and the dogs with transposition minus adrenal and renal blood were each

studied for two months. At autopsy, the per cent of liver to total body weight was 1.8 and 1.9 per cent in the respective groups.

The dogs with standard as well as those with modified portacaval transposition had hepatic deglycogenation and striking elevations in cyclic 3', 5'-adenosine monophosphate (Table III).

Kinetic Studies

Partial portacaval transposition. In four of five dogs of groups 1a (Fig. 1a or b), the rate of cyclic 3', 5'-

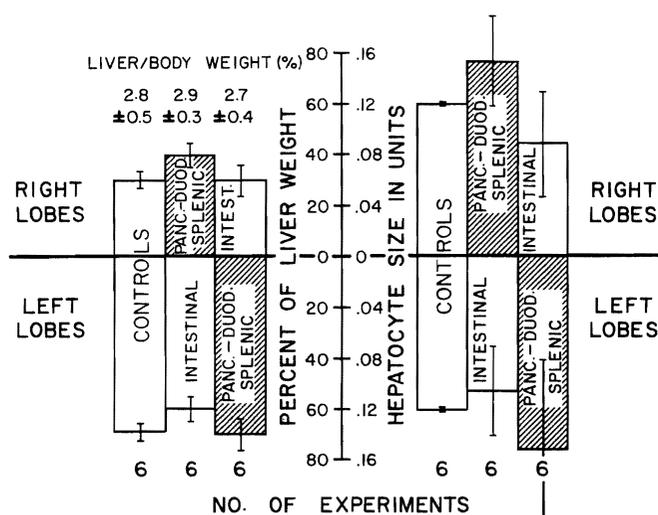


FIG. 8. The morphologic consequences of splanchnic venous flow division in the dogs in group 2 compared with normal dogs after 28 to 173 days, average 73. The liver fractions which were perfused with venous blood from the pancreatic, gastroduodenal, and splenic areas are shaded. Note that these portions gained weight and underwent an increase in hepatocyte size relative to the other side while the total liver weight to body weight ratios were little altered. One standard deviation is depicted graphically on the bar graphs and written out for the weight percentages.

adenosine monophosphate formation, as unmasked by the aminophylline test, was greater in the liver tissue receiving vena caval blood than in the contralateral lobes receiving splanchnic venous blood (Fig. 10).

In the single successful experiment in which partial transposition was with vena caval blood minus adrenal and renal inflow (Fig. 1d), the cyclic 3', 5'-adenosine monophosphate response showed a different pattern in that the side of the liver supplied with vena caval blood had a much slower rate of cyclic 3', 5'-adenosine monophosphate accumulation than the lobes receiving splanchnic blood (Fig. 11). These results were similar to those in three control experiments in which one side of the liver did not have a portal vein inflow at all due to clotting. This side accumulated cyclic 3', 5'-adenosine monophosphate much more slowly than in the fully vascularized lobes (Fig. 12).

With the tolbutamide-glucagon test, the lobes receiving nonhepatic splanchnic blood showed a response similar to that of the control dogs, whereas the contralateral lobes supplied with supra-adrenal vena caval blood showed a rapid accumulation of cyclic 3', 5'-adenosine monophosphate (Fig. 13).

Splanchnic flow division. With the aminophylline infusion test, it was found in four experiments that the lobes receiving splenic-gastroduodenal-pan-

creatic blood had a more rapid rate of cyclic 3', 5'-adenosine monophosphate synthesis than the contralateral lobes supplied with intestinal blood (Fig. 14).

However, the lobes with splenic-gastroduodenal-pancreatic venous inflow were found by the tolbutamide test to be under insulin control in that three of five experiments showed a much slower rate of cyclic 3', 5'-adenosine monophosphate accumulation in response to exogenous glucagon than in the lobes receiving mesenteric blood (Fig. 15).

DISCUSSION

Little doubt remains that there are substances in splanchnic venous as opposed to systemic venous blood that are important for the maintenance of hepatic structure and function. The first steps by which this concept was developed and validated have already been mentioned. The extent of present day acceptance of the concept can be appreciated by the fact that Fisher and Lee and their associates (15-17, 24), who until recently were the most outspoken critics of the hepatotrophic hypothesis, have lately supported the idea. By adapting the double liver fragment principle introduced during investigation of auxiliary liver transplantation in our laboratory (28, 30, 51), they and their col-

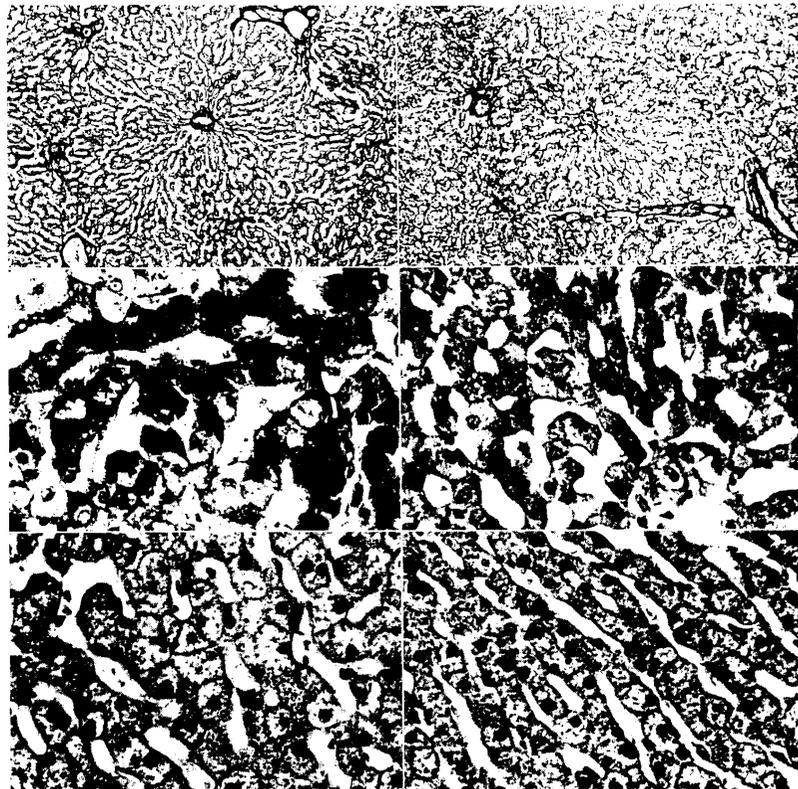


FIG. 9. Photomicrographs of sections from a liver which had been subjected to splanchnic flow division. The part of the liver supplied by the pancreaticogastro-duodenal-splenic blood, panels on left, shows enlargement of the liver lobules, abundant glycogen, and hypertrophy and hyperplasia of hepatocytes when compared with the part supplied by intestinal blood, panels on right. Upper, Reticulin stain, $\times 10$. Middle, Periodic acid-Schiff, $\times 87$. Lower, Hematoxylin and eosin, $\times 87$.

leagues have added convincing evidence of their own supporting the hepatotrophic theory (9, 18, 25).

The advantage of the various double or split liver preparations for the investigation of hepatotrophic mechanisms is that the hepatotrophic factors are apparently exhausted by exposure to one hepatic fragment and, therefore, are unavailable to other competing liver tissue if the latter is endowed with a blood supply deficient in such substances. With a single liver, as historically was used in studies of Eck fistula or portacaval transposition, the unmasking and precise study of splanchnic hepatotrophic effects was difficult or impossible after the portal by-pass procedures since biologically active substances in the diverted splanchnic venous effluent were presumably returned to the liver by way of the systemic blood although in diluted concentrations. Parenthetically, a recirculation effect could account for the superior liver state of dogs with Child's transposition compared with animals with Eck fistula since the quantity of di-

luted hepatotrophic substances reaching the liver would be proportional to the total hepatic blood flow which is more than twice as great with transposition than with Eck fistula. Then, the classical error in interpretation followed, namely to assume that the quantity of blood flow was infinitely more important than the quality of the blood in maintaining hepatic structure and function.

Conceding the qualitative specialness of portal venous blood, the experiments in this study were designed to answer two sets of additional questions. The first concerned the source and the nature of the hepatotrophic factors in splanchnic venous blood. The second was involved with the mechanism of action of this factor or complex of factors.

The origin of the hepatotrophic factors was determined from studies of the morphologic and biochemical changes induced in the liver by modifications of the portal venous inflow. These were of three types: total portacaval transposition, partial portacaval transposition, and splanchnic flow division.

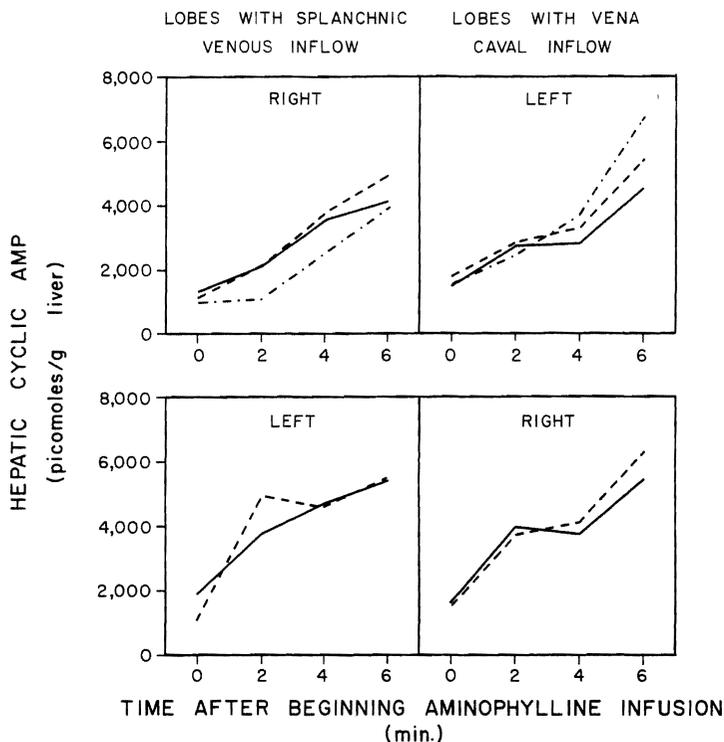


FIG. 10. Aminophylline infusion tests in five dogs with partial portacaval transposition. In all but one, the rate of formation of cyclic 3', 5'-adenosine monophosphate was greater in the lobes deriving their portal inflow from the inferior vena cava although these differences were usually not obvious until several minutes had gone by.

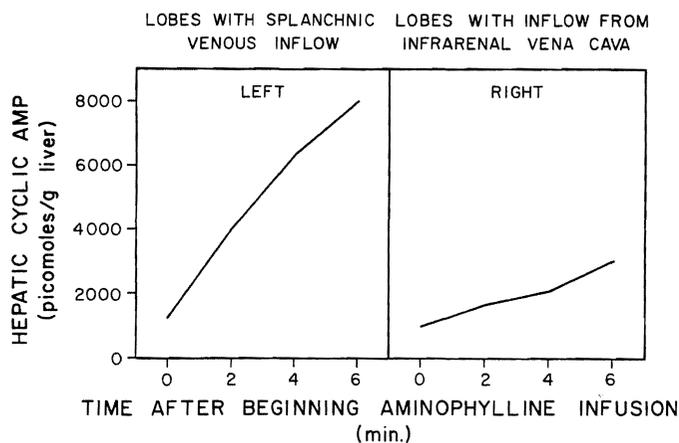


FIG. 11. Rate of cyclic 3', 5'-adenosine monophosphate formation, as measured by the aminophylline infusion test, in the single successful experiment of group 1d.

The observations of Marchioro and his associates (28, 30) were first confirmed in experiments with partial transposition. Within four to eight weeks, liver lobes supplied with splanchnic inflow had hypertrophic glycogen-rich hepatocytes

which in addition often had findings of hyperplasia. In contrast, the other lobes of the liver supplied by vena caval blood underwent involutional changes. The hepatocytes became smaller, and the glycogen concentration decreased.

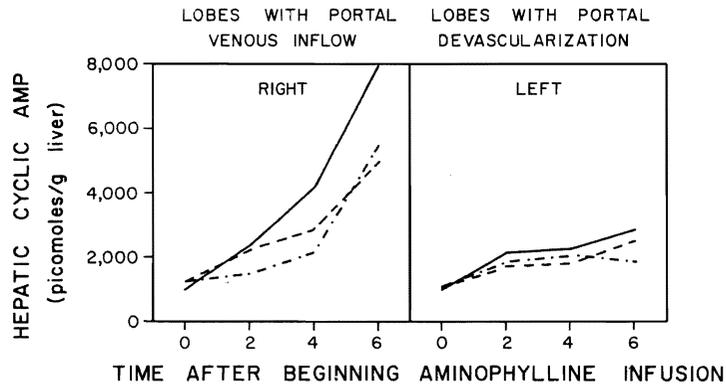


FIG. 12. Aminophylline infusion tests in three dogs taken from groups 1a or 1c in which the left portal branch clotted, leaving these lobes supplied only with arterial blood, whereas the right lobes received both arterial and splanchnic venous blood. Note that the rate of synthesis of hepatic cyclic 3', 5'-adenosine monophosphate was greater in the right lobes having a portal inflow than in the left lobes suffering from total portal devascularization. The time units are in minutes.

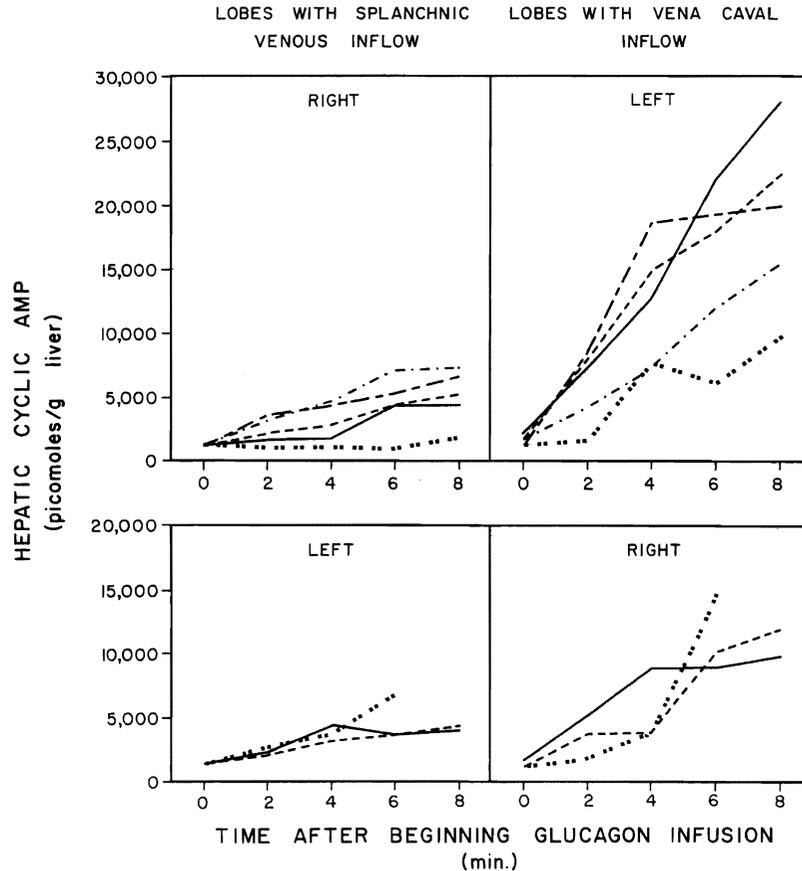


FIG. 13. Results of tolbutamide-glucagon tests in eight dogs with partial porta-caval transposition, demonstrating the effect of endogenous insulin in the lobes receiving splanchnic venous blood. These insulin-controlled lobes had a restrained cyclic 3', 5'-adenosine monophosphate response to the exogenous glucagon whereas the response in the other lobes was uninhibited.

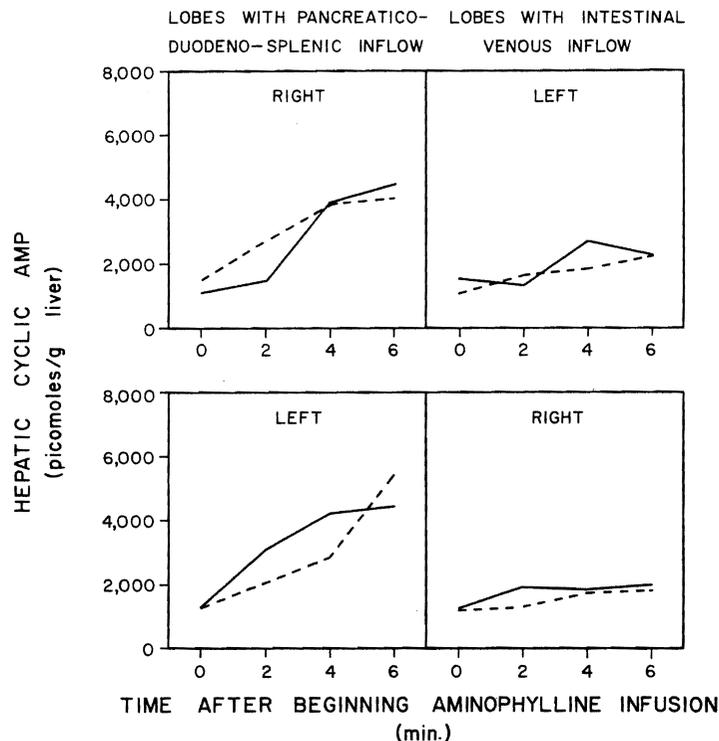


FIG. 14. Aminophylline infusion tests two months after splanchnic flow division in four dogs. Cyclic 3', 5'-adenosine monophosphate synthesis was much more rapid in the hepatic lobes receiving pancreatic-gastro-duodenal-splenic blood than in the contralateral lobes supplied with intestinal venous effluent.

Next, surgical techniques were used that partitioned the splanchnic flow. The major part of the hepatotropic influence as manifested both by morphologic and biochemical criteria was unequivocally shown to be in the blood returning from the pancreas, proximal part of the duodenum, stomach, and spleen. In contrast, the other hepatic lobes fed by nutritionally rich venous blood from the small intestine underwent involutional changes, including atrophy (Fig. 16) and deglycogenation nearly as profound as if a vena caval supply had been used.

For several years, there has been good reason to suspect that the upper splanchnic organ complex and specifically the pancreas was the source of the hepatotropic factors. In the studies of the blood flow requirements for auxiliary hepatic transplantation by Marchioro and his colleagues (29), atrophy of the native liver could be minimized by provision of pancreatic-gastroduodenal-splenic blood even though all the rest of the splanchnic venous blood went to a co-existing liver homograft. Pouyet and his associates (34) came to the same general conclusion with carefully documented

splanchnic division experiments similar to those in group 2 of this study. The correctness of these observations was later confirmed in a beagle homo-transplantation model used by Ranson and his associates (37). In all these studies as well as in those reported herein, a contribution to the hepatotropic support of the liver could hypothetically have been made from the stomach, duodenum, or spleen although some of Pouyet's experiments essentially ruled out the spleen and stomach. Nevertheless, it was logical as Price and his associates (35) have suggested to look for a hormonal explanation. This was done in the present study with a series of biochemical evaluations.

The first step was to analyze the hepatic changes caused by the classical portacaval transposition of Child and his associates (11), a procedure which profoundly deglycogenates the liver (43, 52) as was confirmed in the present study, deprives it of access to pancreatic hormones until after recirculation by way of the hepatic artery or systemic venous blood, and subjects the whole organ more or less continuously to endogenous epinephrine. Since Sutherland and Rall (53) and Murad and his associates (33)

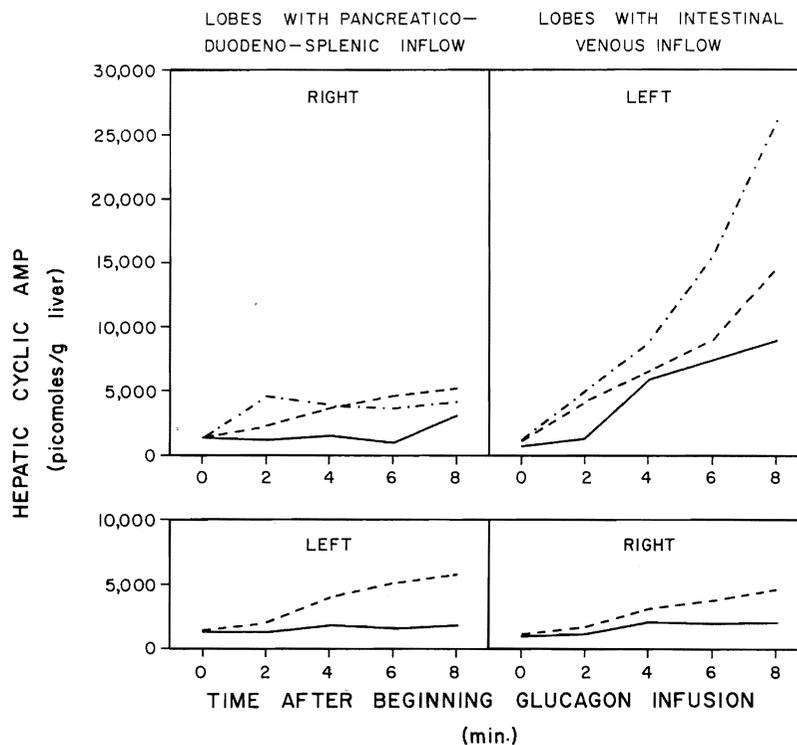


FIG. 15. Tolbutamide-glucagon infusion tests in five dogs two months after splanchnic flow division. In the two experiments in which pancreatic-gastroduodenal splenic blood passed to the left lobes, group 2b, there were no significant differences in the hepatic cyclic 3', 5'-adenosine monophosphate concentrations on the two sides of the liver. However, in the three dogs of group 2a, top, there was a runaway response in the lobes receiving intestinal venous blood compared with a restrained response in the lobes nourished by pancreatic-gastroduodenal-splenic venous blood.

have shown that epinephrine works by the activation of adenylyl cyclase and the consequent formation of cyclic 3', 5'-adenosine monophosphate, the finding of elevated concentrations of cyclic 3', 5'-adenosine monophosphate in the total transposition livers of the dogs in group 3a was consistent with the concept that epinephrine was being excreted in large enough quantities to play a significant role in the deglycogenation. Nevertheless, proof that direct hepatic perfusion by endogenous epinephrine was not the only factor promoting these changes was provided by the experiments of group 3b in which transposition minus the adrenal and renal blood was successfully carried out in two dogs. Falls in hepatic glycogen concentration and rises in cyclic 3', 5'-adenosine monophosphate occurred of almost the same magnitude as in the standard transposition. Here, it might be suggested that adrenal secretions which by-passed the liver were recirculated to the organ after passing through the cardiac mixing chamber and even the peripheral capillary beds. Obvious atrophy was not produced in these

transposition livers with or without direct provision of adrenal venous blood.

In the split transposition experiments of group 1a, increases in cyclic 3', 5'-adenosine monophosphate similar to those caused by a standard transposition were found in the deglycogenated and atrophic lobes of the liver receiving supra-adrenal vena caval blood as compared with the lobes supplied with splanchnic venous blood. In these experiments, the increases of activated glycogen phosphorylase and the decreases in triglyceride concentration in the lobes perfused with vena caval blood were consistent with the metabolic consequences of epinephrine infusion and increased cyclic 3', 5'-adenosine monophosphate, as summarized by Himms-Hagen (22). The fact that the concentration of endogenous epinephrine was physiologically significant was also suggested by some of the portal angiograms in the split transposition experiments which showed relative vasoconstriction in the lobes being perfused by supra-adrenal vena caval blood. Thus, it was not surprising in the

one successful split transposition experiment in group 1b that exclusion of the adrenal and renal venous blood from the lobes supplied by the inferior vena cava curtailed both the rise in cyclic 3', 5'-adenosine monophosphate and the fall of glycogen concentration although atrophy was not thereby prevented. These last findings were in contrast to the observations discussed in the preceding paragraph in the livers of two dogs that had total transposition minus the adrenal and renal blood. The apparent disparities indicated once more that influences other than the adrenal secretions were of importance in regulating cyclic 3', 5'-adenosine monophosphate levels and in determining atrophy or hypertrophy. They focused attention upon the crucial role of the pancreas.

Such a pancreatic role may be assumed to be due to the interactions of glucagon and insulin for which cyclic 3', 5'-adenosine monophosphate also represents a secondary messenger system. Like epinephrine, pancreatic glucagon increases cyclic 3', 5'-adenosine monophosphate as demonstrated by the classical studies of Sutherland and Rall (53), setting in motion multiple chemical processes. Bergen (3) and Weintraub (61) and their associates described the resulting glycogenolysis. Gluconeogenesis has been documented by Exton and Park (14), lipolysis by Butcher and his associates (6), and ketogenesis by Menahan and Wieland (31) as well as by Exton and his group (12).

As reviewed by Exton and Park (13), Sutherland and Robison (54), and Robison and his colleagues (38), insulin promotes many converse metabolic events by depression of the basal level of cyclic 3', 5'-adenosine monophosphate, thus qualifying as an anabolic hormone. Besides aiding glycogen synthesis by the cyclic 3', 5'-adenosine monophosphate mechanism, Salas and his co-authors (40) have shown that insulin supports glycogen metabolism by increasing hepatic glucokinase, and Larner (23) has demonstrated activation of glucose transferase. Consequently, in the liver partition experiments of groups 1 and 2, it was not surprising that glucokinase levels were elevated on the side of pancreas inflow and reduced on the side receiving either vena caval or intestinal flow. Butcher (7) and Robison (38) and their colleagues have shown that insulin regulates lipid synthesis by inactivating lipolytic enzymes through a lowering of cyclic 3', 5'-adenosine monophosphate levels. Insulin also controls protein synthesis by a mechanism that is not understood.

The fact that glucagon and insulin have partially cancelling effects helps explain why the cyclic 3', 5'-adenosine monophosphate levels in liver lobes

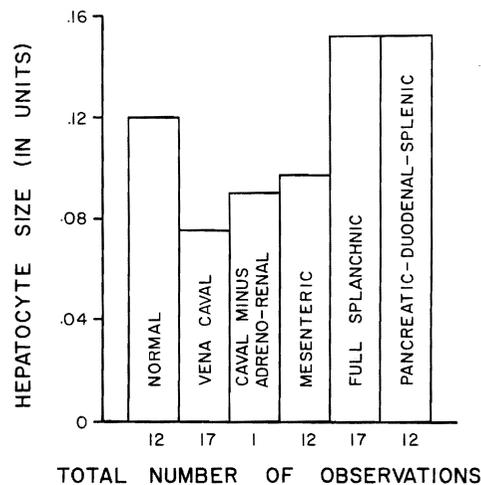


FIG. 16. Summary from the different experiments depicted in Figures 1 and 2 shows the influence of the type of portal inflow upon hepatocyte size. The data are taken from Figures 7 and 8 and are compared with values obtained from the livers of normal dogs. Note that the presence of hypertrophy or atrophy was almost exclusively under the control of pancreatic-gastroduodenal-splenic blood and that the addition or subtraction of blood from other sources was of little further consequence.

with an inflow of pancreatic venous blood tended neither to be very high nor very low. In turn, it helps explain why in the experiments of group 2 significant differences could not be demonstrated between the cyclic 3', 5'-adenosine monophosphate levels of liver lobes receiving pancreatic venous blood versus those receiving the hormone-poor effluent from the small intestine.

The aforementioned biochemical studies provided insight about how various hormones could affect the liver. However, since cyclic 3', 5'-adenosine monophosphate is destroyed so rapidly, mainly by the enzyme cyclic 3', 5'-adenosine monophosphate phosphodiesterase, accurate evaluation of the rate of its production by the simple measures of its tissue concentration was not possible. Consequently, in several special experiments on the dogs of group 1, aminophylline was used to block the phosphodiesterase. The considerable extent to which the rate of cyclic 3', 5'-adenosine monophosphate production was increased in the hepatic tissue exposed to adrenal venous blood could then be determined.

The same kind of information was obtained in the livers of group 2 dogs in which neither hepatic side received adrenal venous blood directly. The aminophylline test uncovered a striking difference between the two sets of liver lobes. The rate of cyclic 3', 5'-adenosine monophosphate production was retarded in the liver lobes receiving blood from

the intestine but was essentially normal in the lobes receiving pancreatic-gastroduodenal-splenic blood. It might be suggested that the lobes receiving blood from the intestines in such experiments should have higher cyclic 3', 5'-adenosine monophosphate levels if there were an unopposed presence of intestinal glucagon. However, Unger (58) and Valverde (59) and their associates have shown that this substance which has some physiologic qualities similar to pancreatic glucagon and which in addition cross reacts with antiglucagon antibodies does not affect the level of hepatic cyclic 3', 5'-adenosine monophosphate when injected into the portal circulation of the dog or into the perfusate nourishing an isolated rat liver.

The role of insulin in controlling the level of cyclic 3', 5'-adenosine monophosphate was further documented by the tolbutamide-glucagon infusion test. In this experiment, measurable increases in endogenous portal insulin were caused by an injection of tolbutamide. This was followed by the intravenous administration of exogenous glucagon. In all the experiments performed, there was a striking difference in the production of cyclic 3', 5'-adenosine monophosphate between the liver sides according to the type of blood supply, indicating once again a profound metabolic dissociation. Hepatic lobes perfused with pancreatic blood and, thus, under insulin influence had modest or no rises in cyclic 3', 5'-adenosine monophosphate in spite of the exogenous glucagon whereas the other lobes had uninhibited increases.

The data accumulated in this study leave little doubt that the previously nebulous hepatotrophic factors in splanchnic venous blood are hormones and that the major anabolic hormone is insulin counterbalanced to an unknown degree by the opposing effects of glucagon and possibly epinephrine as well. The interrelationship of these hormones determines the chemical environment of the liver thereby influencing its function and structure. In most of the experiments herein reported, the competing liver fragment preparation developed during research on auxiliary hepatic transplantation was the experimental tool whereby the metabolic dissociation and morphologic changes caused by different hormone exposure easily could be exposed and examined. But the findings in this special experimental model have major implications in normal as well as abnormal hepatic physiology.

For example, the reason for the liver atrophy caused in animals by the performance of Eck fistula obviously involves hormone deprivation. Explanations of a similar kind can be easily formulated for

other portoprival states, including those caused in man by portacaval anastomosis of a portal vein which has significant residual hepatopetal flow. Why portal diversion procedures have been of such great value for children with certain of the glycogen storage diseases (52) becomes evident. Even in attempts at liver preservation, it may be suggested that long term conservation by perfusion is not apt to be successful unless the chemical and hormonal constituency of the perfusate is made to simulate at least some of the features of portal blood.

Although numerous other examples could be cited of the potential importance of hormonal mechanisms affecting the liver, attention will be focused only upon the virtual certainty that such hormonal factors are central to an understanding of hepatic regeneration. The way in which the portal hormone constituents can direct liver regeneration will require further detailed study from which clarification of some confusing past claims should emerge. A number of authorities on hepatic regeneration, including Sigel (48), Fisher (18), and Price (35) and their colleagues, have suggested that hypertrophy and hyperplasia are dissociated phenomena during regeneration. Price and his associates (35) have stated that there actually is a reciprocal relationship between hypertrophy and hyperplasia. The latter authors believe that hypertrophy is controlled by endogenous glucagon, and that hyperplasia is determined by some factor released by the liver itself.

Almost all the experiments purporting to show a dissociation between hepatocyte hypertrophy and hyperplasia have involved studies of deoxyribonucleic acid or ribonucleic acid synthesis or cell mitosis counts within a few hours or days after a major traumatic procedure. In our own studies which permitted chronic observations, hypertrophy and hyperplasia occurred together, although not necessarily in absolute parallel, in hepatic fragments supplied by either total splanchnic blood or blood from organs in the upper part of the abdomen whereas atrophy and the absence of hyperplasia were found in liver tissue deprived of the appropriate splanchnic influence. These observations indicate that hypertrophy and hyperplasia are associated rather than dissociated, a point of view more in accord with the majority of regeneration studies which were recently reviewed by Bucher (5).

The findings are also consistent with the hypothesis that the hormonal hepatotrophic substances which have been the subject of this article are, in fact, the essential control factors of or at least play a vital role in hepatic regeneration.

SUMMARY

The origin of hepatotrophic factors in splanchnic venous blood was investigated by modifying the portal venous inflow to different parts of the canine liver while leaving the arterial blood supply and biliary drainage intact.

In one variety of experiment, termed partial transposition, the liver portion perfused with the total splanchnic venous blood underwent weight gain and hepatocyte hypertrophy, hyperplasia, and glycogenation compared with the portion perfused with venous blood from the hindquarters, kidneys, and adrenal glands, but the combined weight of the total liver remained constant in spite of the rapidly evolving regional disproportions. The lobar changes were well developed within one to two months. At this time, the hepatic lobes supplied with splanchnic venous blood had higher concentrations of glucokinase and lower concentrations of cyclic 3', 5'-adenosine monophosphate and active phosphorylase than the lobes receiving hindlimb and adrenorenal venous blood, indicating that the biochemical environment of the different liver regions was drastically different by virtue of being under specific hormonal control.

The dissociation was even more dramatically illustrated by dynamic studies in which the destruction of cyclic 3', 5'-adenosine monophosphate by phosphodiesterase was blocked with aminophylline thereby permitting estimation of the rate of formation of cyclic 3', 5'-adenosine monophosphate. In addition, the modifying effect of tolbutamide-induced endogenous insulin upon exogenously administered glucagon was evaluated by serial determinations of cyclic 3', 5'-adenosine monophosphate. These investigations with the aminophylline and tolbutamide-glucagon tests demonstrated the anabolic role of insulin and the opposing roles of both glucagon and epinephrine in contributing to liver homeostasis. Epinephrine and glucagon caused striking increases in cyclic 3', 5'-adenosine monophosphate, and insulin had the converse effect.

Another type of preparation involving partition of the splanchnic venous blood between the liver portions was termed splanchnic flow division. The substances responsible for the hepatic hypertrophy, hyperplasia, glycogenation, and weight gain were shown to emanate mainly, if not virtually exclusively, from the pancreatic-gastroduodenal-splenic venous drainage. In contrast, intestinal nutritional substrate and hormones from the intestine or adrenal gland were not profoundly influential in either promoting or preventing the morphologic or glycogen concentration changes. The concentrations of

cyclic 3', 5'-adenosine monophosphate, phosphorylase, and glucokinase in the two sides of the liver did not follow as distinctive a pattern as in the partial transposition experiments. However, the aminophylline and tolbutamide-glucagon tests revealed the same type of major dissociation of cyclic 3', 5'-adenosine monophosphate as with the partial transpositions. Particularly impressive was the way in which trace doses of tolbutamide-induced endogenous insulin on the side nourished by pancreatic venous blood restrained the cyclic 3', 5'-adenosine monophosphate response to exogenous glucagon, whereas the other liver fragment which was not so covered by insulin had completely uninhibited rises in cyclic 3', 5'-adenosine monophosphate.

The conclusion from these experiments is that the hepatotrophic factors previously reported from our laboratories and by other investigators to be in splanchnic venous blood are pancreatic hormones and specifically insulin and glucagon. Of these, insulin is anabolic and glucagon is mainly catabolic but not exclusively so, since glucagon also has the anabolic effect of stimulating gluconeogenesis. The insulin-glucagon relationship and the interrelationship of these hormones to others, such as epinephrine, in the moment to moment regulation of nutrient and hepatic homeostasis is a central fact of liver physiology that should reconcile a number of previously divergent opinions about portoprival syndromes, mechanisms of hepatic atrophy and hyperplasia, and the control of liver regeneration.

REFERENCES

1. ALBANO, O., and FRANCAVILLA, A. Effect of CCl_4 intoxication in the synthesis of β -lipoprotein fractions in rat liver. *Clin. Chim. Acta*, 1968, 19: 363.
2. BARRON, E. J., and HANAHAN, D. J. Observations on the silicic acid chromatography of the neutral lipides of rat liver, beef liver, and yeast. *J. Biol. Chem.*, 1958, 231: 493.
3. BERGEN, S. S., HILTON, J. G., and VAN ITALLIE, T. B. Glycogenolytic effect of adenosine 3'-5'-monophosphate in the canine liver. *Endocrinology*, 1966, 79: 1065.
4. BLOOM, W. L., LEWIS, G. T., SCHUMPERT, M. Z., and SHEN, T.-M. Glycogen fractions of liver and muscle. *J. Biol. Chem.*, 1951, 188: 631.
5. BUCHER, N. L. R. Experimental aspects of hepatic regeneration. *N. Engl. J. Med.*, 1967, 277: 686.
6. BUTCHER, R. W., BAIRD, C. E., and SUTHERLAND, E. W. Effects of lipolytic and antilipolytic substances on adenosine 3'-5'-monophosphate levels in isolated fat cells. *J. Biol. Chem.*, 1968, 243: 1705.
7. BUTCHER, R. W., SNEYD, J. G. T., PARK, C. R., and SUTHERLAND, E. W. Effect of insulin on adenosine 3'-5'-monophosphate in the rat epididymal fat pad. *J. Biol. Chem.*, 1966, 241: 1651.
8. BUTCHER, R. W., and SUTHERLAND, E. W. Adenosine 3'-5'-phosphate in biological materials—I, purification and properties of cyclic 3'-5'-nucleotide phosphodiesterase and use of this enzyme to characterize adenosine 3'-5'-phosphate in human urine. *J. Biol. Chem.*, 1962, 237: 1244.

9. CHANDLER, J. G., LEE, S., KRUBEL, R., and others. The roles of inter-liver competition and portal blood in regeneration of auxiliary liver transplants. *Surg. Forum*, 1971, 22: 341.
10. CHEUNG, W. Y. Properties of cyclic 3'-5'-nucleotide phosphodiesterase from rat brain. *Biochemistry*, 1967, 6: 1079.
11. CHILD, C. G., BARR, D., HOLSWADE, G. R., and HARRISON, C. S. Liver regeneration following portacaval transposition in dogs. *Ann. Surg.*, 1953, 138: 600.
12. EXTON, J. H., CORBIN, J. G., and PARK, C. R. Control of gluconeogenesis in liver—IV, differential effects of fatty acids and glucagon on ketogenesis and gluconeogenesis in the perfused rat liver. *J. Biol. Chem.*, 1969, 244: 4095.
13. EXTON, J. H., and PARK, C. R. The role of cyclic AMP in the control of liver metabolism. *Adv. Enzyme Regul.*, 1968, 6: 391.
14. Idem. Control of gluconeogenesis in liver—III, effect of L-lactate, pyruvate, fructose, glucagon, epinephrine and adenosine 3'-5'-monophosphate on gluconeogenesis intermediates in the perfused rat liver. *J. Biol. Chem.*, 1969, 244: 1424.
15. FISHER, B., FISHER, E. R., and LEE, S. Experimental evaluation of liver atrophy and portacaval shunt. *Surg. Gynecol. Obstet.*, 1967, 125: 1253.
16. FISHER, B., FISHER, E. R., and SAFFER, E. Investigations concerning the role of a humoral factor in liver regeneration. *Cancer Res.*, 1963, 23: 914.
17. FISHER, B., RUSS, C., UPDEGRAFF, H., and FISHER, E. R. Effect of increased hepatic blood flow upon liver regeneration. *Arch. Surg.*, 1954, 69: 263.
18. FISHER, B., SZUCH, P., and FISHER, E. R. Evaluation of a humoral factor in liver regeneration utilizing liver transplants. *Cancer Res.*, 1971, 31: 322.
19. GILMAN, A. G. A protein binding assay for adenosine 3'-5'-cyclic monophosphate. *Proc. Natl. Acad. Sci. U. S. A.*, 1970, 67: 305.
20. HALGRIMSON, C. G., MARCHIORO, T. L., FARIS, T. D., and others. Auxiliary liver homotransplantation; effect of host portacaval shunt. *Arch. Surg.*, 1966, 93: 107.
21. HENRY, R., SOBEL, C., and BECKMAN, S. Interferences with biuret methods for serum proteins. *Anal. Chem.*, 1957, 29: 1491.
22. HIMMS-HAGEN, J. Sympathetic regulation of metabolism. *Pharmacol. Rev.*, 1967, 19: 367.
23. LARNER, J. Hormonal and nonhormonal control of glycogen metabolism. *Trans. N. Y. Acad. Sci.*, 1966, 29: 192.
24. LEE, S., and EDGINGTON, T. S. Liver transplantation in the rat. *Surg. Forum*, 1966, 17: 220.
25. LEE, S., KEITER, J. E., ROSEN, H., and others. Influence of blood supply on regeneration of liver transplants. *Surg. Forum*, 1969, 20: 369.
26. MACKENZIE, C. G., MACKENZIE, J. B., and REISS, O. K. Regulation of cell lipid metabolism and accumulation—III, the lipid content of mammalian cells and the response to the lipogenic activity of rabbit serum. *Exp. Cell Res.*, 1964, 36: 533.
27. MANN, F. C. Restoration and pathological reactions of the liver. *J. Mount Sinai Hosp. N. Y.*, 1944, 11: 65.
28. MARCHIORO, T. L., PORTER, K. A., BROWN, B. I., and others. The effect of partial portacaval transposition on the canine liver. *Surgery*, 1967, 61: 723.
29. MARCHIORO, T. L., PORTER, K. A., DICKINSON, T. C., and others. Physiologic requirements for auxiliary liver homotransplantation. *Surg. Gynecol. Obstet.*, 1965, 121: 17.
30. MARCHIORO, T. L., PORTER, K. A., ILLINGWORTH, B. I., and others. The specific influence of non-hepatic splanchnic venous blood flow upon the liver. *Surg. Forum*, 1965, 16: 280.
31. MENAHAN, L. A., and WIELAND, O. The role of endogenous lipid in gluconeogenesis and ketogenesis of perfused rat liver. *Eur. J. Biochem.*, 1969, 9: 182.
32. MORGAN, C. R., and LAZAROW, A. Immunoassay of insulin; two antibody system—plasma insulin levels of normal, subdiabetic and diabetic rats. *Diabetes*, 1963, 12: 115.
33. MURAD, F., CHI, Y., RALL, T. W., and SUTHERLAND, E. W. Adenyl cyclase—III, the effect of catecholamines and choline esters on the formation of adenosine 3'-5'-phosphate by preparations from cardiac muscle and liver. *J. Biol. Chem.*, 1962, 237: 1223.
34. POUYET, M., BERARD, Ph., RUCKEBUSCH, Y., and others. Derivations portohepatiques selectives origine pancreatique du facteur hepatotrophique portal. *Ann. Chir.*, 1969, 23: 393.
35. PRICE, J. B., JR., TAKESHIGE, K., MAX, M. H., and VOORHEES, A. B., JR. Glucagon as the portal factor modifying hepatic regeneration. *Surgery*, 1972, 72: 74.
36. PRICE, J. B., JR., VOORHEES, A. B., JR., and BRITTON, R. C. The role of portal blood in regeneration and function of completely revascularized partial hepatic autografts. *Surgery*, 1967, 62: 195.
37. RANSON, J. H. C., GARCIA-MORAN, M., BECKER, F., and LOCALIO, S. A. Influence of the source of portal vein blood on dog auxiliary hepatic allograft function in recipients with hepatic insufficiency. *Surg. Forum*, 1971, 22: 345.
38. ROBISON, G. A., BUTCHER, R. W., and SUTHERLAND, E. W. Cyclic AMP. New York: Academic Press, 1971.
39. ROUS, P., and LARIMORE, L. D. Relation of the portal blood to liver maintenance; a demonstration of liver atrophy conditional on compensation. *J. Exp. Med.*, 1920, 31: 609.
40. SALAS, M., VINUELA, E., and SOLS, A. Insulin-dependent synthesis of liver glucokinase in the rat. *J. Biol. Chem.*, 1963, 238: 3535.
41. SCHNEIDER, W. C., and HOGEBOM, G. H. Intracellular distribution of enzymes—V, further studies on the distribution of cytochrome C in rat liver homogenates. *J. Biol. Chem.*, 1950, 183: 123.
42. SEIFTER, S., SEYMOUR, D., NOVIC, B., and MUNTWYLER, E. The estimation of glycogen with the anthrone reagent. *Arch. Biochem.*, 1950, 25: 191.
43. SEXTON, A. W., MARCHIORO, T. L., WADDELL, W. R., and STARZL, T. E. Liver deglycogenation after portacaval transposition. *Surg. Forum*, 1964, 15: 120.
44. SHIMAZU, T., and AMAKAWA, A. Regulation of glycogen metabolism in liver by the autonomic nervous system—II. *Biochem. Biophys. Acta*, 1968, 165: 335.
45. SHIMAZU, T., and FUKADA, A. Increased activities of glycogenolytic enzymes in liver after splanchnic nerve stimulation. *Science*, 1965, 150: 1607.
46. SIEKEVITZ, P. Uptake of radioactive alanine *in vitro* into the proteins of rat liver fractions. *J. Biol. Chem.*, 1952, 195: 549.
47. SIGEL, B., BALDIA, L., and DUNN, M. R. Studies of liver lobes autotransplanted outside the abdominal cavity. *Surg. Gynecol. Obstet.*, 1967, 124: 525.
48. SIGEL, B., BALDIA, L. B., MENDUKE, H., and FEIGL, P. Independence of hyperplastic and hypertrophic responses in liver regeneration. *Surg. Gynecol. Obstet.*, 1967, 125: 95.
49. SIGEL, B., DUNN, M. R., and BUTTERFIELD, J. Effect of partial hepatectomy and Eck fistula on autotransplanted liver tissue. *Surg. Forum*, 1963, 14: 72.
50. STARZL, T. E. (with PUTNAM, C.W.). Metabolic considerations of auxiliary transplantation in animals. In: *Experience in Hepatic Transplantation*. Pp. 475-491, Philadelphia: W. B. Saunders Co., 1969.
51. STARZL, T. E., MARCHIORO, T. L., ROWLANDS, D. T., JR., and others. Immunosuppression after experimental and clinical homotransplantation of the liver. *Ann. Surg.*, 1964, 160: 411.
52. STARZL, T. E., MARCHIORO, T. L., SEXTON, A. W., and others. The effect of portacaval transposition on carbohydrate metabolism; experimental and clinical observations. *Surgery*, 1965, 57: 687.

53. SUTHERLAND, E. W., and RALL, T. W. The relation of adenosine-3', 5'-phosphate and phosphorylase to the actions of catecholamines and other hormones. *Pharmacol. Rev.*, 1960, 12: 265.
54. SUTHERLAND, E. W., and ROBISON, G. A. The role of cyclic AMP in the control of carbohydrate metabolism. *Diabetes*, 1969, 18: 797.
55. TAKAHASHI, T. Inorganic phosphate determination. *Tampa Kushitsu Kakusar Koso* (Tokyo), 1957, 2: 464.
56. THOMFORD, N. R., SHORTER, R. G., and HALLENBECK, G. A. Homotransplantation of the canine liver. *Arch. Surg.*, 1965, 90: 527.
57. TRETBAR, L. L., BEVEN, E. G., and HERMANN, R. E. The effects of portacaval shunt and portal flow occlusion on canine auxiliary liver homotransplants. *Surgery*, 1967, 61: 733.
58. UNGER, R. H., OHNEDA, A., VALVERDE, I., and others. Characterization of the responses of circulating glucagon-like immunoreactivity to intraduodenal and intravenous administration of glucose. *J. Clin. Invest.*, 1968, 47: 48.
59. VALVERDE, I., RIGOPOULOU, D., EXTON, J., and others. Demonstration and characterization of a second fraction of glucagon-like immunoreactivity in jejunal extracts. *Am. J. Med. Sci.*, 1968, 255: 415.
60. WASTILA, W. B., STULL, J. T., MAYER, S. E., and WALSH, D. A. Measurement of cyclic 3', 5'-adenosine monophosphate by the activation of skeletal muscle protein kinase. *J. Biol. Chem.*, 1971, 246: 1996.
61. WEINTRAUB, B., SARCIONE, E. J., and SOKAL, J. F. Effect of glucagon on phosphorylase activity of the isolated perfused liver. *Am. J. Physiol.*, 1969, 216: 521.