A Portal Blood Factor as the Humoral Agent in Liver Regeneration

Abstract. *It was demonstrated, by use of extracorporeal cross-circulation, that a humoral factor responsible for liver regeneration does not arise from the liver remnant. While intact livers of normal rats incorporated [methyl-^3H]-thymidine in proportion to the amount of liver removed in the partner, the greatest response occurred after a total hepatectomy. Evidence from portacaval-shunted, partially hepatectomized animals connected to normal members indicates that the factor is in portal blood and that the onset of regeneration is the result of a quantitative imbalance between the available portal blood factor and the number of liver cells present.*

After Christensen and Jacobsen (*1*) reported in 1949 that partial hepatectomy of one parabiotic rat resulted in an increase in hepatic mitoses in the intact partner, extensive investigation has been directed toward ascertaining the role of a blood-borne factor in liver regeneration. Since findings have been so conflicting, the importance of such an agent in hepatic restoration has remained uncertain. Recently, however, Moolten and Bucher (*2*), employing extracorporeal cross-circulation techniques, have provided convincing evidence to affirm a humoral mechanism. They observed that synthesis of DNA by parenchymal cells was stimulated in the intact liver of a cross-circulated rat when a partial hepatectomy was performed on the other member of the pair. The response was greater after an 85 percent resection than after a 68 percent one. Such findings differ from those of other investigators (*3*) who used similar methodology, and, to our knowledge, have so far not been confirmed. Moreover, Moolten and Bucher failed to reveal whether results were due to accumulation of a stimulatory blood-borne factor arising from the hepatic remnant or were a consequence of loss of an inhibitor present in intact liver. In order to disclose such information we have likewise utilized pairs of animals maintained in cross-circulation.

Isogeneic Buffalo female rats (3 months old, weighing approximately 200 g) were used. They were denied food but not water from the start of the experiment. Both single and cross-circulated rats were maintained in semirestraining cages without sedation. Either a 70 percent (median and left lateral lobe), an 86 percent (median and left lateral lobes in addition to four-fifths of the right lateral lobe), or a 100 percent hepatectomy was performed. When livers were totally removed, animals were first subjected to end-to-side portacaval shunt to divert the portal blood supply. After ligation of the hepatic artery and common bile duct, a 70 percent hepatectomy was performed. Then the rest of the liver was removed by ligating and resecting the anterior portion of the right lateral lobe followed by removal of the posterior segment of that lobe with the caudate lobe. Animals that had partial liver resections were permitted to recuperate for approximately 90 minutes prior to the start of cross-circulation. Those having total hepatectomies were cross-circulated immediately after surgery.

Cross-circulation was established so that the blood flow was from the left carotid artery of each rat to the right jugular vein of its partner. Cannulas consisting of lengths of polyethylene tubing (PE-50; Clay-Adams) were introduced into the artery and vein of each animal and were connected at the other end by inserting them into a short sleeve of tubing (PE-90) so that the ends of the catheters were in perfect apposition. The cannulas were filled with heparinized saline (70 units of heparin per milliliter) before insertion, and an additional 0.2 to 0.4 ml of heparinized saline was injected through the arterial one after its insertion. All rats received 10 ml of isotonic saline subcutaneously prior to cannulation and 1.5 to 2.0 ml via the venous catheter prior to beginning cross-circulation. Totally hepatectomized animals were infused subcutaneously with 5 percent glucose in saline (0.3 ml/hour) during cross-circulation. Blood flow through the extracorporeal circuit was 2 to 2.5 ml/min, as determined by timing the delivery of 0.5 ml from each arterial catheter at the termination of cross-circulation. At that time blood pressures were monitored with a Sanborn Polyviso, and only when it was shown that both members of a pair were not hypotensive and that pressures coincided (that is, within 20 mm-Hg) were they used. Cross-circulation was maintained for 23 hours between pairs in which one member was totally hepatectomized. When one member was partially hepatectomized, such flow was maintained for 21½ hours before separation of partners.

To study DNA synthesis, [methyl-^{3}H]thymidine (10 μc; specific activity, 3 c/mmole) was injected into the femoral vein of the animals immediately after their separation. All animals were killed 1 hour later (24 hours after hepatectomy) by exsanguination (between 2 and 4 p.m.). The right lateral lobe was used for determination of DNA and preparation of autoradiographs. DNA was extracted from livers with hot 5 percent trichloroacetic acid

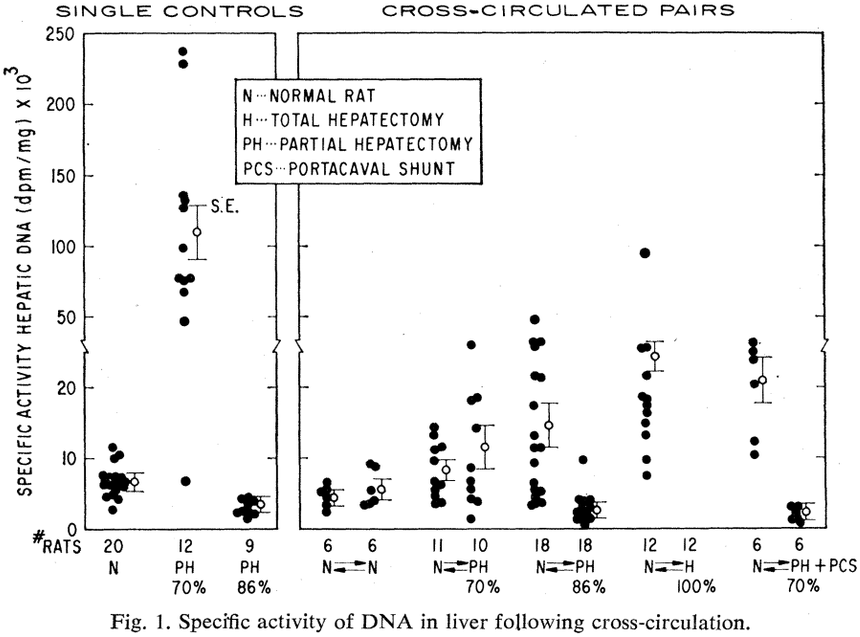

Fig. 1. Specific activity of DNA in liver following cross-circulation.

(*4*) and the extract was assayed for DNA content by the diphenylamine procedure (*5*). Radioactivity was determined with a Packard Tri-Carb scintillation counter, model 3003. Activity is expressed as disintegrations per minute (dpm) per milligram of DNA. Autoradiographs showed that all activity was confined to parenchymal cells.

A statistical analysis of data was carried out by use of a nonparametric procedure. The Wilcoxon two-sample rank test was employed, and values were considered statistically significant at the .05 level of significance.

Seventy percent partial hepatectomy of normal single rats resulted in approximately a 15-fold increase in specific activity of DNA from hepatic parenchymal cells (Fig. 1). After 86 percent hepatectomy in such animals, however, this value was less than in nonhepatectomized single rats, which is in keeping with the observation of Weinbren and Woodward (*6*) that a resection of that magnitude greatly delays hepatic synthesis of DNA. Cross-circulation of rats with intact livers slightly, but statistically significantly, decreased the DNA activity of hepatic cells from that observed in single normal animals. This alteration would seem to be of little, if any, biological significance. The DNA activity of livers of normal members significantly increased in proportion to the amount of liver removed from partners in cross-circulation. After 70 percent hepatectomy, a value slightly greater than that of livers of normal partners was obtained. After 86 percent hepatectomy there was a threefold increase and, following 100 percent removal, almost a fivefold increase of DNA specific activity in intact livers of partners. It is of particular interest that, when one of the partners was subjected to an end-to-side portacaval shunt in addition to a 70 percent hepatectomy, the intact liver in the other member showed almost as great an increase in activity as that observed after total hepatectomy. The 70 percent hepatectomized, shunted livers, however, demonstrated significantly less DNA activity than did livers in nonshunted, 70 percent hepatectomized, cross-circulated animals, which in turn had much less activity than similar livers in non-cross-circulated animals did. The activity in liver remnants after shunt and 70 percent hepatectomy was in fact significantly less than that observed in any other situation.

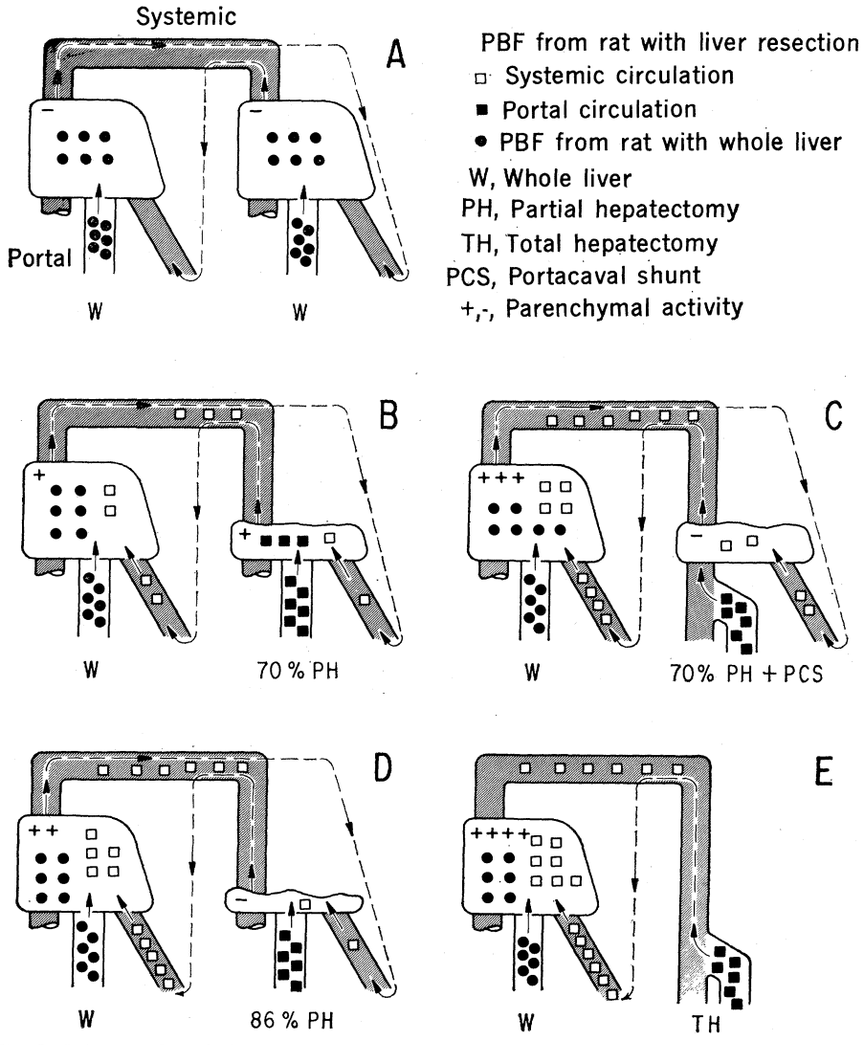

Fig. 2. Scheme of availability of portal blood factor (PBF) for parenchymal cell activity in cross-circulated animals.

Our findings indicate that there is a blood-borne factor that is capable of stimulating DNA synthesis in hepatic parenchymal cells. Since the response was greatest when normal rats were connected by cross-circulation to totally hepatectomized animals with no impairment of their portal flow, it is concluded that such a factor is not the product of a partially hepatectomized liver but arises elsewhere. Results from portacaval-shunted, 70 percent hepatectomized animals have provoked the view that there is a portal blood factor (PBF) that, when diverted away from the partially removed liver into the systemic circulation, becomes more available to the intact liver of the partner and, when added to the PBF normally being produced by the partner, provides an amount adequate to stimulate DNA activity in the intact liver. Such findings indicate that PBF is neither destroyed nor inhibited in systemic blood; they are also in keeping with our previous observations (*7*) that livers in portacaval-shunted, partially hepatectomized single animals responded to PBF in a manner similar to partially hepatectomized livers in nonshunted rats. These findings minimize the consideration that the initiating factor in liver regeneration is the loss of an inhibitor, as hypothesized by Glinos (*8*). The results of this study, in conjunction with those we obtained (*9*) by using auxiliary livers transplanted into isologous rats by means of blood-vessel anastomosis, are more indicative that the initiation of liver regeneration is related to an alteration of the quantitative balance between available PBF and the number of liver cells present.

In this light it is likely that in single animals and cross-circulated pairs with intact livers PBF was "removed" by the liver as portal flow traversed it (Fig. 2A). Its concentration per num-

ber of liver cells was inadequate to produce a response. However, after removal of 70 percent of the liver in the single animal, which resulted in a disturbance of the relationship between the PBF and parenchymal cells, the amount of PBF that reached the liver directly via portal blood and by recycling was adequate to stimulate DNA activity in the remnant. When cross-circulation was maintained between an animal with 70 percent hepatectomy (as well as one with 86 percent hepatectomy) and a normal member, the amount of PBF that would ordinarily return to the partially hepatectomized liver from the systemic circulation was reduced by the quantity reaching the whole liver and being removed by it (Fig. 2, B and D). Such a reduction resulted in a diminished response in the partially hepatectomized liver as compared with the activity of similar livers in single animals, yet the addition of the PBF to that going to the liver via its own portal blood was sufficient to produce some stimulation of the liver cells of the whole liver. The addition of a portacaval shunt to partial hepatectomy in one partner would be expected to divert more of the PBF of that animal to the intact liver of the other member, which would result in a greater parenchymal cell response in the other member, as was the case (Fig. 2C). Since the partially hepatectomized liver had a markedly reduced blood flow, that is, only the flow via its hepatic artery, the amount of PBF available to it was inadequate to effect a response similar to the one that would occur in such a liver in a single animal. Indeed, the DNA activity was less than that observed in intact livers of single or cross-circulated animals. Following total hepatectomy all of the PBF entered the systemic circulation to reach the intact liver of its partner (Fig. 2E). When this PBF was added to the PBF being produced by the partner, a maximum response resulted. Further investigations into the nature, site of origin, mechanism of action, and regulation of concentration of PBF are needed.

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Specialization of Rabbit Reticulocyte Transfer RNA Content for Hemoglobin Synthesis

Abstract. *The amount of acceptance of each amino acid per absorbancy unit of rabbit reticulocyte transfer RNA was determined. The results were compared with the amino acid composition of rabbit hemoglobin and with a similar determination of the transfer RNA content of rabbit liver. The histidine and isoleucine transfer RNA content of reticulocytes is specialized for the synthesis of hemoglobin, in which histidine is unusually common and isoleucine unusually scarce compared to most proteins.*

Reticulocytes, the anucleate precursors of circulating erythrocytes, are among the most specialized cells from the point of view of protein synthesis since 90 percent of the protein made in them is of one kind, hemoglobin (*1*). The amino acid composition of the mammalian hemoglobins has some unusual features (*2, 3*), the most notable being the abundance of histidine, a generally uncommon amino acid, and the scarcity of isoleucine, a generally common amino acid. Isoleucine is entirely absent in many hemoglobins including adult human hemoglobin, and it accounts for very few residues in any mammalian hemoglobin.

Many studies have been done seeking to relate the transfer RNA (tRNA) content of various kinds of cells to biological processes such as differentiation, viral infection, and neoplasia. If tRNA content is specialized to serve the translation of proteins characteristic of particular kinds of cells, then reticulocytes with their unusual protein product should provide one of the most unambiguous animal cell systems for examination. Moreover, globin synthesis is subject to a degree of posttranscriptional regulation in that it is coupled to hemin synthesis and to the availability of iron (*4*). It has long been considered likely that tRNA may have a role in the control of protein synthesis at this level. The possibility that the amount of hemoglobin synthesized is closely dependent on the tRNA content of reticulocytes was first raised by Itano (*5*) to explain the observation that, in heterozygotes for most of the hemoglobinopathies, the protein product of the mutant allele compared to that of the corresponding normal allele is present in circulating red cells in smaller quantities.

We have determined the relative content of tRNA accepting each amino acid for rabbit reticulocytes, and the results are considered in view of the amino acid composition of rabbit hemoglobin as well as in comparison with the tRNA content of rabbit liver. The liver was chosen for this comparison because the variety of proteins it synthesizes for both internal use and for extracellular transport would suggest that it is relatively nonspecialized in its utilization of amino acids and therefore in its tRNA content. Evidence is presented that the histidine and isoleucine tRNA content of reticulocytes is specialized for hemoglobin synthesis.

The relative content of tRNA's accepting each of the amino acids in liver and reticulocytes was determined by measuring the enzymatic aminoacylation of limiting amounts of tRNA with radioactive amino acids (*6*). Assays were performed as described in Table 1. For each amino acid, an optimum Mg^{2+} concentration and an optimum ratio of Mg^{2+} to adenosine triphosphate (ATP) were determined. To avoid spurious results due to contaminated radioactive amino acids, acceptance of each amino acid was first determined in the presence of an approximately tenfold excess of the same amino acid in nonradioactive form. When the acceptance was not depressed to an appropriate level, a mixture of all nonradioactive amino acids in tenfold molar excess except the one being assayed was added. In general, the enzyme preparation used for the assays was from the same source as