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Communications of Liver Cells

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Inter-organ communications: with particular reference to hepatotrophic factors and intrinsic liver growth factors

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In the natural state, the liver obtains more than three-quarters of its blood supply through the portal vein. The possible potential endocrinological significance of this circumstance was barely perceived until recently. Now it is understood that the gastrointestinal tract is (amongst other things) a complex of endocrine foci which contains and can release a dozen or more biologically active polypeptides[1,2] into the portal circulation. From there, these polypeptides are exposed to the liver on first pass, and thus could, at least potentially, influence hepatic structure, function and the capacity for regeneration. There is no question that this happens.

PORTAL BLOOD DEPRIVATION AND THE NON-REGENERATING LIVER

When portacaval shunt (Eck fistula) is performed, the liver which now depends upon its arterial circulation, undergoes major atrophic changes within 3 or 4 days[3]. Ultrastructurally, the most specific changes are depletion and disruption of the rough endoplasmic reticulum and reduction in the membrane-bound ribosomes. In the face of acute atrophy, there is moderate hyperplasia, cell renewal being increased about 3 times[3]. It was for a long time thought that these changes were caused by the decreased quantity of the total hepatic blood flow[4]. However, evidence which was at first circumstantial[5,6] and later direct[3] has shown that the most important loss was not the volume of portal flow but some substance(s) within the portal blood that could not be replaced by equal volumes of systemic blood.

The single most important ingredient of portal blood proved to be insulin. The crucial experiment was exceptionally simple[3]. After complete portacaval shunt in dogs, non-hypoglycaemic doses of insulin were infused into the tied-off left portal vein. The acute atrophy and ultrastructural deterioration normally caused by Eck fistula was greatly reduced in those lobes supplied by the left portal vein. There was no spillover effect to the non-infused right liver

lobes. An additional effect of the insulin was major hepatocyte hyperplasia which was of the magnitude of a regeneration response after hepatectomy. Infusions of glucagon alone or added to insulin had no demonstrable effect.

The striking morphological consequences of portal diversion are accompanied by major, although subtle, changes in function. Because they have clinical significance, much attention has been paid to the consequent alterations in lipid metabolism. Hepatic cholesterol and triglyceride synthesis are markedly diminished by portacaval shunt in animals[7] and humans[8] and there is a coincidental fall in serum cholesterol[7]. Many patients with idiopathic Type II hyperlipidaemia have now been treated by portacaval shunt, and usually with a striking fall of serum cholesterol and low-density lipoprotein[9]. The complex metabolic changes after portal diversion have also been exploited as a palliative to patients with glycogen storage disease[10].

There is the possibility that the liver damage of α_1-antitrypsin deficiency can be slowed by portacaval shunt. In this disorder, the abnormal α_1-antitrypsin elaborated by the liver cannot be excreted and thus accumulates in the hepatocytes. Two of our paediatric patients have been followed for $1\frac{1}{2}$ and $3\frac{1}{4}$ years following portacaval shunt. Both have had stabilization of their previously deteriorating clinical state. In one, serial liver biopsies have demonstrated by semi-quantitative organelle analyses a progressive diminution of the α_1-antitrypsin within hepatocytes. Our assumption is that portacaval shunt diminished the synthesis of the glycoprotein (presumably by altering the rough endoplasmic reticulum and polyribosomes) without commensurately reducing the transport of this α-protein.

PORTAL FACTORS AND LIVER REGENERATION

We turn now to the influence of portal blood factors upon hepatic regeneration. It is clear that regeneration cannot proceed normally in the absence of non-hepatic splanchnic viscera. But which of the splanchnic organs is the touchstone for liver renewal? In dogs submitted to excision of all the non-hepatic splanchnic organs, no regeneration whatever can be seen, at least within the first 2 or 3 days after concomitant partial hepatectomy[11]. The loss of the pancreas with the evisceration is not the entire explanation. When only total pancreatectomy is performed at the time of partial hepatectomy, a subdued but easily identifiable regeneration proceeds in the diabetic dogs[11]. The mirror-image procedure in which all the viscera except the pancreas are removed also markedly inhibits, but does not abolish, regeneration[11]. The evidence thus suggests that several splanchnic organs contribute multiple growth factors, and that the pancreas, albeit important, is far from the only source of regenerative factors.

The essential role of the so-called portal hepatotrophic factors (including insulin) in liver regeneration seems indisputable. However, the controversial issue is whether these factors merely permit regeneration to go forward, or whether changes in these factors actually initiate regeneration by biochemical pathways involving mainly adenyl cyclase activation and deactivation. MacManus and his associates of Canada[12] have produced evidence in tissue culture systems and after partial hepatectomy in rats which is compatible with

cellular growth control by hormone interactions. Early biphasic rises in cyclic AMP with a return towards normal as DNA synthesis begins have been confirmed in rats[13,14] and in dogs[15]. The experiments of Short, Gaza and Lieberman[16–18] are also compatible with the hormone control hypothesis.

However, the hypothesis has not been proved. Attacks on the hormone control theory of liver regeneration have been frequent but indecisive. An example is a recent paper by Price and his associates[19]. In cross-circulated rats, they removed all of the intra-abdominal viscera, including the liver, from one animal and observed liver regeneration in the normal partner. Their conclusion was that they had disproved the splanchnic initiation of regeneration. The experimental design was flawed. Conceptually, what had been performed was a technically complicated 50% hepatectomy whereby two animals with a shared circulation had half of their combined liver mass removed. The other liver half, which was now essentially a fragment with an intact splanchnic organ–liver axis, regenerated vigorously.

INTRINSIC LIVER GROWTH FACTORS

The issue of portal hepatotrophic control of liver regeneration thus remains unsettled. An additional possibility is that something in the liver fragment after hepatectomy can contribute to, or even initiate, its own regrowth. Recent work from our laboratories[20] and by LaBrecque and Pesch[21] has raised interesting questions about this concept. By ultracentrifugation at 140 000 *g*, we made canine hepatic cytosol extracts that were free of cell membranes, organelles and viruses. Such cytosol contains proteins and other constituents that are soluble, but no insulin and very little glucagon. The cytosol was tested in the same Eck fistula model described earlier by injecting it as a 4–6 h bolus into the tied-off left portal vein, just after completion of a portacaval shunt.

The results were unequivocal[20]. Cytosol from normal adult livers had no effect upon the events that follow Eck fistula, nor did the cytosol prepared from liver fragments remaining after 72% hepatectomy performed 1 day earlier. However, 2-day regenerating liver changed the pattern of results and 3-day regenerating liver was even more potent. In the directly infused left liver lobes the active cytosol extracts initiated a burst of regeneration which had a delayed onset. The increased mitoses were not clearly seen until 2 days after the pulse of cytosol had been given, with a response that was doubled again at 3 days and almost completely confined to the directly treated lobes[22].

Proliferation was not the only cytosol effect. The atrophy characteristic of Eck fistula proceeded for 2 days *after* the delivery of the active liver extracts, but between the second and third days there was a dramatic reversal. Now the hepatocytes returned to normal size at the same time as other structural and ultrastructural features of these cells were restored to or towards normal[20]. The result at 3 days was a liver in which healthy hepatocytes in the left but not the right liver lobes were undergoing changes indistinguishable from vigorous regeneration.

The cytosol has been tested in another canine system. Dogs were submitted to 44% hepatectomy, a procedure which in otherwise unaltered animals

evokes a moderate regeneration. Cytosol from 48 and 72 h regenerating livers was given intraportally over a 6 h period to the test dogs immediately after completion of their 44% hepatectomies. The expected regeneration response was significantly amplified by the cytosol from regenerating livers. No change in the expected response was caused either by cytosol from normal dog liver or by a 6 h infusion of intraportal insulin. When given intraportally to normal dogs, the active cytosols did not cause any change in the baseline mitotic index.

It seems clear that regenerating livers come to possess some kind of stimulatory growth factor. The significance of this growth factor is by no means certain, nor is its relationship to the so-called portal hepatotrophic factors. Does this stimulatory liver factor merely reflect the fact that regeneration is going on? Is its development dependent upon input of the so-called portal hepatotrophic factors? And does it influence regeneration in the liver fragment in which it resides? Experiments are in progress to answer such questions. However, a real understanding of this liver growth factor will be dependent upon its isolation and biochemical characterization.

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