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Insulin, Glucagon, and the Control of Hepatic Structure, Function, and Capacity for Regeneration

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WE WISH to present evidence that hormones (especially insulin) that are released by the splanchnic organs into the portal venous system can influence the liver in many ways that were only vaguely suspected until recently.

If the liver is deprived of the hormone-rich portal venous blood, it becomes abnormal. The most extreme portaprival state is when all the splanchnic venous return is diverted around the liver via a portacaval shunt (Eck fistula), leaving the liver with only an arterial supply. Dogs with Eck fistula develop weight loss, liver atrophy, and hepatic encephalopathy.

Hepatocyte atrophy, fatty infiltration, and other changes caused in the liver by Eck fistula are easily perceived by light microscopy in the dog and in all other species so far studied, including man. Ultrastructurally, the most specific alterations are depletion and disruption of the rough endoplasmic reticulum and reduction in the membrane bound polyribosomes.[1] These and other changes are about 90% complete within 4 days after portal diversion.[2]

CONTRIBUTIONS FROM AUXILIARY LIVER TRANSPLANTATION

The reasons for these striking changes began to unfold about 13 yr ago with experiments in dogs to define the necessary conditions for auxiliary liver transplantation. If the extra canine liver was deprived of splanchnic blood, it promptly underwent severe shrinkage even though the lost portal flow was replaced with equal volumes of systemic blood.[3] Conversely, if the graft was given the splanchnic venous return, the acute atrophy now affected the native liver.[4] The organ with first access to the splanchnic venous blood apparently was efficiently extracting something (later work has shown this to be mainly insulin), the absence of which was profoundly damaging to the second organ.

THE USE OF PARTIAL (SPLIT) PORTACAVAL TRANSPOSITION

The same effect has been well documented in a nontransplant model which has been termed a split or partial transposition which in effect divides the dog

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liver into two fragments. With this operation, splanchnic venous blood goes to one portal branch of the liver, whereas the other portal branch is detached and supplied with systemic blood from the inferior vena cava. The *quantity* of flow was measured in many of these experiments and generally found to be actually greater on whichever side was perfused by vena caval blood;[6] yet, atrophy was seen on that side. The side receiving splanchnic venous return had dramatic hypertrophy.

The two liver sides had other easily quantifiable differences in experiments in which the splanchnic venous blood went to the right lobes for 60 days.[7] The right or splanchnic fed lobes had more glycogen and glucokinase and lower concentrations of cyclic AMP and active phosphorylase. The biochemical dissociation was shown in many other ways[7] that will not be detailed here. But a reasonable generalization was that the two liver sides were living in different metabolic worlds in which hormone control, especially that by insulin, played the dominant role.

The significance of the pancreatic hormones in these differential effects was further studied in partial transposition experiments in which some of the dogs were made diabetic with alloxan or by total pancreatectomy and then treated with subcutaneous insulin.[8] The exogenous insulin now would be distributed without obvious preference to both sides. The right lobes were receiving the total splanchnic venous return and the left had systemic blood.

An exquisitely accurate way to measure liver cell atrophy was developed for such experiments. With light microscope tracing, hepatocytes were drawn on a standard thickness paper and weighed. The weights were called size units. These measures correlated well with the true size of single cells as measured directly with planimetry and other techniques. The cell size data could then be summarized in graphs and by other methods.

In the split transposition experiments which ran for 60 days, the hepatocytes in the right lobes of nondiabetic dogs receiving the total splanchnic venous return were twice as large as their left-sided companions receiving vena caval blood. The cell size advantage was lost by the superimposition of alloxan diabetes, or of total pancreatectomy.

In nondiabetic dogs these same right lobes receiving the total splanchnic blood also had a higher rate of cell mitosis as measured by autoradiography, and both sides were higher than normal. The right-sided advantage was only partly removed by alloxan and pancreatectomy diabetes.[8] These dogs were being treated with subcutaneous insulin which was distributed to both sides. We think the residual difference in right and left hepatocyte proliferation with retention of some right-sided advantage even after diabetes represented an influence on cell renewal of factors other than insulin, a point to which we will return later.

SPLANCHNIC DIVISION EXPERIMENTS

Eventually, another kind of double fragment model provided much more decisive information.[7] In these experiments, one portion of the liver was fed by the effluent of hormone-rich blood returning from the pancreas, duodenum, stom-

ach and spleen. The opposite lobes were perfused via a graft with nutrition-rich blood returning from the intestine.

The morphological results in 60-day experiments were dramatic. The lobules receiving pancreaticoduodenal venous effluent became big compared to those perfused with nutrient-rich intestinal blood. The individual hepatocytes on that side were strikingly bigger, had evidence of hyperplasia, and contained much glycogen compared with the cells on the other side. Differences in chemical composition were also noted.

The probability that insulin was the major cause for the differences between the two sides was strengthened by additional 60-day experiments, in which alloxan diabetes and pancreatectomy were superimposed upon splanchnic division.[8] In these dogs, pancreaticoduodenogastrosplenic blood was directed to the right lobes and intestinal blood to the left lobes. In nondiabetic dogs after splanchnic division, the liver cells in the hormone enriched right lobes became hypertrophic as expected; the left lobes atrophied. These effects were cancelled about equally by alloxan diabetes or pancreatectomy. In all such experiments, the nearly equal effects of alloxan poisoning and pancreatectomy have tended to minimize any major role of glucagon as a hepatotrophic factor. We emphasize again that these diabetic animals had to be treated with insulin which was delivered to both hepatic sides.

The insulin effect on cell proliferation was also convincingly unmasked.[8] In nondiabetic animals with splanchnic division, the right liver lobes receiving pancreatic blood had unequivocal autoradiographic evidence of hepatocyte hyperplasia relative to the left lobes, although both sides had greater cell renewal than normal. The right lobar dominance was eliminated, indeed it was transferred to the left side by either alloxan or pancreatectomy diabetes in the insulin treated animals.

Shifts of DNA synthesis were also brought about by diabetes.[8] In nondiabetic dogs after splanchnic division, the right lobes which were perfused with pancreatic blood had the dominant DNA synthesis. This dominance changed over to the left with the addition of either treated alloxan or pancreatectomy diabetes.

The effects of these various manipulations on lipid metabolism were also marked.[9] In normal unaltered dogs, cholesterol synthesis was equal on both liver sides. After splanchnic division in nondiabetic animals, the liver portion being perfused with blood from the pancreas and upper splanchnic organs had a much greater cholesterol synthesis than the other liver portion being perfused with venous return from the intestine. This advantage of cholesterol synthesis was eliminated, and reversed, both by alloxan diabetes and total pancreatectomy in insulin treated dogs. Triglyceride synthesis followed the same pattern.

Although the foregoing remarks are based on 60-day splanchnic division experiments, it should be added that most of the described changes have now been shown in this same model (and also after partial transposition) to be nearly complete within 4 days.[10] The 4-day experiments convincingly confirmed the role of insulin in controlling both cell size and cell proliferation in the acute regeneration that follows hepatic resections. A prominent role for glu-

cagon could not be identified in either the 4-day experiments or in the 60-day ones.[10]

THE ECK FISTULA

If the liver disease of the Eck fistula were caused by deprivation of the liver of direct access to endogenous insulin, as all our studies suggested, it should be possible to minimize these changes if nonhypoglycemic infusions of insulin with or without glucagon were made into the tied off left portal vein after portacaval shunt. Study of the uninfused right lobes should reveal if there was any spillover therapeutic effects on these right lobes. Such a study was performed over a 4-day interval.

The results were unequivocal,[2,11] In the treated left lobes, insulin greatly reduced the acute atrophy that otherwise halved the size units of the cells in control groups. Insulin also preserved hepatocyte ultrastructure. The protection was limited to the directly infused left lobes, there being no spillover effect on the right lobes. In small doses (2:1 I/G molar ratio) glucagon did not potentiate the action of insulin and in 2:100 I/G doses, it may have reduced the insulin benefit. Glucagon alone in either small or large doses had no effect.

The effect of insulin on hepatocyte proliferation was also striking.[2] After Eck fistula in untreated dogs, the mitotic rate was increased to about three times normal, from 1.5 to 4.5/1000 hepatocyte cells. Insulin more than tripled this cell renewal in the infused left lobes, again with no spillover to the contralateral lobes. Glucagon alone in a wide dose range had no effect, nor did glucagon potentiate the action of insulin in 2:1 or 2:100 I/G ratios.

CLINICAL IMPLICATIONS

Thus, it was established that relative "hepatic insulinopenia" is the most important element in the liver injury of Eck fistula. However, the by-passing of other substances of enteric origin may be contributory inasmuch as the protection by insulin infusions in our experiments was not quite complete.

Even so, the prospect seems promising of favorably influencing regeneration and recovery after acute liver injury in experimental animals and ultimately in man by the simple expedient of intraportal insulin therapy. None of our work has identified a beneficial additive role of glucagon.

There are many other clinical implications in appreciating the extent to which the liver is a *target* and not just a monitor and integrator of hormonal messages.

The fact that man is resistant to the more serious metabolic consequences of Eck fistula has made it feasible to perform the procedure with benefit to patients suffering from hepatic based inborn errors of metabolism, including glycogen storage disease.[12] These patients have had growth spurts and correction of a number of preexisting metabolic abnormalities.

In homozygous type II hyperlipidemia,[13] which leads to lethal cardiovascular complications in early life, a variable lowering of serum lipids may be effected by portacaval shunt. Only two outright failures of response have been recorded (one from Europe and one from Africa) and in both patients the portacaval shunts were proved to have clotted with subsequent liver revascularization by collaterals. In our original case[13] the serum cholesterol concentration fell from

800 mg/100 ml to nearly normal, probably at least in part from reduced hepatic cholesterol synthesis which we, in dogs,[9] and Bilheimer et al. in one of our patients[14] have shown to be caused by portal diversion. Whatever the mechanism, xanthoma-cholesterol deposits in the skin and tendons melted away as the months went by. The reversal of angina in a number of these patients, and the diminution of aortic stenosis in a few others have suggested that resorption of the same material is occurring from the damaged vascular system.

These applications in glycogen storage disease, and hyperlipidemia accept a trade-off of distinctly suboptimal conditions of liver perfusion in return for metabolic improvements that are almost certainly derivative from the suboptimal conditions as could be shown histopathologically in our first child with hyperlipidemia.[13] At the time of portacaval shunt, a liver biopsy was obtained which was normal. After operation the hepatocytes shrank and glycogen granules became scarce. Rough endoplasmic reticulum 6 months later decreased to $\frac{1}{4}$ or $\frac{1}{3}$ of its original amount as judged by the quantitative technique of Loud. These changes were indistinguishable from those caused in dogs by Eck fistula. The potential penalties for this kind of treatment are clear, although we have followed our patients with glycogen storage disease or hyperlipidemia for as long as 13 yr without overt clinical portaprival complications.

In evaluating shunting operations for the traditional indications in patients with esophageal varices who retain hepatopetal portal flow it is clear that there should be a similar weighing of gains and losses. An obvious argument can be mounted for a Warren type operation which preserves much of the residual splanchnic flow to the liver including that returning from the pancreas.

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Discussion

Lipoprotein Synthesis and Liver Regeneration

Dr. Claus, Dr. Richman, Dr. Pilkis, and Dr. Friedman: Studies of Drs. Starzl, Leffert, Bucher, and others have pointed to the possible interplay of several effectors on the regeneration of liver. One such factor that has not been studied with regard to liver, to our knowledge, is epidermal growth factor (EGF). Figure 1 shows data from an experiment on the effect of glucagon, insulin and EGF on the incorporation of tritiated thymidine into DNA of isolated hepatocytes from partially hepatectomized rats in culture for 3 days. The height of each bar represents the average of duplicate flasks. The two individual values are represented as the bars above and below the average.

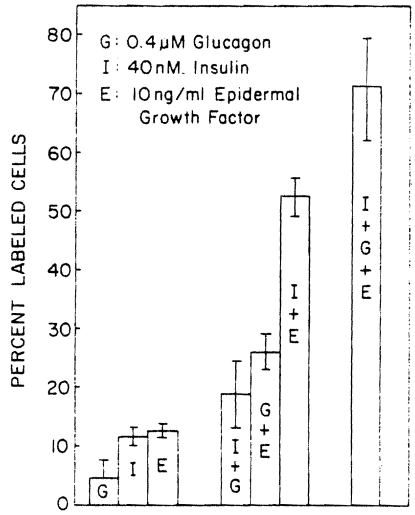

Fig. 1.

In the absence of any hormone additions (data not shown) only 2% of the hepatocytes were labeled after 3 days in culture. The addition of 4×10^{-7} *M* glucagon for 3 days increased the number of labeled hepatocytes to 5%. EGF, a gift of Dr. S. Cohen of Vanderbilt University, and insulin each increased the number to 10%-12%. The effects of insulin and glucagon were additive. However, combination of either glucagon or insulin, but particularly insulin, with EGF produced dramatic synergistic effects. Similar effects of insulin plus EGF in fibroblasts have been reported by others. Combination of all three hormones led to a still greater percentage of hepatocytes that were labeled (70%). Similar results were obtained if, instead of doing autoradiography, the DNA was isolated and counted.

Despite the stimulation of DNA synthesis by these hormones, cell number progressively decreased with time, and cell division occurred infrequently. Photographs of several microscopic fields taken every 6 hr for 3 days revealed some examples of cell division on the third day when all the hormones were present. The failure to observe significant cell division may be due to a lack of the proper conditions required to initiate mitosis.