Hepatotrophic Effect of Insulin

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A completely diverting portocaval shunt (Eck's fistula) profoundly alters the structure and function of the liver in several species. Until recently, these changes were thought in what has been termed the "flow hypothesis" (1) to be caused by and to be proportionate to the reduction in total hepatic blood-flow which the procedure causes. We have suggested instead, that disruption of normal endogenous insulin delivery to the liver for determining this lesions was mainly responsible. We showed that infusion of commercial insulin into the tied-off central portal vein of dogs prevented most of the acute atrophic changes that are ordinarily very advanced by light microscopy within four days after portocaval shunt.

Much of the evidence supporting the importance of insulin as an hepatotrophic factor has to do with what happens when the liver is deprived of portal venous blood or that portion of portal blood that emanates from the pancreas.

The light microscopic changes in the liver caused by portal blood deprivation include atrophy, fatty infiltration, and deglycogenation (10) Ultrastructurally, the most striking and specific changes are depletion and disruption of the rough endoplasmic reticulum, and reduction in the membrane-bound ribosomes. All these events occur with surprising speed, being about 90% complete within four days. We also know from other work recently summarized by Putnam el al. (7) that the same general light and electron microscopic changes have been seen after portal diversion in the livers of rats, dogs, swine, baboons, and humans with some variations in degree. Thus, the hepatic injury of Eck fistula is common to all species so far studied. The metabolic consequences have been the mildest in rats and man.

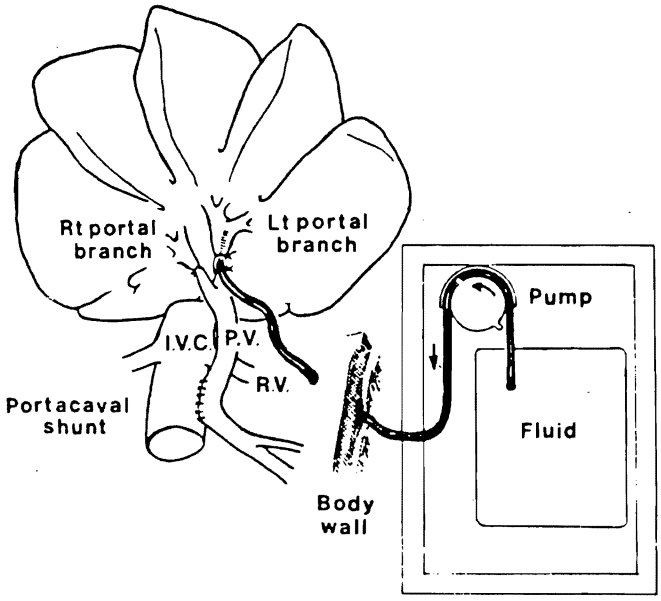

Fig.1. Experiments in which Eck fistula is performed and post-operative infusions are made into the left portal vein. (From Starzl et al. (1976) by permission of The Lancet.)

Fig. 1 represents the direct test to demonstrate that the liver alteration of Eck fistula is caused by deprivation of liver of direct access to endogenous insulin. Seventy dogs were infused with a fine infusion catheter placed into the tied-off left portal branch and led trough the body wall and trough along subcutaneous tunnel to a small calibrated finger pump that was incorporated into a light body cast. Table 1 reports the group of animals and the kind of hormonal infusion.

The size of hepatocytes and the percent of labelled nuclei were determined by biopsy specimens obtained from the left lobe at the time of the portocaval shunt (L_0), and from the left and the right lobes (L_4 and R_4) at the time of killing (5, 11, 12, 13).The results reported in table 2 demonstrate that insulin greatly reduced the acute atrophy that otherwise halved the size of the cells and preserved hepatocyte ultrastructure. In small doses, glucagon did not potentiate the action of insulin, and, in large doses, it may have reduced the insulin benefit. Glucagon alone in either small or large doses had no effect under the conditions of this experimental model.

TABLE 1.

Group N°	N° of exp	Type of infusion	Insulin* (units/Kg/day mean ± SD)	Glucagon (mg/Kg/day mean ± SD)
1	11	No treatment	0	0
2	6	Hepar. saline	0	0
3	13	Large dose ins.	0.43 ± 0.05	0
4	4	Small dose ins.	0.16 ± 0.11	0
5	8	Purified ins.	0.42 ± 9.08	0
6	8	Small dose gluc.	0	0.0053 ± 0.0011
7	6	Large dose gluc.	0	0.60 ± 0.10
8	7	2/1 ins./gluc.	0.45 ± 0.03	0.0053 ± 0.0005
9	7	2/100 ins./gluc.	0.42 ± 0.01	0.5 ± 0.02

*All insulin was commercial regular insulin, except in Group 5

Table 2.

Group N°	Cell size units (mean ± SD)			P values* (L_4 vs R_4)
	L_0	L_4	R_4	
1	0.197 ± 0.04	0.108 ± 0.02	0.108 ± 0.02	N.S.
2	0.200 ± 0.04	0.105 ± 0.02	0.104 ± 0.01	N.S.
3	0.189 ± 0.03	0.160 ± 0.02	0.100 ± 0.02	<0.001
4	0.194 ± 0.04	0.143 ± 0.02	0.094 ± 0.01	<0.05
5	0.196 ± 0.04	0.158 ± 0.01	0.095 ± 0.02	<0.001
6	0.189 ± 0.01	0.103 ± 0.01	0.103 ± 0.01	N.S.
7	0.165 ± 0.03	0.085 ± 0.01	0.082 ± 0.01	N.S.
8	0.211 ± 0.05	0.156 ± 0.05	0.094 ± 0.02	<0.05
9	0.215 ± 0.04	0.114 ± 0.03	0.073 ± 0.02	<0.05

* Comparisons were by Student's t test.

The effect of insulin on hepatocyte proliferation was also striking, as reported in table 3. After Eck fistula, the mitotic rate was increased to about three times normal, from 1.5 to 4.5 per 1000 cells. Insulin more than tripled this cell renewal, with no spill-over to the controlateral lobes. Glucagon alone had no effect, nor did it potentiate the action of insulin.

Table 3.

Group N°	N° of labelled hepatocytes per 1000 epatocytes (mean ± SD) L_4	R_4	P values* (L_4 vs R_4)
1	4.9 ± 1.0	4.7 ± 0.9	N.S.
2	4.6 ± 0.8	4.7 ± 0.9	N.S.
3	13.0 ± 3.9	4.6 ± 0.9	< 0.001
4	15.6 ± 2.0	5.3 ± 1.0	< 0.001
5	14.4 ± 1.1	4.8 ± 1.0	< 0.001
6	4.9 ± 0.9	4.3 ± 0.6	< 0.05
7	4.2 ± 1.5	4.3 ± 1.1	N.S.
8	11.8 ± 1.2	4.5 ± 0.8	< 0.001
9	14.8 ± 1.0	4.5 ± 0.9	< 0.001

*Comparisons were by Student's t test.

In unaltered or sham-operated dogs the left and right lobar values respectively were 1.6 ± 0.4 and 1.6 ± 0.5. The increase caused by Eck fistula is significant ($P < 0.001$).

CONCLUSIONS

Non-hypoglicaemic insulin infusions for four days into the tied-off left portal vein after Eck fistula greatly reduced the left lobar hepatocyte atrophy, permitted the ultrastructure of the protected liver cells to remain essentially normal, and caused a threefold increase in the number of left lobar hepatocytes undergoing mitosis. There was no spill-over effect in the right lobes. Glucagon by itself did not cause any of these changes, and it did not potentiate them when added to insulin at a 2/1 molar insulin/glucagon ratio. At a dose 100 times greater, glucagon may actually have reduced the benefit from insulin.

Thus, it has been established that "hepatic insulinopenia" is the most important element in the liver injury of Eck fistula. It is ironic that this answer was so close to detection all the time. Hahn et al. (2) definitely emphasized more than 80 years ago that, unless the portal vein was ligated above the last portal branch during the performance of Eck fistula, their dogs remained quite normal. This highest vessel drains the pancreas, but of course the endocrinological significance of that fact was not then known. Because of that same ignorance of hormones, speculation by Rous and Larimore (9) was vague about the possibility, as they saw it in 1920, that portal blood might have special liver-

supporting constituents. A perplexing observation of our own from almost 20 years ago contained a strong clue (6). It was noticed that dogs with Eck fistula plus insulin-treated alloxan diabetes remained in much better health than animals with Eck fistula alone in that the diabetic dogs gained weight and were spared all evidence of encephalopathy. The now-obvious explanation is that peripheral insulin concentrations of two to three times normal are required in diabetics to maintain normoglycaemia when insulin is given systemically, thus inadvertently providing a compensatory hepatic arterial increase in the hormone. Conversely, in untreated alloxan-diabetic rats with unaltered hepatic circulation. Reaven, Peterson, and Reaven (8) have demonstrated acute ultrastructural changes in the liver cells that were remarkably like those caused by Eck fistula.

Another aspect which is related with this result is the role of insulin in the area of cell growth control and regeneration. The provision of exogenous insulin in the present studies provoked a sustained burst of proliferation beyond the already heightened mitotic background.

These results were consistent with those in the important study by Younger, King, and Steiner (15) who allowed rats to be alloxan-diabetic for a month before treating them with insulin. Although the livers were thought to already contain a higher than normal number of hepatocytes, the proliferative response to insulin was spectacular, being similar to that after a 68% liver resection. However, Younger and his colleagues also showed that diabetic rats retained a potent although subnormal ability to regenerate their livers after an actual hepatic resection, even if insulin treatment was withheld. Accordingly, it is unlikely that any single control factor will be the sole explanation of regeneration. Holley (3) and Leffert (4) have summarized the dozens of substances, hormonal (including insulin) and others, that can initiate and regulate cell growth in tissue-culture systems. There is no reason to doubt the relevance of their comments concerning the complexities of growth control to in-vivo situations. The conclusion on the basis of this clear experiment is that insulin is the most important factor in controlling hepatocyte structure and function, and that, furthermore, it shows some activity in liver regeneration not yet fully understood (14).

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