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Regulation of Liver Size and Regeneration: Importance in Liver Transplantation

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THE LIVER, under normal conditions, presents only one mitosis per 1,000 hepatocytes, and the cells contain a fair amount of glycogen, lipids, and proteins.

Table 1 indicates the two types of factors controlling normal liver physiology. It is very difficult to define the exact role of each of these factors because they are very closely interrelated. The typical alterations induced by a portacaval shunt constitute the best demonstration of the existence of these two types of factors.[1,2] In fact, in addition to the typical signs of atrophy in this model, the presence of regeneration with increases in the number of mitosis from 1.14 to 6 can be observed. The appearance of atrophy and ultrastructural deterioration demonstrates a lowering of factors controlling the size and morphology of the liver, and the latter indicates the appearance of regulating factors of regeneration.

In a series of investigations during 1971 to 1977,[3-5] we have established that insulin is the main factor that regulates the size, morphology, and structure of the liver. In fact, a nonhypoglycemic insulin infusion into the ligated left portal vein for four days after an Eck fistula greatly reduced the left lobular hepatocyte atrophy, preserved the hepatocyte ultrastructure, and increased the cell renewal (mitosis rose from six to 15 per 1,000 cells). This effect was not changed by the addition of glucagon, regardless of the dose. The finding that insulin increased the number of mitoses raised the question of the importance of hormones in the regeneration process.

After this study, several authors[6-10] have described hormonal changes in both rats and dogs, ie, decreases in insulin, triiodothyronine, thyroxine, and calcitonin levels, whereas glucagon and corticosterone levels increase. Furthermore, we have recently reported that sexual hormones are involved in liver regeneration after 70% hepatectomy in rats.[11-13] Estrogen levels increase in the serum with a corresponding increase in estrogen receptors, which correlates with the peak of the mitotic index. On the other hand, serum testosterone and androgen receptors display a very significant decrease.

On the basis of these hormonal changes, many speculations have been proposed to attempt to define the role of hormones in liver regeneration. Nevertheless, none of these hypotheses were conclusive for the following reasons: (1) it is possible to prevent the typical insulin-glucagon change after hepatectomy by glucose infusion without affecting liver regeneration in a major way; (2) insulin and

Table 1. Factors Regulating Liver Size and Regeneration

Factors for Liver Throphism	Factors for Liver Regeneration
Hepatocyte content	Rapid activation of hepato-
Glycogen	cyte spontaneous re-
Lipids	newal to maintain liver
Vitamins	mass constant
Electrolytes	
Hepatocyte structures	

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Table 2. Tissue and Serum Growth Factors

Investigator	Time Period	Name of Substance	Purification (fold)	Molecular Weight (kd)
Tissue factors				
Blomquist	1957			
LaBrecque	1975-1987	HSS	110,000	14-15
Hatase	1979			30
Starzl	1979			
Goldberg	1980-1985	Hepatopoietin	13,000	38
Terblanche	1980			
Francavilla	1981-1987	HSS	38,000	15-50
Schwarz	1985			14-25
Weberman	1984			
Fleig	1986			
Serum factors				
Morley	1973		400,000	26
Michalopoulos	1984-1986	HPTA		120
		HPTB		3
Russell	1984			65
Nakamura	1984	Hepatotrophin	20,000	120
Goldberg	1980-1985	Hepatopoietin	13,000	38
Diaz-Gil	1986	HP	83,000	64

Abbreviations: HPTA, hepatopoietin A; HPTB, hepatopoietin B; HP, hepatic DNA-synthesis promoter.

glucagon in vitro do not affect hepatocyte proliferation; (3) triiodothyronine, thyroxine, calcitonin, and sex hormones in vitro do not show particular activity on hepatocyte proliferation; (4) the administration of all these hormones in animals after hepatectomy has never provided consistent data on liver regeneration; and finally (5) and even of further importance are the results we have obtained by transplanting small livers into much larger recipients. A rapid growth of small livers occurs during the first day after transplantation, which is similar to that observed after partial hepatectomy. None of the typical hormonal alterations observed after hepatectomy occurred during this process.

Because hormonal changes did not provide any conclusive answers as related to liver regeneration, in 1975 several investigations on growth factors in serum and tissue were initiated.

Table 2 illustrates the growth factors that have been partially isolated from serum[14-19] and liver.[19-32] As reported in Table 2, we have been working on the extraction and purification of hepatic stimulator substance (HSS)

Table 3. Steps in the Purification of HSS and Chemical and Physicochemical Properties of Fraction F_{150} Obtained From Weanling Rat Liver

Material	Protein Injected in Each Rat (mg/2 mL)	DNA Synthesis (cpm/mg DNA)*	Resistant to			Purification (fold)
			Heat	Trypsin	Neuroaminidase	
Cytosol	75	43,350 ± 8,820†			—	—
65°C supernatant	20	56,720 ± 10,240†			—	6
OH-F	10	66,350 ± 11,350†			—	15
30 Kd	2.75	63,520 ± 13,220†			—	102
F_{150}	0.003	54,380 ± 10,200	100%	30%	100%	38,100

*[^{3}H]thymidine incorporation in a 40% hepatectomized rat injected with phosphate-buffered saline was 16,500 cpm/mg DNA. The numbers are the averages from no less than 20 different rats ± SD.

†Significantly different from controls, $P < .05$.

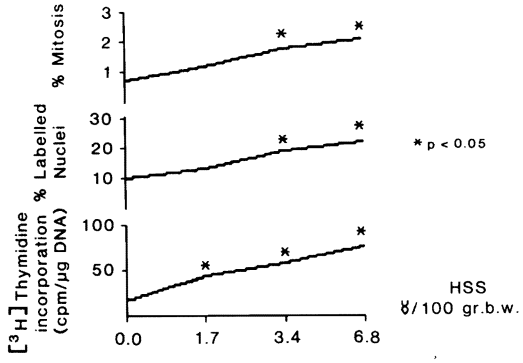

Fig 1. Dose response curve in 40% hepatectomized rats injected with F_{150}. F_{150} was injected intraperitoneally six hours after operation. [^{3}H]Thymidine incorporation, percentage of labeled nuclei and percentage of mitosis were determined 24 hours after operation.* $P < .05$.

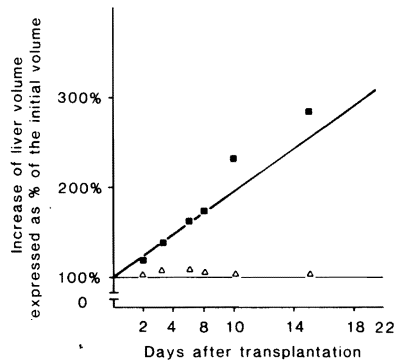

Fig 2. Change in liver volume after transplantation. The growth of a small-for-size liver transplanted in a large recipient (■) and the growth of a normal liver transplanted in a recipient of similar size (△) are expressed as a percentage of the initial volume.

since 1980. HSS was first described by LaBrecque and Pesch[20] in the cytosol of partially hepatectomized rats and subsequently identified in canine liver by Starzl et al.[22]

Table 3 reports the steps in the purification and the physicochemical characteristics of the active chromatographic fraction, referred to as F_{150}, that is obtained from weanling rat liver cytosol using fast protein liquid chromatography (FPLC) apparatus. This fraction stimulated DNA synthesis in hepatocytes in a dose-dependent regimen when injected in 40% hepatectomized rats (Fig 1). The activity of this fraction is organ specific but not species specific.[33]

Similar to the findings with hormones, the considerable knowledge obtained on growth factors has not yet resulted in any conclusive theory on liver regeneration. A better understanding of this process is clinically very important, not only for cases of liver damage but also for hepatic transplantation.

It has been observed that in clinical liver transplantation a liver from a small donor, occasionally transplanted into a much larger recipient, rapidly increases in size and achieves a size comparable to that of a normal liver for that particular recipient in a period of 2 weeks. These phenomena have been extensively studied in dogs in which small-for-size livers were transplanted into larger recipients.[34]

In Fig 2, the increase in the volume of a small liver transplanted in a larger animal is compared with that of a normal-for-size liver transplanted into a dog of suitable size.

A great increase in thymidine kinase, ornithine, and decarboxylase levels and in the percentage of mitoses was detected only in the

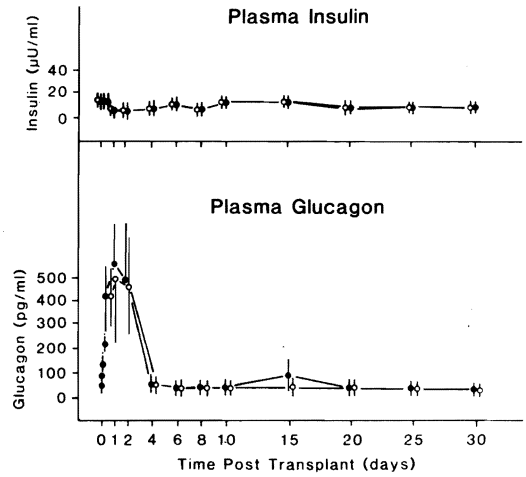

Fig 3. Plasma insulin and plasma glucagon levels in the two groups of animals: ●, small-for-size liver transplanted in large recipient; o, normal liver transplanted in a recipient of similar size.

small liver transplanted in a host of a larger size. This indicates an active hepatocyte proliferation. Figure 3 compares the insulin glucagon changes observed in both models. Despite a different rate of hepatocyte proliferation, the hormonal changes observed were similar but at the same time different from the levels observed in a regenerating liver induced by partial hepatectomy.[3]

In this experiment, the lack of specific hormonal changes suggests that the role of hormones in liver regeneration has to be reevaluated, and it raises the question of the existence of inhibitors as additional factors of liver growth regulation. A small liver, when transplanted into a large animal, might be unable to produce enough inhibitor substance to keep a normal plasma concentration. Therefore, regeneration could be seen as a complex mechanism in which the initiating key is the plasma concentration of inhibitors. This possibility even further complicates the already complex view of the regeneration process. In fact, we know only a few pieces of the puzzle that are responsible for the complex system of regeneration, and even more discouraging, we do not fully understand how all these pieces fit together.

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