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Chapter
9

Regeneration*

A. Francavilla, T. E. Starzl, D. H. Van Thiel, M. Barone*, and L. Polimeno**
University of Pittsburgh
Veterans Administration Medical Center
Pittsburgh, Pennsylvania
**University of Bari*
Bari, Italy

THE ORIGIN OF MAN'S INTEREST IN HEPATIC REGENERATION

The first mention of hepatic regeneration can be traced to the Greek legend of Prometheus, which was developed centuries before the birth of Christ (Esiodo, B.C.). However, it was only toward the end of the 19th century that the first experimental observations performed on laboratory animals were reported demonstrating complete restoration of hepatic mass following either a partial hepatectomy or a sublethal hepatic injury induced by chloroform (Von Podwyssozki, 1886; Ponfick, 1890).

A landmark event in the field of hepatic regeneration occurred when Higgins and Anderson (1931) standardized the procedure of partial hepatectomy (PH) using the rat. As a means of experimentally examining the process of liver regeneration, this model rapidly became the most frequently utilized *in vivo* system for such studies and the characterization of factors responsible for and controlling hepatic regeneration. The second series of landmark observations in this field were the experiments of Christensen (Christensen and Jacobsen 1949), Bucher et al. (1951), and Fisher (Fisher et al. 1971) who all utilized parabiotic rats, and the studies of Leong et al. (1964) and Segal et al., (1968) who used autografts in which the factors initiating the process

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of hepatic regeneration were found to be blood borne. Earlier experimental studies by Teir and Ravanti (1953) and Blomqvist (1957), in which homogenates of regenerating liver were used as a reagent source, suggested that the factors initiating hepatic regeneration might be synthesized by the liver itself. These observations led to a variety of theories concerning the regulation of hepatic regeneration. The most important of these suggested the existence of both growth stimulating factors (GSFs) (Blomqvist, 1957; Marshak and Walker, 1945; Teir, 1952; Paschkis, 1958) and growth inhibiting factors (GIFs; (Weiss, 1952; Weiss, 1955; Weiss and Kavanau, 1957; Smythe and Moore, 1958; Stich and Florian, 1958; Glinos, 1958; Bullough, 1962). With these concepts in mind, many attempts have been made to isolate both GSFs (LaBrecque and Pesch, 1975; Starzl et al., 1979; Terblanche et al., 1980; Michalopoulos et al., 1984; Nakamura et al., 1984; Goldberg, 1985; Schwarz et al., 1985; Fleig et al., 1986; Diaz-Gil et al., 1986; Gohda et al., 1986; Francavilla et al., 1987) and hepatic GIFs (Chany and Frayssinet, 1971; Chopra and Simnett, 1971; Verly et al., 1971) from a wide variety of sources and tissues.

The major alternative hypotheses to the concept of GSFs and GIFs concerning the regulation of hepatic regeneration were (1) the metabolic overload theory (Goss, 1964; Alston and Thomson, 1966), in which the liver was believed to increase its mass to satisfy a specific need, and (2) the blood flow theory, in which the amount of blood delivered to the liver, particularly via the portal vein, determined the hepatic mass (Mann, 1944; Child et al., 1953; Fisher et al., 1967; Weinbren et al., 1972; Weinbren et al., 1975). Numerous studies, in particular those directed at assessing the latter hypothesis, led to the recognition of insulin as an important hepatotrophic substance responsible for liver regeneration. The widespread development and use of various portacaval-shunt (PCS) animal models has confirmed the importance of insulin as an important growth regulating substance in determining the degree of hepatic regeneration (Starzl et al., 1973, 1975a, and 1976) and represents a third landmark in the long history of hepatic regeneration and its control.

Following the recognition of insulin as an important hepatic growth factor, several other hormones were also shown to have some effect, albeit less than that of insulin in stimulating liver regeneration (Leffert et al., 1975, 1979; Richman et al., 1976; McGowan et al., 1981). In particular, Leffert et al. (1979) emphasized the role of blood-borne hormones as being critical for the induction of hepatocellular proliferation and suggested that hepatic regeneration was controlled by a "concerted endocrine regulation" (McGowan et al., 1981; Bissell, 1976).

In the mid-1970s, the development of primary hepatocyte cultures provided an important new *in vitro* model for the study of hepatic regeneration under highly controlled laboratory conditions (Leffert et al., 1978; Maher, 1988). Using cell culture systems, it was quickly noted that the processes regulating liver cell growth observed *in vivo* and those noted *in vitro* were different, presumably because liver cells in culture lack the complex interactions which occur *in vivo* between hepatocytes and nonparenchymal cells

(NPC) (Guguen-Guillouzo et al., 1983, 1984; Begue et al., 1984; Clement et al., 1984; Fraslin et al., 1985; Morin and Normand, 1986). Subsequent studies have demonstrated that critical interactions also occur between liver cells and the matrix upon which the liver cells are plated (Rojkind et al., 1980; Reid et al., 1986; Sawada et al., 1986; Bissell et al., 1987; Schuets et al., 1988; Bissel and Choun, 1988), and that these interactions influence the functional and replicative activity of hepatocytes maintained in culture (Bissel and Guzelian, 1980; Ichiara et al., 1980; Guguen-Guillouzo and Guillouzo, 1983b; Crane and Miller, 1983; Jefferson et al., 1985; Nawa et al., 1986; Hutson et al., 1987; Flaim et al., 1987; Lloyd et al., 1987).

In the 1980s the techniques of molecular biology had allowed investigators to define more clearly the molecular events occurring within proliferating hepatocytes, i.e., alterations in oncogene expression, the timing of transforming growth factor-α and -β (TGF-α, and -β) production and both qualitative and quantitative changes in the intracellular content of proteins important in the process of cell growth and replication as assessed by quantitation of their specific mRNAs (Atryzek and Fausto, 1979; Fausto and Shank, 1983, 1987; Fausto, 1984; Mead and Fausto, 1989; Fausto and Mead, 1989).

Most recently, new data concerning liver regeneration in man and the relationship between the immune system and hepatic growth control have been added to the knowledge base.

The major factors regulating the regenerative response of the liver in man are similar to those known to regulate hepatic regeneration in animals. The differences between man and animals relate to the timing of specific events (Francavilla et al., 1989a, 1990a).

The immunosuppressive drugs, cyclosporine (CyA), FK 506, and rapamycin (RPM), which modify signal transmission in cells of the immune system, also modulate the process of hepatic regeneration (Makowka et al., 1986; Francavilla et al., 1989, 1992a).

KINETICS OF HEPATIC REGENERATION

Under normal circumstances, the proliferative activity of hepatocytes in adult animals is very low, and only a few cells can be shown to proliferate. These cells are typically found near the portal tracts (Harkness, 1952; Grisham, 1962; Fabrikant, 1967; Verly, 1976), closer to the incoming portal blood.

Under basal conditions the labeling index of hepatocytes 2 h after an i.p. administration of [^{3}H]-thymidine in rats is about 0.3% (Grisham, 1962; Schultze and Oehlert, 1960; Edwards and Koch, 1964). In dogs, this value falls to 0.16% (Francavilla et al., 1978). Under steady-state conditions nonparenchymal cells have an even lower proliferation rate than that observed for hepatocytes. In fact, after 60 h of continuous administration of [^{3}H]-thymidine under basal conditions, Kupffer cells and endothelial cells have a labeling

index of only 3 and 7%, respectively (Volkman, 1977; Crofton et al., 1978; Bouwens et al., 1986).

This situation changes dramatically, however, when a surgical resection or hepatic injury, induced either by a viral illness or chemical injury reduces the functional mass of the liver. Following each of these processes, residual liver cells experience an enhanced proliferative response, the magnitude of which varies as a function of the amount of hepatic mass lost as a result of the prior injury. This phenomenon is referred to as hepatic regeneration. This term is also widely used to refer to the process which occurs after a partial hepatectomy but, in this case, should more properly be described as compensatory hepatic hyperplasia because the excised hepatic lobes do not actually grow back. Instead, the residual liver mass undergoes a hyperplastic reaction which compensates for the lost tissue (Weinbren, 1959).

During the regenerative response that follows any major hepatic injury, virtually all of the surviving hepatocytes undergo mitosis. In fact, if the residual liver of a partially hepatectomized rat is infused with [^{3}H]-thymidine for the 7 to 8 d required for the hepatic mass to be restored fully, 95% of the liver cells will be found to contain the label (Fabrikant, 1969; Tsanev, 1975; Wright and Alison, 1984; Fausto, 1990; Farber, 1956; Lombardi, 1982; Fausto et al. 1986; Sell, 1990).

In rats, the hepatic regenerative response following partial hepatectomy starts in an almost synchronous manner in the peripheral part of the lobule, producing a peak of DNA synthesis after about 20 h. Almost all the remaining hepatocytes of the lobule progressively proliferate, generating a sort of wave-like front of DNA synthesis moving toward the central vein (Bucher and Malt, 1971; Rabes et al., 1975). This observation suggests that under physiologic conditions resting liver cells are arrested in the G_0 phase of the cell cycle (Tsanev, 1975; Wright and Alison, 1984; Farber, 1956; Lombardi, 1982; Fausto et al., 1986; Bucher and Malt, 1971).

In rats following a partial hepatectomy, hepatocyte DNA synthesis starts after a lag phase of 12 h and reaches a peak within 24 h. In contrast, the peak mitotic activity of nonparenchymal cells is delayed, and occurs at 48 and 96 (Widmann and Fahimi, 1975).

A post-hepatectomy-induced proliferative response leads to full restoration of the hepatic mass in rats in about 10 d. For larger animals and man, full restoration of hepatic mass requires a longer period of time, i.e., 2 and 3 weeks, respectively (Fausto et al., 1986; Van Thiel et al., 1985; Bucher and Malt, 1971).

The kinetics of the regenerative process described above are those observed in young adult animals. In weanling rats, the peak of DNA synthesis is achieved 3 h earlier, while in older rats it is delayed by about five h (Bucher et al., 1964). Moreover, a regenerative response occurs only when at least 30% of the liver mass is removed in young adult rats. In very young and in aged rats, a 10% reduction in hepatic mass suffices to trigger a regenerative response probably because the functional reserve of the liver is different for each of these age groups (Bucher et al., 1964; Bucher, 1967). The proliferative response is influenced also by the feeding or light exposure pattern of the animal as both of these factors modulate the circadian rhythms of the animals (Bucher and Malt, 1971).

CONTROL FACTORS IN HEPATIC REGENERATION

The nature of the various factors involved in the process of liver proliferation is addressed in the next section of this review. Subsequently an attempt is made to define the relationships between these factors and the observed hepatic regeneration.

Hormones

Numerous changes in circulating hormone levels have been reported in rats following PH (Leffert et al., 1975, 1979; Bucher and Malt, 1971; Echave-Llanos et al., 1971; Thrower and Ord, 1974; Leffert and Alexander, 1976; Bucher and Weir, 1976; Leffert, 1977; Bucher et al., 1978a; Royse and Morley, 1984). Many of these changes have been characterized and appear to correlate with measurable alterations in the hepatocyte expression of receptors for these hormones (Leffert et al., 1975; Porter et al., 1984; Francavilla et al., 1984, 1986a; Eagon et al., 1985; Cruise et al., 1989). However, *in vitro* only some of the hormones can be shown to amplify the proliferative response when added to a culture system containing medium with growth factors (Richman et al., 1976; McGowan et al., 1981; Michalopoulos et al., 1982; Russell and Bucher, 1983; Cruise et al., 1985; Francavilla et al., 1986b; Takai et al., 1988).

Insulin and Glucagon

The role of insulin and glucagon in the control of hepatic regeneration has been the subject of numerous studies utilizing a wide variety of different experimental models. The one that has contributed the most to the current understanding of the relationship between pancreatic hormones and hepatic regeneration is the splanchnic flow division model of Starzl et al. (1973) (Figure 1). In short, the two portal vein branches are isolated. One is left undisturbed while the other is detached and anastomosed by means of an iliac vein graft to the common mesenteric vein below the level of the splenic and pancreatic venous input. Functionally this model splits the liver into two distinct areas: the left lobe, which receives portal blood rich in pancreatic hormones, and the right lobe, which receives blood from the intestine. Table 1 shows the changes in terms of glycogen and cyclic adenosine-5′-monophosphate (cAMP) content of the two sides of the liver 2 months after such a shunting procedure has been performed. The lobe perfused with nutrient-rich intestine venous blood becomes atrophic, while the contralateral lobe

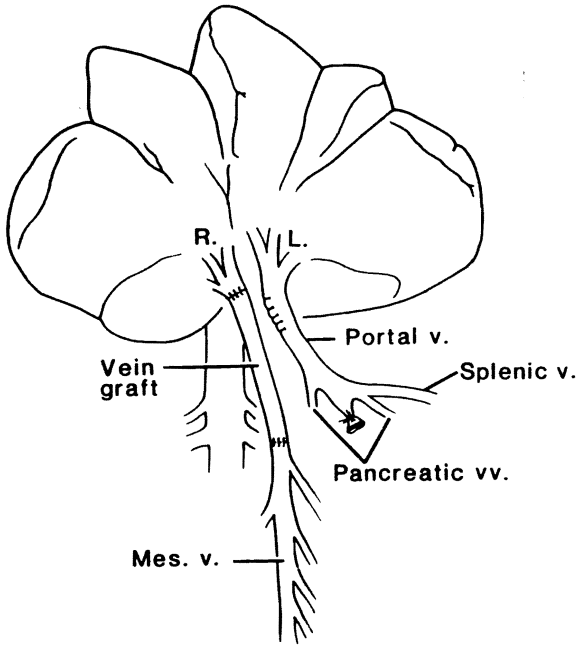

Figure 1. Schematic representation of division of splanchnic venous flow into a pancreatico-gastroduodenal-splenic compartment and an intestinal compartment.

TABLE 1
The Effect in Splanchnic Division Experiments of Perfusing the Liver with Pancreatic Duodenal Splenic vs. Intestinal Blood

	Splanchnic Flow to Left Lobes (2 months)			
	No.	Right	Left	*p*
Glycogen, mg/g of liver				
Total	6	2.83 ± 1.87	4.07 ± 1.64	<0.01
Trichloroacetic acid, soluble	6	2.00 ± 1.31	3.23 ± 1.41	<0.001
Cyclic adenosine monophosphate, pmol/g of liver	6	1077 ± 237	1164 ± 218	<0.1
Phosphorylase, nmmol/min/mg of liver protein				
Total	6	121.2 ± 13.2	113.2 ± 11.6	NS
Active	6	49.2 ± 7.9	48.5 ± 7.3	NS
Glucokinase, μmol/g/min	4	2.07 ± 1.13	2.67 ± 0.88	NS
Protein concentration, mg/g of liver	6	192.7 ± 33.1	198.2 ± 33.9	NS
Protein synthesis, cpm/g of liver	3	2340 ± 970	2509 ± 341	NS

Note: NS, not significant.

which is supplied with pancreatic hormone-rich portal venous blood maintains an almost normal appearance. In addition, the latter has an increased number of liver cells undergoing mitoses (Starzl et al., 1973). These differences between the two lobes disappear when the animal is made diabetic with alloxan (Starzl et al., 1975a, b, and d). Additional evidence for an important role played by insulin in the process of hepatic regeneration include: (1) the regenerative response following PH is reduced when anti-insulin antibodies are injected into portal venous blood (Thrower and Ord, 1974) and (2) the administration of insulin to rats with experimental diabetes causes an increase in the number of mitoses found in the liver which returns to a level similar to that observed after a partial hepatectomy in nondiabetic normal animals (Yaunger et al., 1966).

Despite this considerable experimental evidence, the precise role of insulin in the process of hepatic regeneration remains controversial: (1) insulin does not stimulate hepatocyte proliferation *in vitro* (Leffert et al., 1979; Michalopoulos et al., 1982; McGowan et al., 1981), (2) the effect of insulin on hepatocyte proliferation appears to depend upon its metabolic effects, (3) the difficulties encountered in defining the role of glucagon, either alone or in conjunction with insulin, as a hepato-proliferative factor (Simek et al., 1967; Bucher et al., 1977; Caruana et al., 1981).

Nonetheless, the introduction of the portacaval shunt model of Starzl et al., that is a further development of the Eck fistula (Figure 2) (Starzl et al., 1976, 1983) has contributed substantially to define the role of insulin *in vivo* in modulating hepatocyte proliferation. After a portacaval anastomosis, the liver becomes remarkably atrophic despite the fact that the number of proliferating hepatocytes (those with mitosis) within the liver increases from 0.16% of the total to a value of 0.47%. This change in mitotic activity occurs within 48 h and persists indefinitely in the dog. Importantly, this level of proliferative activity is comparable to that obtained in a dog 72 h after a 40% hepatectomy (Francavilla et al., 1978).

Infusion of minute quantities of either hormones or growth factors in either one of the two lobes over a 4-d period results in a restoration of cell size, cell ultrastructural appearance, and an increase in the number of mitotes only in the infused lobe (Starzl et al., 1976, 1983; Francavilla et al., 1991a). The advantages of this unique model are

1. There is no biological variability within the experimental and control groups because by splitting the liver into two experimental parts every animal becomes its own control.
2. The effect of both growth stimulating and growth inhibiting substances can be assessed with the model.
3. The method has a high degree of reproducibility and sensitivity (1 to 100 ng/kg/d of either growth factors or hormones are able to produce a biological response in the perfused hepatic lobe).

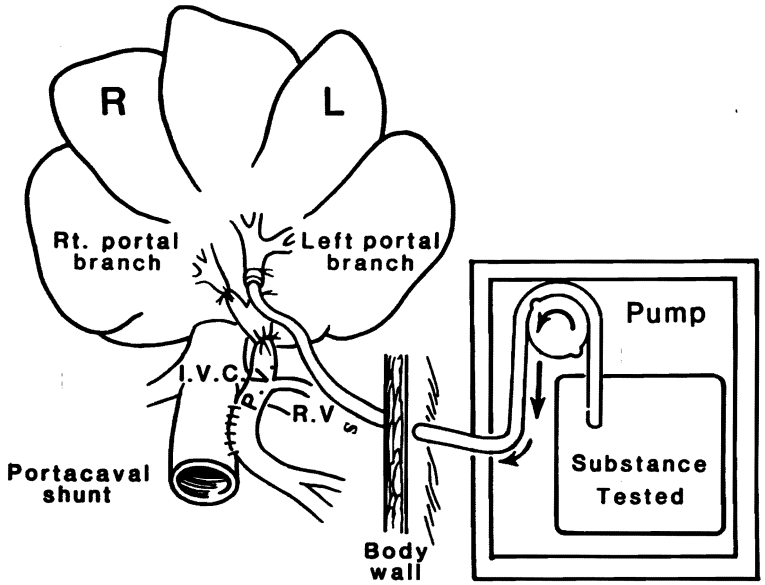

Figure 2. Portacava shunt model. The shunt is obtained by a large side-to-side anastomosis between the portal vein and the inferior vena cava and completely diverting portal blood by tieing off the main right and left portal trunks. The tip of a fine infusion catheter is then placed into the tied-off left portal branch and led through the body wall and through a long subcutaneous tunnel to a battery-driven automatic pump placed into an animal jacket. The solutions to be tested are infused in the left portal branch at the rate of 25 ml/d, for 4 d, beginning just after the intervention.

TABLE 2
Effect of Insulin and/or Glucagon Infusion on Cell Division in PCS

Group No.	Insulin Dose (μs/kg/d) (mean ± S.D.)	Glucagon Dose (mg/kg/d) (mean ± S.D.)	No. of Labeled Hepatocytes/1000 Hepatocytes (mean ± S.D.) Left	Right	*p* Values L vs. R
1	0[a]	0[a]	4.6 ± 0.8	4.7 ± 0.9	NS
2	0.42	0	13.0 ± 3.9	4.6 ± 0.9	<0.001
3	0	0.60	4.2 ± 1.5	4.3 ± 1.1	NS
4	0.45	0.0053	11.8 ± 1.2	4.5 ± 0.8	<0.001
5	0.42	0.50	14.8 ± 1.0	4.5 ± 0.3	<0.001

Note: NS, no significance.

Table 2 shows the results obtained by infusing pancreatic hormones into the left lobe of the liver of dogs with such a PCS (Starzl et al., 1976). Unlike glucagon, which has no effect even in large unphysiologic doses, insulin stimulates hepatocyte proliferation at physiologic lvels. Importantly, the simultaneous administration of insulin and glucagon does not alter the response from that observed with insulin alone.

Sex Steroid Hormones

The relationship between sex steroid hormones and liver cell proliferation has been examined in the last several years, both at a basic cellular level and at the macroscopic clinical level. Several neoproliferative hepatic diseases such as adenomas and angiosarcomas have been reported to occur in association with either endogenous or exogenous hyperestrogenism (Baum et al., 1973; O'Sullivan and Wilding, 1974; Ameriks et al., 1975; Sherlock, 1975; Christopherson et al., 1975; Glassberg and Rosenbaum, 1976; Neuberger et al., 1980; Wanless and Medline, 1982). The uniqueness of these observations is the fact that such neoproliferative hepatic diseases have never been described to occur in association with hyperinsulinism, hyperthyroidism, or any other condition recognized as modulating circulating levels of factors known to affect liver cell proliferation either *in vitro* or *in vivo*. Moreover, estrogens appear to modulate both normal and neoplastic proliferation in other organs of the digestive tract (Polimeno et al., submitted; Sica et al., 1984).

The relation between liver regeneration and steroidal sex hormones has been studied extensively using the PH rat model. Following a PH, striking alterations in the blood levels of sex hormones occur and accompanying changes in the hepatic content of sex hormone receptors appear within the liver (Figure 3). Serum estradiol levels increase, while the hepatic content of E_2 receptors also increases. In contrast, serum androgen levels decline in concert with a rapid disappearance of androgen receptors within the liver. These changes in the hormone receptor level in the liver occur only in male rats, and are accompanied by a reduction in the hepatic levels of other testosterone-inducible enzymes which metabolize estrogens. This process of the increase in serum estradiol level, an increase of ER and a reduction of E_2 metabolizing enzyme system has been termed hepatic "feminization" (Eagon et al., 1985; Francavilla et al., 1986a) and appears to be a critical step in the overall process of hepatic regeneration for male animals.

Estrogen-induced hepatocyte proliferation both *in vitro* and *in vivo* can be inhibited with the addition of tamoxifen, an anti-estrogen drug. When this drug is given i.p. to rats 6 h after a 70% PH, it completely inhibits DNA synthesis, whereas the simultaneous administration of estradiol completely blocks the growth inhibiting effect of tamoxifen administration (Francavilla et al., 1989).

It must be pointed out, however, that the relationship between steroidal sex hormones and hepatic regeneration remains difficult to understand fully, as the effect of these agents is seen only when hepatocytes from male rats are studied. The best current hypothesis is that in males, estrogens modulate epidermal growth factor- (EGF) -receptor expression to a level similar to that present in females and the interaction between EGF and its hepatic receptor is the crucial step in effecting a proliferative response (Francavilla et al., 1987).

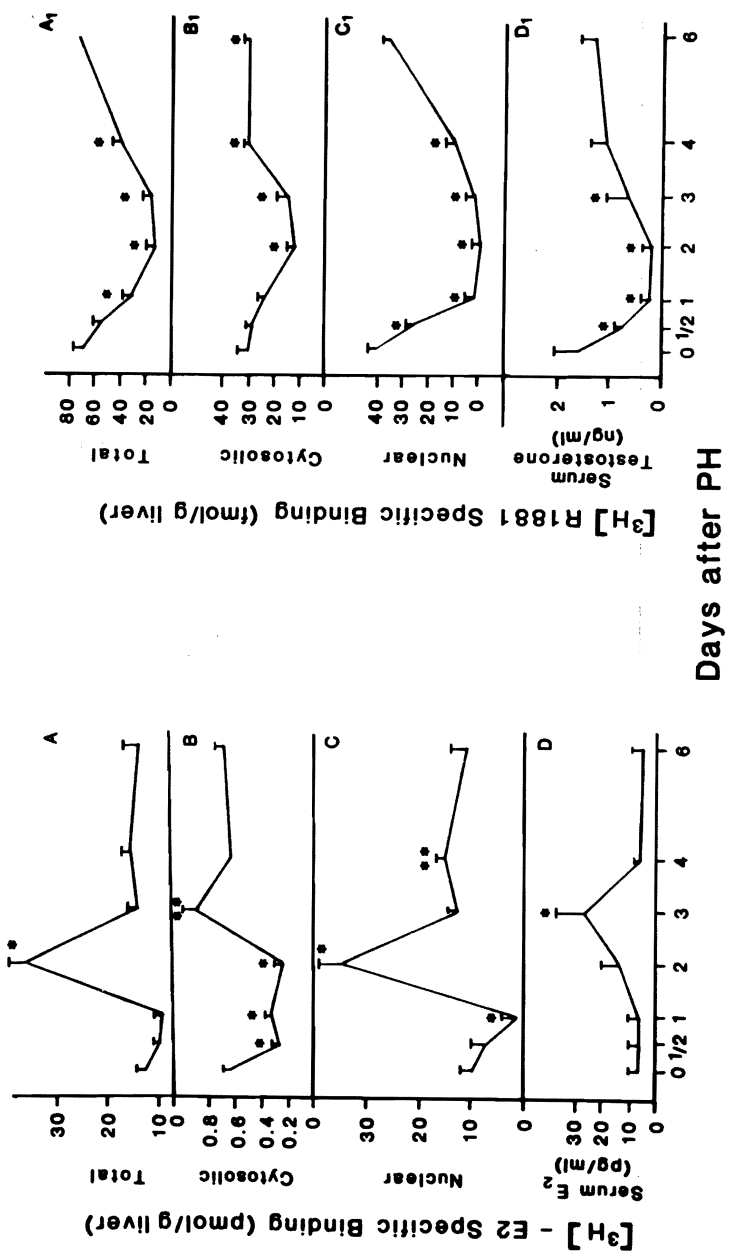

Figure 3. Variations of estrogen and androgen blood levels and relative liver receptors after partial hepatectomy in male rats. * Significantly different from control ($p < 0.05$); ** significantly different from control ($p < 0.01$).

A similar relationship between EGF, its receptor, and estrogen have been shown to exist using breast cancer cells. In breast cancer cells, high estrogen receptor (ER) levels occur in association with low levels of EGF-Rs and reduced ER levels are associated with high EGF-R levels (Sainsbury et al., 1985). Moreover, experimental data obtained from patients with hepatocellular carcinoma suggest that alterations in the estrogen/androgen (E/T) receptor ratio is actually more important than are the absolute changes in hormone levels or their receptors (Eagon et al., 1991; Francavilla et al., 1991b). An increase in the E/T ratio occurs during normal liver cell proliferation, whereas a reduction in this ratio is seen in neoplastic conditions.

Thyroid Hormone, Norepinephrine, and Vasopressin

When administered i.p., triiodothyronine (T_3) stimulates liver cell mitoses in intact animals (Short et al., 1972), whereas thyroidectomy, performed 7 d prior to PH, results in a reduction in the subsequent regenerative response to a PH (Canzanelli et al., 1949). In addition, T_3 increases the number of mitoses present in the infused hepatic lobe with the PCS model of Starzl (Francavilla et al., 1991a). However, T_3, when added to serum-free media *in vitro*, does not alter the proliferative activity of cultured liver cells.

Norepinephrine is not mitogenic on its own as assessed using primary liver cells in culture. However, its interaction with α1-adrenergic receptors increases the hepatic regenerative response observed following EGF-stimulated DNA synthesis, and is associated with a down-regulation of the EGF receptor on the surface of the liver cell (Cruise and Michalopoulos, 1985; Cruise et al., 1986). Moreover, this particular neurotransmitter reduces the inhibitory effect of TGF-β experienced by hepatocytes acting through an EGF-independent pathway (Michalopoulos, 1990; Houck et al., 1988).

The *in vivo* administration of prazosin, an α1 blocker, delays the peak increase in DNA synthesis experienced following PH and is associated with an increase in the number of EGF receptors present on the surface of liver cells (Cruise et al., 1987).

Finally, it should be noted that 2 h after a PH, norepinephrine blood levels increase substantially. Importantly, the increase in the norepinephrine level is considerably greater than that seen in sham-operated animals (Cruise et al., 1987).

Vasopressin, both *in vitro* and *in vivo* acts synergistically with other hormones to promote hepatocyte DNA synthesis (Russell and Bucher, 1983). Its hepatocyte-stimulatory activity depends upon binding of the hormone to specific receptors (Russell and Bucher, 1983b). After receptor binding, the vasopressin-receptor complex is internalized and the number of cell surface receptors for vasopressin is restored within 10 min, provided there are no additional hormonal stimuli. Intracellular transduction of the resultant vasopressin-induced signal is dependent upon the phosphatidylinositol system (Fishman et al., 1985; Kirk et al., 1977; Rozengurt et al., 1979; Kirck et al., 1984; Exton, 1988).

The role of vasopressin in the control of liver cell regeneration is confirmed further by the fact that the regenerative response following PH is impaired in the Battleboro rat, which is vasopressin deficient, and that the administration of exogenous vasopressin to Battleboro rats enhances the hepatic regenerative response to PH (Russell and Bucher, 1983b).

Hepatic Growth Factors

The hypothesis that hepatic regeneration is under the control of specific growth factors has stimulated considerable effort toward the identification and isolation of such factors. In this section, various factors which have the capability of stimulating (GSFs) or inhibiting (GIFs) hepatocyte proliferation are examined.

Growth Stimulating Factors (GSFs)

Hepatocyte Growth Factor (HGF)

The identification and purification of this particular GF has been achieved almost simultaneously by Zarnegar and Michalopoulos (1989) and by Nakamura et al. (1986, 1987). HGF is known to be a protein composed of two subunits, a heavy and a light, which have molecular weights of 69 and 34 kDa, respectively. It has been isolated from the serum of rats and man (Strain et al., 1982; Michalopoulos et al., 1983; Russell et al., 1984; Thaler and Michalopoulos, 1985; Ghoda et al., 1988). The heavy chain is composed of four Kriglers, i.e., peptides with a double loop structure held together by three disulfide bonds (S–S) (Miyazawa et al., 1989). The amino acid sequence of HGF has extensive homology with that of plasminogen and with other catalytic and proteolytic substances present in blood (Strain et al., 1982). Immunohistochemical studies have shown the presence of HGF in the thyroid gland, the brain, the pancreas, Bunner's glands of the duodenum and in the residual partially hepatectomized rat liver (Zarnegar et al., 1990).

In vitro HGF is ten times more powerful as a growth promoter than is EGF. Moreover, it has been shown to produce morphological changes in hepatocytes consistent with hypertrophy (Francavilla et al., 1991a). Its growth promoting effect has been shown to be additive to that of EGF, and to be inhibitable by TGF-β (Michalopoulos, 1990). Recently, it has been shown to be active also *in vivo* using the portacaval shunt model (Francavilla et al., 1991a).

Hepatopoietin B (HPTB)

HPTB is a glycopeptide with a mol wt of 500 Da. It was first identified by Michalopoulos (Michalopoulos et al., 1984). It has been shown to stimulate hepatocyte proliferation *in vitro* by amplifying the proliferative response to EGF. No studies have been performed *in vivo* using this growth factor.

Heparin Binding Growth Factor-1 (HBGF-1)

HBGF-1 is a heat-resistant substance having a mol wt of 16 kDa. It is produced by both parenchymal and nonparenchymal liver cells, and is active *in vitro* but not *in vivo*, and only in the presence of heparin (Kan et al., 1989). This characteristic identifies it as being completely different from hepatic stimulatory substance (HSS), which has a similar molecular weight and is also heat resistant, but is not active *in vitro* despite the presence of heparin (Francavilla et al., 1987a, 1991c).

Transforming Growth Factor-α

TGF-α is a 5.6-kDa protein consisting of a single chain of 50 amino acids (Marquardt et al., 1984). Initially it was identified in cultures of tumor cells. More recently, TGF-α has been shown to be produced by normal cells both *in vivo* and *in vitro* (Mead and Fausto, 1989). It stimulates cell proliferation through its interaction with the EGF receptor. In fact, its biological effect and the primary structure of TGF-α are quite similar if not identical to that of EGF (Marquardt et al., 1984; Marquardt et al., 1983; Derynck et al., 1984; Derynck, 1988). TGF-α-mRNA expression increases in hepatocytes 4 h after PH and reaches a maximal value 24 h after PH (Mead and Fausto, 1989), points in time that correspond to the onset and peak mitotic activity seen after PH.

Considering the role of TGF-α in the control of cell proliferation, Mead and Fausto (1989) have advanced the hypothesis that TGF-α is a major physiological stimulator of liver cell regeneration, and that it acts via an autocrine mechanism. Recently, using the portacaval shunt model TGF-α has been shown to be a potent *in vivo* stimulator of liver cell regeneration (Francavilla et al., 1991a).

Epidermal Growth Factor (EGF)

Undoubtedly EGF is the best studied growth factor in terms of its ability to stimulate hepatocyte proliferation (Richman et al., 1976; Earp and O'Keefe, 1981; McGowan et al., 1981; Rubin et al., 1982; Francavilla et al., 1986b; O'Connor-McCourt et al., 1986; Goustin et al., 1986; Olsen et al., 1988; Marti et al., 1989). After the first demonstration of this behavior by Richman (Richman et al., 1976) many subsequent *in vivo* and *in vitro* studies have been performed to assess the importance of this factor in the process of liver regeneration.

EGF is a mitogenic factor for most if not all epithelial tissues which are particularly rich in their expression of EGF-specific receptors. The ability of EGF to stimulate liver cell proliferation *in vitro* is enhanced by the presence of insulin and the amino acid proline (McGowan et al., 1981; Houck and Michalopoulos, 1985). Moreover, its effect is amplified when other GFs are added such as HGF, HBGF-1, and hematopoietin B (HPTB) (Michalopoulos, 1990).

In vitro EGF induces an internalization of its high-affinity cell surface receptor and the expression of low-affinity EGF receptors on the cell surface

(Wollenberg et al., 1989). This finding suggests that EGF and its low affinity receptor are important in the regenerative process. It also suggests the high affinity EGF receptor may serve only as a means of removing a surplus of EGF from the bloodstream and controlling (inhibiting) the mitogenic response of EGF (Wollenberg et al., 1989).

The latter argument is supported by the clear-cut differences noted between male and female rats in terms of their hepatic EGF-Rs (Francavilla et al., 1987). In fact, the pattern of EGF cell surface receptors in male rats after PH becomes similar to that of the female, where only low-affinity receptors are demonstrated (see the section on feminization).

The importance of the observation of a reduction in the regenerative response of PH rats treated with anti-EGF antibodies or following the surgical removal of EGF-producing organs such as the duodenum (Olsen et al., 1988) has been deemphasized somewhat by the recent demonstration that the structure and biological characteristics of EGF and TGF-α are similar, EGF has been shown to have a rapid turnover, and EGF has been shown to be produced by many different organs (Elder et al., 1978; Gregory et al., 1979; Oka and Orth, 1983; Shikata et al., 1984; Fallon et al., 1984).

Hepatic Stimulating Substance (HSS)

HSS is a protein that has a mol wt ranging between 12 and 16 kDa. It is found in cytosolic extracts of rat (LaBrecque and Pesch, 1975; LaBrecque and Bachur, 1982; Francavilla et al., 1987a, 1991c; LaBrecque et al., 1987; LaBrecque et al. 1987) rabbit (Fleig et al., 1986), and dog livers (Starzl et al., 1979) undergoing a proliferative response.

Its molecular structure has not yet been fully defined. This fact and the possibility that different investigators have used different species of the substance may explain why the results of different investigations studying this material occasionally appear to diverge.

Both the HSS fraction purified 9000-fold by La Brecque et al. (1984) and the fractions isolated by Fleig et al. (1986), which have a lesser degree of purification, are active using normal hepatocytes or hepatoma cells studied *in vitro*.

An HSS fraction purified about 380,000-fold was isolated by Francavilla et al. (1987a, 1991c). This fraction is active only when administered *in vivo* and has been shown to be active both in the 40% hepatectomized rat model and in the PCS model (Francavilla et al., 1991a). In the latter model, it induces a proliferative response that exceeds that induced by insulin and other well-recognized growth factors, at a dose of 20 ng/kg/d. This highly purified fraction does not stimulate the proliferation of hepatocytes *in vitro* in the presence or absence of either EGF or heparin (Francavilla et al., 1987a).

Table 3 describes the physicochemical characteristics of HSS as currently recognized (Francavilla et al., 1991c).

TABLE 3
Physicochemical Characteristics of HSS

Mol Wt	14–16 kDa
Enzymatic sensibility	
Neuraminidase	—
Trypsin	+
Chymotrypsin	+
Heat resistance	95°C for 10 min

Cyclosporin and FK 506

Very recently, CyA and FK 506, two immunosuppressive agents which specifically inhibit T lymphocytes have been shown to be able to enhance hepatocyte proliferation *in vivo*. Makowka et al. (1986) and Kim et al. (1988) have reported that CyA enhances the regenerative response induced by PH. Subsequently, it has been shown that FK 506 also stimulates hepatic regeneration following a PH in rats (Francavilla et al., 1989b). The stimulatory activity achieved with FK 506 is greater than that obtained with CyA and is organ specific, as it does not alter the proliferative response of the kidney following a unilateral nephrectomy or that of a remnant intestine after a 40% bowel resection (Francavilla et al., 1990b). It appears as if at least some of the beneficial properties of FK 506 as compared to CyA in liver transplantation are likely to be a result of the effect of FK 506 on hepatocyte proliferation.

The hepatocyte stimulatory effect of CyA and FK 506 does not depend on the immunosuppressive properties of these agents (Francavilla et al., 1991d). Whatever the mechanism for the proliferative effect of these agents might be, it clearly is not a result of a direct effect on hepatocytes as both agents do not stimulate proliferation of cultured hepatocytes either in the presence or absence of EGF (Francavilla et al., 1990b).

Growth Inhibiting Factors

Only a small number of substances have been identified as being inhibitors of hepatocyte proliferation.

Transforming Growth Factor-β

TGF-β is a 25-kDa homodimer produced by endothelial cells (Braun et al., 1988). It inhibits both EGF and TGF-α stimulated hepatocyte proliferation *in vitro* (Nakamura et al., 1985; Carr et al., 1986; McMahon et al., 1987).

An injection of TGF-β into rats at the time of PH and 11 h later completely inhibits hepatocyte DNA synthesis for 24 h (Russell et al., 1988). Figure 4 shows the TGF-β mRNA content in rat liver after a PH.

The gradual increase in TGF-β level noted after PH, together with its potent inhibitory activity on hepatocyte proliferation *in vitro*, led Fausto and Mead (1989) to hypothesize that this substance is a physiological inhibitor of

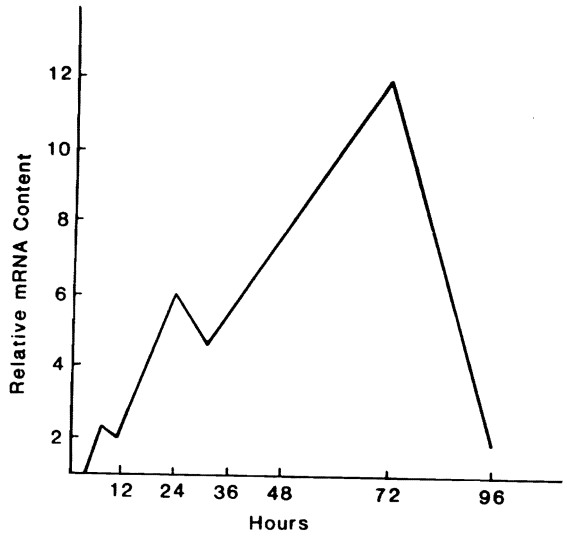

Figure 4. TGF-β mRNA in regenerating rat liver. The relative content refers to the amount of TGF-β 1 mRNA present in liver from sham-operated rats.

liver cell regeneration which exerts its inhibitory effect via a paracrine mechanism.

TGF-β is also a potent inhibitor of the regenerative response of cells found in other organs, including those of the immune system (Goustin et al., 1986; Roberts et al., 1985).

It has been reported that hepatocytes become TGF-β resistant after a PH. This topic was debated exhaustively in two monographs (Fausto, 1990; Michalopoulos, 1990).

Considering the emerging relationships between the immune system and liver cell regeneration, it is possible that TGF-β represents a link(s) between these two organ systems.

Kupffer Cell Hepatocyte Inhibitor (KCHI)

This factor is produced by Kupffer cells *in vitro* and is released into the culture medium 6 h postplating. It induces a 50% reduction in the proliferative activity of hepatocytes incubated in the presence of insulin independent of the presence or absence of EGF.

This hepatocyte-inhibitory effect is reversible with removal of the material from the medium. Preliminary studies on the physicochemical characteristics of KCHI have shown that it has a mol wt ranging between 10 and 50 kDa and that it is sensitive to heat and a pH >8. These characteristics distinguish it from TGF-β, tumor necrosis factor (TNF), and interleukin-1 (IL-1) (Francavilla et al., 1988).

IL-1 and IL-6

IL-1 inhibits *in vivo* hepatocyte DNA synthesis and enhances the expression of genes responsible for acute phase protein synthesis. Thus, IL-1 prevents liver cell mitosis and enhances reprogramming of the anabolic activity of the cell toward the production of acute phase proteins (Nakamura et al., 1988). The activity of IL-6 as a growth inhibitor is a matter of considerable debate (Henney, 1989; Mizel, 1989).

Hepatocyte Proliferation Inhibitor (HPI)

This protein has a mol wt of 15 kDa. It differs physiochemically from TGF-β in that it inhibits hepatocyte proliferation *in vivo* as well as the proliferation of other epithelial cells (Huggett et al., 1987).

Rapamycin

RPM is a potent immunosuppressive drug that disrupts normal signal transduction processes and inhibits hepatocyte proliferation *in vivo* and *in vitro* (Francavilla et al., 1992b, 1992c) at levels not associated with inherent cytotoxicity for rat hepatocytes cultured either in conventional medium or in a medium enriched with EGF (Francavilla et al., 1992a). The antiproliferative effect of RPM is dose dependent and long lasting after only a brief exposure. Moreover, the effect is unaltered by the concomitant presence of FK 506 in the medium and is uninfluenced by the presence of EGF in the medium, suggesting that there is a separate RPM binding protein may be involved in its action.

DIETARY MANIPULATIONS AND LIVER CELL REGENERATION

It is well known that feeding habits influence liver cell proliferative activity acting through their effect upon circadian rhythms. Moreover, experimental dietary manipulations have been shown to induce profound changes in the proliferative response of cells.

Rats fed a protein-free diet for 3 d and then given an oral amino acid bolus (casein hydrolysate 2.5 g/5 ml tap water per rat) demonstrate a remarkable increase in DNA synthesis activity (15-fold over that seen in normal animals), which is followed by a peak in mitotic activity (Bucher et al., 1978b). This increase in DNA synthesis is similar to the increment in DNA synthesis induced by PH. The only difference is that the peak of DNA in animal synthesis refeeding with amino acids occurs 4 h earlier than that seen after a PH. This is probably due to the fact that hepatocytes fed a protein-free diet for 3 d are in G_1 phase as demonstrated by the behavior of the protooncogenes *c-myc* and *c-fos*, markers of this particular phase of the cell cycle (Horikawa et al., 1986).

HEPATIC REGENERATION AND LIVER TRANSPLANTATION

With every liver transplant, some hepatic injury is experienced by the donor organ. For successful engraftment to occur, the hepatic injury must be balanced by an equal amount of hepatic regeneration. This regeneration is required to replace liver cells lost in the graft as a consequence of any one or more of the following hepatic injuries: hypoxia, reperfusion, ischemia, or rejection.

Moreover, because of a variety of surgical considerations, the donor organ is often smaller than the diseased resected native liver. As a result, the liver graft is often too small for the recipient's long-term metabolic needs and compensatory hyperplasia occurs with the liver graft growing at a rate of 100 to 140 g/d (Kam et al., 1987). This hepatic growth which is seen following liver transplantation is probably the result of many different factors including the following: α-adrenergic stimulation, release of endogenous hepatic growth factors that act via autocrine and paracrine pathways, and CyA- or FK 506-induced growth stimulation.

Evidence for chronic low grade but nonetheless continued regeneration of liver grafts following transplantation is demonstrated by the findings of thickened hepatic plates and nodular hepatic regeneration occurring in the absence of hepatic or portal fibrosis in well-tolerated long-term successful grafts (Van Thiel et al., 1987). Grafts adversely affected by rejection show even greater degrees of hepatic regeneration with nodule formation, bile ductular proliferation, and bridging scars that can simulate cirrhotic nodules (Van Thiel et al., 1987).

Thus, some degree of hepatic regeneration occurs with every liver transplant. The degree to which this regeneration process is manifest, however, is often inversely related to the degree of adversity experienced by the graft.

Hepatic Regeneration in Humans

We have recently shown that thymidine kinase (TK) and ornithine decarboxylase (ODC) activities in plasma reflect changes occurring during liver regeneration. Following a 70% PH in rats, the levels of these two enzymes provide a practical and noninvasive method for monitoring liver regeneration (Polimeno et al., 1991). Similar observations have been made in humans (Francavilla et al., 1990a). In the patients studied, a significant increase in the serum level of ODC, an enzyme required for increased polyamine synthesis (McCann, 1980; Bachrach, 1980; Russell, 1980), was seen 24 h after partial hepatectomy (McCann, 1980; Bachrach, 1980; Russell, 1980). An increase in ODC activity was seen 3 or 4 d later followed by a significant increase in the activity of TK, a marker of DNA synthesis. The sequential appearance in blood of ODC and TK was similar but delayed in comparison with these in experimental animals. In rats, the interval between the early activity of

ODC and the subsequent increases of TK is only 8 h (Polimeno et al., 1991). In dogs (Francavilla et al., 1978) and pigs (Kahn et al., 1980) the progression of these changes is slower, and in humans, 3 or 4 d seem to exist between the resection and the initial regenerative events, signaled by the increase in ODC activity and the wave of DNA synthesis assessed by the level of TK in serum. This slower pace of liver regeneration in man can be documented also by CT scan studies that show that hepatic regeneration following a major hepatic resection is not complete until 3 weeks after the surgery, as opposed to 8 to 10 d in the rat (Bucher and Malt, 1971) and 14 d in the dog (Francavilla et al., 1978).

Changes in pancreatic hormone levels such as insulin and glucagon follow a more protracted schedule in humans as opposed to animals subjected to a PH. Avid insulin-binding to hepatocytes, with a subsequent decline in plasma insulin levels, occurs within 12 h in rats (Pezzino et al., 1981), but is not seen in patients, probably because the baseline glucagon level is four times greater in patients undergoing major hepatic surgery than those noted in normal. In contrast, changes in the sex hormone levels in the blood of men studied after PH occurs briskly are evident within a few hours (Francavilla et al., 1989a) and compatible with that reported in experimental animals (Francavilla et al., 1986a).

GENERAL CONSIDERATIONS

Current knowledge does not enable us to provide a comprehensive and fully integrated picture of the role that different factors play in the process of hepatic regeneration. This is true for the following reasons.

The precise sequence and function of each of the currently recognized GSF and GIFs is not known. Worse yet, the crucial linking steps required for such an integrated understanding may reside in as-yet unrecognized GFs.

Major difficulties are experienced when one selects a specific model for evaluating the behavior of growth factors. Each model has different requirements and the various GSF and GIFs have different mechanisms and play a role at unique time points in the overall sequence of events that occurs after a PH. In any given model, the sequence and therefore the requirement for a given GSF and GIF may differ.

It is currently unknown whether the humoral or the cellular events observed following a partial hepatectomy are more important in initiating and sustaining the observed proliferative response.

It is unclear whether the liver utilizes different proliferative pathways to compensate for a sudden loss of hepatic mass occurring as a result of a PH and the lesser loss of hepatic mass seen clinically after exposure either to a chemical or viral agent that occur over a prolonged period of exposure.

Fausto and Mead (1989) have classified GFs into two major groups: those that act as priming factors, and those that act as progression factors. Priming

TABLE 4
Michalopoulos Classification of Liver Growth Stimulators

Complete Hepatocyte Mitogens	Comitogenic Growth Factors
• Epidermal growth factor (EGF)	• Norepinephrine
• Transforming growth factor α (TGF-α)	• Vasopressin
• Hepatopoietin A (HPTA)	• Estrogen
• Hepatopoietin B (HPTB)	
• Heparin binding growth factor-1 (HBFG-1)	• Insulin

factors induce the transition from G_0 to G_1 in the cell cycle both *in vivo* and *in vitro*. In contrast, progression factors stimulate the transition from G_1 to S and completion of the cell cycle. Although this distinction between types of growth factors is higly speculative, it has enabled investigators to identify two phases within the cell cycle (priming and progression) on the basis of variations in liver cell protooncogene expression (Fausto and Mead, 1989). Increases in the intracellular expression of *myc* and *fos* are features typical of the priming phase. The best model for the demonstration of these phenomena is the 3-d protein-free diet (Horikawa et al., 1986).

A different classification of growth factors has been proposed by Michalopoulos (1990). He made a distinction between complete and incomplete mitogens, based upon the ability of the agent in question to elicit a proliferative response in serum-free medium when studied *in vitro*, or whether the agent simply amplifies the proliferative response initiated by other growth factors that are essential for a response to occur. Table 4 identifies the currently recognized growth factors segregated according to the classification of Michalopoulos. It is important to remember that this classification can be applied only to experiments performed *in vitro*, and is not applicable to *in vivo* models. This fact underlies the most serious limitation of our current understanding of the mechanisms regulating hepatic regeneration, e.g., an ideal *in vivo* model does not exist. Each model has its own unique characteristics and problems.

For instance, even though the PH model has made it possible to identify the various hormonal changes that occur after a PH, it has not allowed us to determine with any confidence the meaning of these events. In fact, the changes observed for insulin and glucagon which have been recognized as being important are actually a result of the 70% hepatectomy per se and are not essential to the process of hepatic regeneration. In fact, the simple administration of glucose prevents PH-induced hypoglycemia in fasted animals, and as a result the hormonal changes typically observed after a PH do not occur,

and yet the pattern of hepatic regeneration remains unaltered (Caruana et al., 1981).

It is generally believed that the increase in plasma norepinephrine levels seen immediately after a PH sensitizes hepatocytes to mitogenic stimulation by various growth factors present in the very early phase of the hepatic regenerative process following PH (Cruise et al., 1987). In contrast, the increase in serum estradiol levels and the reduction in testosterone levels seen after a PH in both male rats and in human men (Francaville et al., 1986a) appears to be required somewhat later in the regenerative process when stabilization of the EGF receptor system is crucial and determines the subsequent regenerative response in the two sexes (Francavilla et al., 1987b). Similarly, a role in the regenerative process is played by T_3 which can be shown to stimulate regeneration in intact animals and following a PCS. In recent experiments performed using the PCS dog model (Figure 2), the activity of all the hormones identified as stimulators of liver regeneration has been determined (Francavilla et al., 1991a). In Table 5 the results for each hormone are shown. In this model, the only hormones active were insulin and T_3. This model demonstrates also that substances such as EGF, HGF, TGF-α, and IGF-II and HSS each stimulate hepatocyte proliferation *in vivo* in a dose-response manner (Table 6).

Most importantly, this model has confirmed the stimulatory activity of HSS, FK 506, and of CyA (Mazzaferro et al., 1990), each of which has been shown to be active in the 40% hepatectomized rat model (Makowka et al., 1986; Francavilla et al., 1987a, 1989b). Insulin, HSS, and the two immunosuppressive agents, CyA and FK 506, are substances which would clearly have gone unrecognized as regeneration promoters without this model. Because of the unique characteristics and behavior of these substances they have been termed by us to be augmenters of hepatic regeneration (Francavilla et al., 1991a).

The negative results obtained using the PCS model with various hormones which have been shown to be active *in vitro* may be a result of the fact that these hormones are initiators of regeneration. As proposed by Fausto and Mead (1989), initiators induce the transition of hepatocytes from G_0 to G_1 which occurs within a few hours of hepatic injury or cell loss. In the PCS model, proliferative activity (hepatic regeneration response) can only be assessed after a 4-d infusion, then it represents a system in which spontaneously activation by endogenous initiating factors present in the liver or the blood have already exerted their effect. Thus, the various ions, nutrients, and regulators which test negatively in the PCS model probably are active as initiators and are undetected by this model.

While it is evident that much remains to be learned about the events governed by growth factors, it is becoming increasingly obvious that the liver still has many remaining secrets; i.e., its ability to recover its mass utilizing different proliferative patterns and its relationship with the immune system are but only two examples.

TABLE 5
Hepatocyte Size and Autoradiographic Labeling after Continuous Infusion of Hormones into the Left Portal Vein Branch of Dogs with Eck Fistula

Groups	Dogs (24)	Dose (ng/kg/d)[c]	No. of Labeled Hepatocytes/1000 Hepatocytes (mean ± SD) Left Lobe	Right Lobe	Cell Size Units (mean ± SD) Left Lobe	Right Lobe
Prolactin	2	200	3.95 ± 0.07	3.95 ± 0.06	0.1002 ± 0.004	0.0971 ± 0.01
Angiotensin II	2	100	4.1 ± 0.05	3.85 ± 0.06	0.1010 ± 0.009	0.0984 ± 0.03
Angiotensin II	1	200	4.2	3.9	0.1142	0.1001
Vasopressin	2	50	3.95 ± 0.07	3.85 ± 0.07	0.0971 ± 0.005	0.0932 ± 0.007
Norepinephrine	3	0.10	3.73 ± 0.7	3.8 ± 0.7	0.1040 ± 0.026	0.1044 ± 0.019
Estradiol	3	700	5.3 ± 0.8	5.1 ± 0.4	0.0999 ± 0.008	0.0996 ± 0.009
T_3	3	100	7.02 ± 0.9[a]	4.1 ± 0.4	0.1155 ± 0.0103	0.0976 ± 0.005
Insulin	5	0.43 U.I.	13 ± 2.8[b]	4.6 ± 0.9	0.160 ± 0.02[a]	0.100 ± 0.02
Glucagon	3	6×10^{-5}	4.2 ± 1.5	4.3 ± 1.1	0.085 ± 0.01	0.082 ± 0.01

[a] $p < 0.005$.
[b] $p < 0.001$.
[c] Except insulin which is expressed in U kg/d.

TABLE 6
Hepatocyte Size and Autoradiographic Labeling after Continuous Infusion of Growth Stimulating Factors into the Left Portal Vein Branch of Dogs with Eck Fistula

Groups	Dogs	Dose (ng/kg/d)	No. of Labeled Hepatocytes/1000 Hepatocytes (mean ± SD) Left Lobe	Right Lobe	Cell Size Units (mean ± SD) Left Lobe	Right Lobe
IGF-II	4	50	12.15 ± 07[a]	4.37 ± 0.51	0.1473 ± 0.01[a]	0.0940 ± 0.006
IGF-II	1	100	12	4.2	0.1521	0.0935
IGF-II	1	150	12.8	4	0.1507	0.0917
EGF	4	50	4.8 ± 0.48[b]	3.92 ± 0.12	0.1147 ± 0.005[b]	0.0945 ± 0.008
EGF	1	100	4.9	4.1	0.1218	0.0910
EGF	1	150	5.2	4.0	0.1195	0.1001
TGF-α	3	50	10.7 ± 0.4[a]	3.9 ± 0.4	0.1363 ± 0.0012[a]	0.093 ± 0.006
HGF	2	200	12 ± 0.1[a]	4.7 ± 0.12	0.146 ± 0.012	0.069 ± 0.02
HSS	5	50	12.3 ± 0.03[a]	4.6 ± 0.008	0.1489 ± 0.015[a]	0.0089 ± 0.0085

Note: IGF-II, insulin growth factor; EGF, epidermal growth factor; TGF-α, transforming growth factor alpha; HGF, hepatocyte growth factor; HSS, hepatic stimulator substance.

[a] $p < 0.001$.
[b] $p < 0.05$.

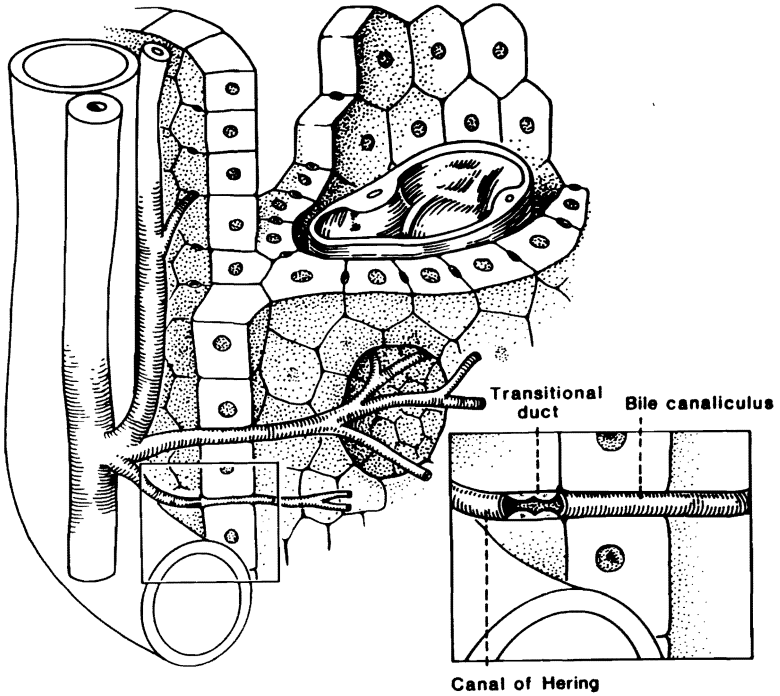

Figure 5. Schematic representation of stem cells (oval cells) at the level of the transitional duct.

Post-hepatectomy regeneration involves all of the hepatocytes within the liver (Fabrikant, 1969; Tsanev, 1975; Wright and Alison, 1984). Specifically all of the remaining liver cells following a PH are altered and express previously unexpressed protooncogenes (*fos*, *myc* P53, *K-ras* and *H-ras*, and heat-shock and drug-resistance genes) (Fausto and Mead, 1989; Cairo et al., 1985), while the hepatic regeneration observed after either a chemical-induced injury or after exposure to a viral agent is characterized by the occurrence of foci of α-fetoprotein and α-fetoprotein RNA-rich cells which express protooncogene markers typical of immature hepatocytes (Sell and Salman, 1984). These cells appear to be derived from a single stem cell (the oval cell) located principally at the junction of Hering's canals and the intralobular bile capillaries (transitional duct) (Figure 5) (Sell, 1990).

The relationship that exists between the immune system and hepatic regeneration was identified as a result of the following experimental observations: DNA synthesis increases in lymphoid tissue after a PH (Sakai et al., 1976); rat serum obtained after a PH stimulates lymphoidal proliferation (Sakai et al., 1976); splenectomy augments hepatic regeneration (Perez-Tamayo and Romero, 1958); the administration of FK 506 and of CyA, two immunosuppressive agents that specifically inhibit T-lymphocytes, augment hepatic regeneration (Makowka et al., 1986; Francavilla et al., 1990b, 1991d).

Experiments performed in nude rats have ruled out a direct effect of immune modulation in the control of the regenerative process by T lymphocytes and natural killer cells as an explanation for the stimulating effect of FK 506 and CyA. As a result an alternative hypothesis has gained currency (Francavilla et al., 1991d). The cytosolic receptors for FK 506 and CyA are small molecular weight proteins collectively called immunophillins (Schreiber, 1991). They modulate a wide variety of calcium-dependent signal transduction pathways (Perez-Tamayo and Romero, 1958; Sakai et al., 1976; Bierer et al., 1990). The interaction of these two agents with their specific receptors modulates immunophillin-mediated signal transduction that contributes to the process of liver cell proliferation. Additional evidence relative to this issue comes from the recently observed effect of RPM on liver regeneration. Instead of promoting liver regeneration as occurs with FK 506 and CyA, RPM is antihepatotrophic (Francavilla et al., 1992a and b). In rats submitted to a partial hepatectomy RPM inhibits liver regeneration instead of augmenting it. This observation constitutes the first physiologic evidence that the immunophillin network may play a key role in the interactions that exist between the immune system and growth control mechanisms (Starzl et al., 1990).

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Chapter
10

Functional Compartmentation of Hepatocytes

Caryn M. Berkowitz, Edgardo Guibert, Bahri Bilir, and Jorge J. Gumucio
The University of Michigan School of Medicine
and
Veterans Administration Medical Center
Ann Arbor, Michigan

INTRODUCTION

The liver parenchyma comprises several cell types. Parenchymatous cells or hepatocytes represent approximately 60% of liver cells. The nonhepatocyte cell population encompasses endothelial cells, Kupffer cells, fat-storing cells (also called stellate cells, adipocytes or Ito cells), Pit cells, and bile duct cells. Each cell population has defined functions. Interestingly, in some instances, the hepatocyte and nonhepatocyte populations may interact, such as in the handling of vitamin A by fat storing cells and hepatocytes (DeWitt, 1988).

In this chapter, we shall address a different type of cell heterogeneity. There is evidence that there are phenotypic differences among hepatocytes (Traber et al., 1988). These differences are due to the predominant, or sometimes exclusive, expression of proteins in certain hepatocytes. As a consequence of the selective expression of proteins, function is compartmentalized. This phenomenon has been called hepatocyte heterogeneity, the subject of this review. Therefore, our objective is to describe the compartmentation of physiological processes in hepatocytes, to discuss its regulation, and finally, to attempt to elucidate its biological meaning.

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