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viewpoints on digestive diseases

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Hepatic Regeneration

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Regeneration of the liver in all mammalian species, including man, is a remarkable and extensively studied, but as yet not fully understood, physiological phenomenon.

The most widely investigated aspect is the response of the normal liver to partial resection. After partial resection there is a lag period and then the liver undergoes a rapid growth spurt as evidenced by DNA synthesis. Histopathologically, the remnant hepatocytes become larger (hypertrophy) and undergo increased mitosis (hyperplasia). Under controlled conditions, the timing of the various events is constant in a given species but it differs quite markedly from species to species. Once regeneration has commenced, the liver mass is very rapidly restored to the normal preresection level and growth then stops. This response to liver resection is even more remarkable when one considers that normal adult liver cells are stable with a very slow turnover rate. However, they clearly retain a latent, but very potent, capacity for growth. Regrowth after one or more liver lobes have been resected is by compensatory hyperplasia (and usually hypertrophy) as the missing lobes do not regrow; it is the residual liver lobes that increase in size until the original liver mass is restored.

The reduction in liver mass that is caused by damage to liver cells by viruses or hepatotoxins (e.g. carbon tetrachloride), and the control of regeneration in this setting, has much wider clinical implications than liver regrowth after resection, but has not been nearly as extensively studied.

Although the understanding of liver regeneration has advanced considerably, we still do not know what initiates the regenerative response once the liver cell mass has been reduced, or what causes the liver to stop growing once the original size has been attained. Another poorly understood aspect of liver cell growth is what causes the normal liver to stop growing once adulthood is reached.

A particularly controversial area is whether there are specific factors that switch on or initiate liver regeneration and other factors that potentiate regeneration or permit it to occur normally, or whether both initiation and potentiation are caused by the same factor or factors. Recent evidence on potentiation of liver regeneration supports the hypothesis that multiple factors are involved (1,2). On the other hand, there is no consensus on what initiates regeneration nor is there agreement on the site of origin of either the initiating and/or the potentiating factor or factors (1).

An understanding of liver regeneration is clearly important in the clinical settings of fulminant hepatic failure and after liver resection. However, studies of liver regeneration might even provide clues to more fundamental problems, such as the control of cell growth in general and why this control is lost in malignancy.

THE CONCEPT OF HEPATOTROHPIC FACTORS

This concept was extensively debated during a recent symposium (2). Blood returning from the nonhepatic splanchnic organs via the portal venous system specifically influences both the morphology and function as well as the regenerative capacity of the liver. The portal blood constituents responsible for these effects have been termed portal hepatotrophic factors. Most of the *in vivo* data on these factors has been accumulated by depriving all or part of the liver of portal venous return, by partial or complete removal of nonhepatic splanchnic viscera or by infusing hormones or other substances into either the portal or the systemic circulation (1). It is important to separate nonregenerative hepatotrophic effects (effects on liver morphology and function) from the effects of the so-called hepatotrophic substances on liver regeneration (1).

Non-Regenerative Hepatotrophic Effects

When the liver is deprived of portal blood (creating a portoprival state) by portacaval shunting or other technical maneuvers, the hepatocytes shrink in size (atrophy). Amongst other morphological changes in the liver cells, the most striking and specific are depletion and disruption of the rough endoplasmic reticulum and reduction in membrane bound polyribosomes. All of these changes occur rapidly, being almost complete within 4 days, and have been noted in the livers of all species studied to date, although the degree may vary.

Liver function is similarly affected to a varying degree in all species. Changes include inefficient clearing of ammonia and other substances and a fall in the different kinds of blood lipids. Because of the striking organelle alterations already mentioned, additional subtle functional effects of portal blood deprivation are likely and are probably wide ranging (1).

Historically, many of the changes caused by portacaval shunt have been ascribed simply to reduction of total hepatic blood flow to which the portal contribution is normally 80%. This "flow hypothesis" emphasized the quantity rather than the quality of blood nourishing the liver (1). However, studies from Denver and elsewhere (1,2) have shown that the quality of portal venous blood is the dominating factor. These investigations in animals have been with heterotopic transplants, portacaval shunts, split or partial transposition and splanchnic division (both of the latter being evaluated with or without alloxan diabetes or total pancreatectomy), and partial or complete nonhepatic splanchnic evisceration (1). In expressing the results of these experiments in practical terms, it was concluded that the most favorable condition for portal perfusion was splanchnic venous blood which contained normal amounts of endogenous insulin. the least favorable condition was perfusing with systemic venous blood. Intermediate in quality was splanchnic venous blood that was deficient in endogenous insulin but which was rich in other as yet unknown elements.

Subsequent experiments in dogs with portacaval shunts and insulin infused over 4 days into the tied off left portal vein, showed that the liver injury produced by portacaval shunting could be markedly ameliorated (3). However, the insulin protection was not complete and this was interpreted as reflecting missing ancillary substances that were not being replaced. This multifactorial theme has been consistent in all the work from Denver on hepatotrophic factors (1,3).

Hepatotrophic Factors and Regeneration

This remains a highly controversial area with conflicting results from many laboratories. An important current debate is whether the portal hepatotrophic factors act as initiators of regeneration or whether they merely play a permissive or potentiating role. As reviewed recently, the investigations of Fisher, Price, Orloff and Bucher and their collaborators, of the Denver group, as well as additional studies by other workers, have clearly demonstrated that portal factors influence the regenerative response (1). A recent study has confirmed that gut factors play an important role in addition to pancreatic factors. In partially hepatectomized dogs, evisceration with preservation of the pancreas caused a greater reduction in hepatic regenerative response than occurred after pancreatectomy alone. This has further strengthened the original multifactorial hypothesis and clearly differentiated pancreatic influences from those originating in the rest of the intra-abdominal gastrointestinal tract (4). None of the above experiments have demonstrated that hepatotrophic substances actually initiate regeneration, although they clearly affect the regenerative response.

INITIATION OF REGENERATION

Portal Factors as Initiators

Is there any evidence favoring an initiator role for portal hepatotrophic factors? The most compelling argument in the past has been the well ordered biphasic changes that occur in the liver in the hormonally controlled "messenger" components, cyclic AMP and adenyl cyclase, prior to and during regeneration (1). Various nonhepatic splanchnic eviscerations, which have a profound adverse effect on regeneration, cause severe pertubations in these "messenger" components (4), as well as in ornithine decarboxylase levels. It remains speculative whether these deviations have a cause and effect relationship or whether they are merely coincidental to already initiated regeneration. We have an open mind about the possibility that those changes that have been noted in the regenerating liver prior to the commencement of DNA synthesis (and the subsequent increase in mitoses) are merely evidence of an early stage of regeneration which has been initiated by some other factor or factors. There is no currently available firm evidence supporting or denying portal hepatotrophic substances as initiators of regeneration. Nevertheless, Bucher and her associates at Harvard have been examining the possibility of hormone therapy (particularly insulin and glucagon) for fulminant hepatic failure.

Non-Portal Factors as Initiators: Liver Factors

If portal factors prove to only have a permissive or potentiating role in liver regeneration, what switches on or initiates the regenerative response? The important additional and by no means contradictory possibility that something in the liver itself after partial hepatectomy or liver cell damage contributes to, or even initiates, its own regrowth merits careful evaluation. The concept of intrinsic hepatic growth control factors has been considered in the literature for almost 50 years (1). The most convincing early evidence was presented by Blomqvist in 1957 (5). He found that liver mash prepared from normal weanling (i.e. young growing) rats, and from the already regenerating remnant of adult rat livers after partial hepatectomy, when given one-time intraperitoneally to adult rats, caused hepatocyte proliferation which was maximal at 48 hours. On the other hand, normal adult liver mash was non-stimulatory (5). This work has been extended and confirmed by La Brecque and Pesch (6) using a more purified liver extract consisting of the supernatant from liver mashes after high speed centrifugation.

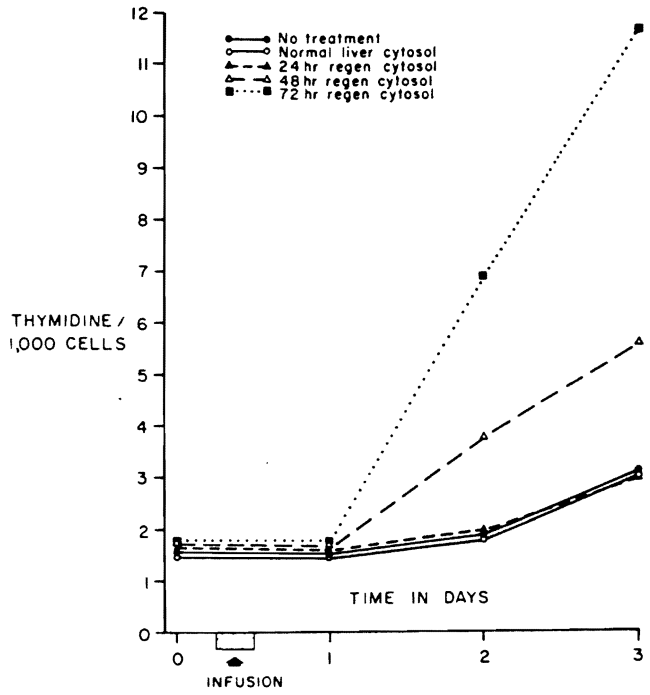

Figure 1

Autoradiography: **Number of thymidine labelled hepatocytes per 1000 hepatocytes. Cytosol from normal and 24 hour regenerating livers does not alter the pattern that occurs after portacaval shunt (no treatment group). The 48 hour and particularly the 72 hour regenerating liver cytosol has a marked stimulatory effect at two and three days.**

In the meanwhile, evidence was accumulating from both *in vivo* and *in vitro* experiments that animals with regenerating livers have a circulating plasma or serum stimulatory factor (1). The data presented above would be compatible with this circulatory factor having originated in the liver itself.

We have recently examined the question of a stimulatory factor in the liver using a previously described portacaval shunt model (3) which permitted extracts of normal and regenerating liver to be introduced into the left tied off branch of the portal vein and tested for regional as well as general hepatic effects (7). Organelle free cytosol extracts from normal dog livers and dog liver after 70% hepatectomy that had been regenerating for 24, 48 and 72 hours were infused for 6 hours only into the tied off left portal vein, 4 to 6 hours after constructing a portacaval shunt. A stimulatory effect was not present in the cytosol from normal or 24 hour regenerating livers but became demonstrable in 48 hour remnants and was highly significant in tissues that had been regenerating for 72 hours (Figure 1). It is presumably no coincidence that both the full development of extract potency from regenerating liver as well as the full response to it in a second animal required 3 days (7), the same time hepatic regeneration reaches a peak after 70% hepatectomy in dogs (8).

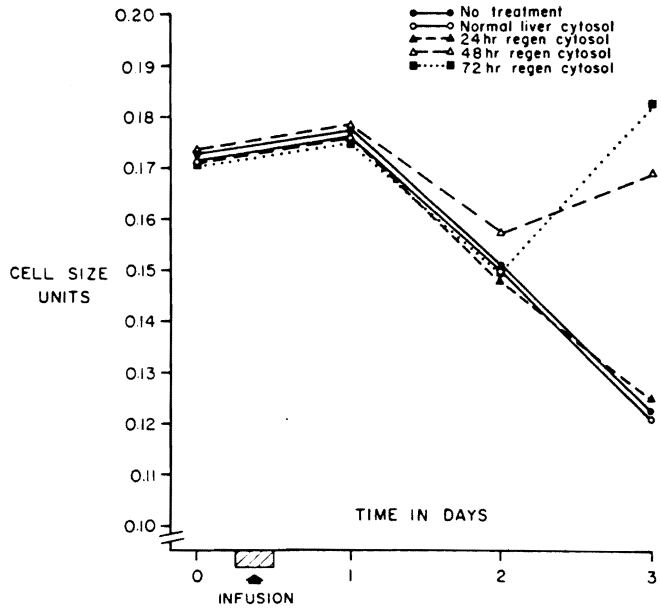

Figure 2

Cell Size: **Cytosol from normal and 24 hour regenerating livers does not alter the atrophy that occurs after portacaval shunt (no treatment group). The 48 hours and particularly the 72 hours regenerating liver cytosol causes reversal of these changes between the second and third days.**

Furthermore, both the 48 and 72 hour regenerating extract reversed the atrophy usually caused by portacaval shunting in 3 days (Figure 2) and partially prevented the ultrastructural hepatocyte deterioration characteristic of portacaval shunt. It was concluded that active liver extracts contained a growth control factor or factors which was (were) not insulin or glucagon.

The brief exposure to the extract shortly after portacaval shunt with a delayed regenerative response is suggestive of a "switch-on" or initiating mechanism. Further experiments are required to confirm and elaborate on these findings. If the active liver substance(s) can be isolated and identified using standard biochemical techniques and confirmed to be potent in this and other models, a stimulator substance will become available which might have important clinical applications. The organ and species specificity of the stimulator substance in liver cytosol still requires further investigation.

INHIBITION OF REGENERATION

Past and recent work has suggested that non-regenerating adult liver and serum contain inhibitory growth factors (1). This was not confirmed in our recent portacaval shunt infusion studies (7); but it was also not excluded, as a greater background mitotic activity might be required to detect inhibition. Infusion of liver cytosol into an already regenerating liver such as a 40% hepatectomy dog model might answer this question, as has already been suggested in rat experiments (6).

CLINICAL IMPLICATIONS

The most important clinical use for a specific hepatic regeneration stimulatory substance might well be in fulminant hepatic failure. Saunders and co-workers have pointed out that the ability of liver to regenerate in the setting of fulminant hepatic failure has been overemphasized in the past (9). As no major breakthrough has been made in the management of this highly lethal condition to date, it is hoped that a better understanding of the controlling mechanisms of regeneration, including both initiators and potentiators, might lead to the development of techniques for stimulating regeneration in these patients (1). Future therapy with as yet unidentified initiators of regeneration is an exciting possibility.

The currently available data on the adverse effects of the portoprival state created by a portacaval shunt makes completely diverting shunt procedures (such as end-to-side portacaval shunt) undesirable in managing patients with portal hypertension. The Warren-Zeppa shunt, which preserves part of the portal flow to the liver, is the one procedure that still holds promise for the future. Alternatively, we should increasingly look for non-shunt procedures, such as periesophageal sclerotherapy of varices (10), to manage patients who have bled from esophageal varices.

On the other hand, the normally adverse metabolic effects of portacaval shunting have been beneficial for some patients with glycogen storage disease or in Type II hyperlipidemia (1). Long term evaluation of patients so treated is of obvious importance.

CONCLUSIONS

The authors current concept is that multifactorial hepatotrophic portal substances play a permissive or potentiating role in hepatic regeneration, and that the possibility still exists that they may actually initiate this process. Although insulin has been shown to be the single most important hepatotrophic factor, other, as yet unidentified, splanchnic factors also play a major role. However, the initiator or initiators (or switch-on mechanism) of hepatic regeneration is (are) as yet unknown. The same applies to the inhibitor or inhibitors (switch-off mechanism) of hepatic growth. Either or both of these mechanisms maintain liver size at a remarkably constant level and stimulate regrowth until the normal mass is once again attained after removal of part of the liver or after liver cell damage. There is a possibility that a hepatic growth control factor or factors originates in the liver itself.

VIEWPOINTS ON DIGESTIVE DISEASES
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Release of CCK during Digestion:

CCK is released from the proximal intestine by partly digested proteins and fatty acids[8,9]. These intraluminal stimulants are teleologically appropriate since they result in secretion of pancreatic proteolytic and lipolytic enzymes and the discharge of bile salts from the gallbladder.

NEURAL CONTROL

Adrenergic innervation of gallbladder muscle is sparse; most of the adrenergic fibers in the human gallbladder are distributed to blood vessels. The extent of vagal cholinergic innervation of gallbladder smooth muscle has yet to be defined; vagotomy is said to decrease nerve fibers in the wall of the gallbladder by 10%. Cholinergic agonists contract and β-adrenergic agonists relax gallbladder smooth muscle but the extent of cholinergic and adrenergic control of gallbladder motor activity is uncertain.

VIP-immunoreactive nerve fibers and cell bodies have recently been demonstrated in muscle and submucosal layers of the gallbladder in man and other mammals and appear to reach the gallbladder via the vagus nerve. VIP relaxes gallbladder muscle and antagonizes the contractile effect of CCK[11]. It seems reasonable to speculate that VIP and CCK function as the neural and hormonal limbs of a peptide system for the control of gallbladder motor activity.

SUMMARY

Hepatic bile is converted in the gallbladder from an isotonic solution of NaCl and $NaHCO_3$ to a concentrated but isotonic solution of bile salts. The gallbladder mucosa is a typical leaky epithelium with high passive permeability to ions and water. The distinctive feature of active ion transport by the gallbladder is the presence of an electrically neutral coupled NaCl influx process at the luminal membrane which insures a one-to-one absorption of Na^+ and Cl^- and is partly responsible for the absent or low transepithelial potential difference. Gallbladder contraction and choledochal relaxation are under the control of a peptide hormone, cholecystokinin. The extent of neural control of gallbladder motor function is uncertain.

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