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Editorial

Hepatic regeneration: Implications in fulminant hepatic failure

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Although the incidence of fulminant hepatic failure
appears to have decreased worldwide, the management
of this dreaded condition remains unsatisfactory (1).
Despite ingenious methods of treatment, the mortality in
patients with grade IV coma is still between 80 and 90%
in most major series (15, 20). Furthermore the mortality
is nearly 100% in patients over the age of 45 who de-
velop grade IV coma (7).

Saunders et al have pointed out that although heroic
measures, such as exchange transfusion, cross circula-
tion and isolated liver perfusion, can reverse the
neurologic and, to an extent, the hematologic complica-
tions of massive liver cell necrosis in some patients,
none have increased the survival rate (15). The same
unfortunately also applies to newer methods of treatment
(1). The hope that an «artificial liver» could be developed
to replace the many intricate functions of the liver, while
waiting for regeneration to take place, has not been
borne out in practice because of the complexity of the
liver itself (2, 7), and because of the surprising finding
that many of these patients do not regenerate their livers
adequately, if at all (10, 15).

In the Cape Town Experience of 119 cases of fulminant
hepatic failure, 68 patients were in grade IV coma. In
this group the survival rate was only 16% despite inten-
sive care and the use of various additional treatment
modalities (15, 20). Autopsies were available in 40 pa-
tients. In 23 some regeneration was noted but in only 5
was this of significant degree. In 7 no regeneration was
detected at all. Event patients who died late after the
onset of the illness frequently showed little evidence of
regeneration (15). This led them to conclude in 1972 that
«although the liver has an enormous regenerative capaci-
ty in certain experimental situations, and sometimes after
massive necrosis, this ability has been overemphasized
in fulminant hepatitis and is not consistent with our ex-

perience in many patients. All the current methods of
treatment will not influence mortality unless suffi-
cient regeneration occurs spontaneously or can be stimu-
lated therapeutically» (15). This lack of regeneration in
many patients with severe fulminant hepatic failure has
been confirmed in other large series (10, 23). The conclu-
sion of a recent conference held at the NIH was that the
best one can offer a patient with fulminant hepatic failure
at this time is meticulous intensive care (1, 2).

If artificial liver support systems are inadequate, and
lack of regeneration a problem, can anything be done to
potentiate or stimulate regeneration in patients with ful-
minant hepatic failure? At this time the answer is no,
largely because of our limited knowledge of what
switches on or controls liver regeneration. In this editori-
al the authors will address themselves to what is current-
ly known about the control of liver regeneration and
speculate how this knowledge might be used in the fu-
ture management of patients with fulminant hepatic fail-
ure.

Liver regeneration

Neither the switch-on nor the switch-off mechanism
that controls normal liver growth so accurately is known.
A current area of controversy is whether there are
specific factors, particularly the so-called portal hepatot-
rophic factors, that potentiate liver regeneration or permit
it to occur normally once it has commenced, and other
factors that actually initiate or switch on the regenerative
response; or alternatively whether both potentiation and
initiation are caused by the same factor or factors. Recent
evidence on potentiation of regeneration supports the
multifactorial concept, particularly with regard to portal
hepatotrophic factors (14), which has been a consistent
hypothesis in all work from Denver (19). On the other
hand, there is no concensus on what actually initiates
regeneration and whether liver cell growth is controlled
by stimulators or inhibitors or both.

The Concept of Potentiation of Regeneration

This has developed as a result of a better understand-
ing of the role played by the «quality» of blood reaching
the liver, or the so-called portal hepatotrophic concept,
which has largely replaced the «flow» or «quantity» of
blood hypothesis that was widely accepted after
Bollman's review in 1961 (4).

Hepatotrophic factors have been studied in vivo using
a number of ingenious animal models and in vitro using
liver cell culture systems. It has been clearly de-
monstrated that portal blood specifically influences both

the morphology and function of the liver on the one hand, and liver regeneration on the other (19). These two effects will be considered separately although they might both be important in the context of liver regeneration after liver cell injury. Most of the *in vivo* data has been accumulated from studies in which all or part of the liver has been deprived of portal venous return, or in which partial or complete removal of non-hepatic splanchnic viscera has been performed and by infusing hormones or other substances into either the systemic or portal venous system (19).

When portal blood is deviated from part or the whole of the liver by various technical maneuvers including complete portacaval shunting, a well defined portoprival state ensues in the liver. Morphological changes include hepatocyte atrophy and electron microscopic aberrations, the most striking and specific of which are depletion and disruption of the rough endoplasmic reticulum and reduction in membrane bound ribosomes. These changes occur rapidly, being almost complete within 4 days after portacaval shunt. Functional changes include inefficient clearing of ammonia and other substances and antilipidemic effects (19).

The striking organelle alterations make it likely that the portoprival state will have other subtle functional effects that are wide ranging and which might even effect the regenerative response.

Investigations from many laboratories, including Denver, have clearly demonstrated that portal factors influence regeneration and that a portoprival state has an adverse effect on regeneration. This work has been reviewed in detail recently (19).

A series of studies have been performed in a variety of *in vivo* experimental models over a number of years in Denver. In expressing the results in practical terms, which were applicable to both regeneration and morphological and functional changes, it was concluded that the most favorable condition for portal perfusion was with splanchnic venous blood which contained normal amounts of endogenous insulin. The least favorable condition was perfusion with systemic blood. Intermediate in quality was splanchnic venous blood that was deficient in endogenous insulin but which was rich in other as yet unknown elements (19). A recent study using differential evisceration has confirmed that gut factors play an important role in addition to pancreatic factors. The hepatic regenerative response after partial hepatectomy in dogs was reduced more by evisceration with preservation of the pancreas than by pancreatectomy alone (17). This further strengthens the multifactorial hypothesis with regard to the hepatotrophic factors' role in potentiating regeneration by clearly differentiating pancreatic influences from those originating in the rest of the intra-abdominal gastrointestinal tract.

The Concept of Initiation of Regeneration

All the evidence presented so far supports the concept that portal hepatotrophic factors are important as potentiators of liver regeneration. The obvious question is whether portal hepatotrophic factors also control regeneration by initiating the process and switching it off once liver mass has been restored. To date the most compelling argument favoring this controlling or initiating concept for hepatotrophic factors has been the changes shown to occur in the hormonally controlled «messenger» components in the liver during regeneration using both *in vivo* and *in vitro* models (19). Cyclic AMP and adenyl cyclase undergo well ordered biphasic changes both prior to the onset of the phenomena used to quantitate regeneration (DNA synthesis and mitosis) and during regeneration (9). The authors presently concede that these changes may be coincidental and that those manifestations that occur prior to DNA synthesis could be merely evidence of an earlier stage of a regenerative response that has already been initiated by some other factor or factors. This hypothesis has at least as much support from currently available evidence as the alternative hypothesis that portal hepatotrophic factors are the initiators of hepatic regeneration.

An important additional, and by no means contradictory, possibility that something in the liver itself, after liver cell damage (or partial hepatectomy), contributes to, or even initiates, its own regrowth merits careful evaluation. This is not a new concept and the literature, which extends back over 50 years, has been reviewed recently (19). Although early work was contradictory, various groups demonstrated that liver mitoses could be stimulated in intact experimental animals using homologous liver mash injected intraperitoneally or intravenously. The first truly convincing evidence of a liver specific mitotic stimulator was presented in 1953 (22) and confirmed in 1957 by Blomqvist in Helsinki (3). He showed that liver mash prepared both from weanling (young) rats, and the already regenerating remnant of an adult rat liver 48 hours after partial hepatectomy, showed striking liver mitotic stimulatory activity when administered one-time intraperitoneally to adult rats. On the other hand, normal adult liver mash was not stimulatory. In the meanwhile, evidence from both *in vivo* and *in vitro* experiments clearly demonstrated that animals with regenerating livers contained circulating plasma or serum liver mitotic stimulatory factors (19). Fisher and co-workers, using cross circulation in rats in 1971, not only showed that partial hepatectomy in one of a pair of cross circulated animals stimulated significant DNA synthesis in the cross circulated partner's intact liver, but also that DNA synthesis was similarly stimulated in the partner's intact liver after total hepatectomy had been performed in the other rat.

They postulated that the source of the humoral factor was not in the liver (8). Although this contention was challenged by Levi and Zeppa (12), their work was not reproducible (13). This confusing and controversial data on the liver as a source of the mitotic stimulatory substance probably influenced the recent major emphasis on portal hepatotrophic factors as initiators of regeneration.

In 1975 La Brecque and Pesch demonstrated a regenerative stimulatory substance in the supernatant after high speed centrifugation of an extract of rat liver mash which was present in very young (weanling) rat livers, but only appeared after partial hepatectomy in adult livers. Their assay system was an already regenerating adult rat liver after 35% hepatectomy (11). Thus attention was once again directed to a liver source for the humoral factors. The authors have pointed out that, if confirmed, this would strongly support a liver-plasma physiological axis being important in liver regeneration (19). It is interesting that this possibility was not discussed during a symposium on hepatotrophic factors held as recently as 1977 (14). The authors examined the possibility of there being a liver based regenerative stimulator factor using a previously described portacaval shunt model (16). This model permitted extracts of normal and regenerating livers to be introduced into the left tied off branch of the portal vein and tested for regional as well as general hepatic effects. Organelle free cytosol extracts from normal dog livers and from dog livers 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. This model once again produced unequivocal results. A liver mitotic stimulatory effect was not present in cytosol extracts from normal or 24 hour regenerating livers but was present in 48 hour liver remnants and became highly significant in the remnants 72 hours after partial hepatectomy, at which time it also reversed the atrophy usually caused by portacaval shunt in 3 days (18). It is presumably no coincidence that both the full development of extract potency from the regenerating liver remnant, as well as the full response to it in a second animal, required 3 days (18), as this is the same time that liver regeneration reaches a peak after partial hepatectomy in the dog (9). It was concluded that the active regenerating liver extracts contained a growth control factor or factors which were not insulin or glucagon (18). The brief exposure to the extract, shortly after constructing the portacaval shunt, and the delayed regenerative response is suggestive of a switch-on or initiating mechanism. Unanswered questions include whether the production of this stimulatory factor is an independent quality of the partially resected liver which then creates a new environment in which the portal hepatotrophic factors can be effective or whether the liver factor is depen-

dent from the outset on hepatotrophic substances including insulin. The next steps will be to isolate and identify the active substance(s) in liver extracts and to determine organ and species specificity (18).

Stimulation of regeneration in the treatment of fulminant hepatic failure

The first practical suggestion of a method to stimulate regeneration in fulminant hepatic failure was based on the hepatotrophic concept by Farivar and co-workers. They investigated insulin and glucagon as important hepatotrophic substances and showed that they were effective in prolonging survival in mice with fulminant murine hepatitis (6). They concluded that the possibility that insulin and glucagon might also beneficially influence the clinical course of acute liver cell injury in man merited investigation. This conclusion is open to criticism in that the histologic picture of murine hepatitis is not the same as human fulminant hepatic failure (5), the mean survival time was not as prolonged and there were fewer survivors (15% versus 40%) when treatment was delayed, DNA labelling, indicative of hepatic regeneration, was depressed equally in treated and control mice at 24 hours, and last but not least patients with fulminant hepatic failure have been shown to have raised levels of both insulin and glucagon anyway (1).

The next practical suggestion is to test the mitotic stimulatory factor, which has been shown to be present in regenerating liver cytosol, in fulminant hepatic failure. This has not been proposed previously but will probably have to await the isolation and identification of the cytosol factor. Ideally, if a purified stimulatory factor can be identified, it should first be tested in a suitable animal model. Unfortunately, this poses problems as a truly suitable animal model of fulminant hepatic failure, meeting all the defined criteria, has not yet been developed (21). Nevertheless, the cytosol extract should at least be tested in the best available animal model. Because of the deficiencies of animal models, the authors believe that a negative result should not prevent the extract being tested in patients. Initial pilot tests should probably be limited to patients with grade IV coma, particularly patients over 45 years of age. Thereafter, it must be subjected to a properly controlled clinical trial, even accepting the multiple difficulties inherent in conducting clinical trials in fulminant hepatic failure (1).

Conclusion

Many patients with severe fulminant hepatic failure fail to regenerate their liver adequately. Although there

are no currently available methods of stimulating liver regeneration in these patients, the possibility of isolating and purifying an active factor from an already regenerating liver and using this to stimulate or even initiate regeneration in this setting is an important practical goal.

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