

# Hepatic regeneration: If it ain't broke, don't fix it

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The capacity for the liver to regenerate after injury or resection has long been recognized, as implied by the legend of Prometheus. Resections of up to 70% of the liver are followed by a sequence of events that generally result in complete restitution of hepatic mass and function. Hypertrophy of hepatocytes begins within hours, with accumulation of amino acids and triglycerides and activation of enzymes that are associated with proliferative activity. Increased DNA synthesis is associated initially with hyperplasia of hepatocytes, and then other cells, which begins in the periportal region and spreads in a wave-like fashion to the pericentral region of the lobule. Quiescent hepatocytes are primed to enter the cell cycle and then proceed through the G<sub>1</sub>/S and G<sub>2</sub>/M restriction points, under the influence of a variety of proteins, growth factors (especially hepatocyte growth factor) and cycle dependent kinases. At each stage there is interplay between growth promoters and inhibitors, including transforming growth factor-beta and GABA. Factors that initiate hepatic regeneration are unknown, and might include hepatic depolarization, increases in blood flow, destruction of liver matrix (with release of growth factors), and increased production or expression of growth promoters compared to inhibitors. Regenerative activity increases with the amount of resection to a point, and then relatively declines. Uncontrolled proliferation of liver tissue after resection or injury is not necessarily beneficial, because it could lead to a diversion of resources from the maintenance of hepatic function and to an increased risk of neoplasia. Therefore, it is unclear whether clinicians should attempt to enhance hepatocyte regeneration. Since both hepatic regeneration and metabolic function require energy from high-energy nucleotide triphosphates, especially adenosine triphosphate (ATP), a reasonable strategy might be to augment energy delivery and ATP production. Mortality rates after limited (fewer than 70%) resections and mild or moderate injuries of previously normal livers are low, and supportive care is often sufficient. The prognosis is unclear; however, in cases of more massive resection, resections in the setting of underlying liver disease or cirrhosis, and fulminant hepatic failure, and liver transplantation is still an important option.

**Key Words:** ATP; CdK; Cyclin; GABA; IL-6; HGF; Liver, Liver disease, Liver regeneration, TGF- $\beta$ ; TNF

## La régénération hépatique : Si ce n'est pas cassé, ne le réparez pas

La capacité du foie à se régénérer après une lésion ou une résection est admise depuis longtemps, comme le sous-tend la légende de Prométhée. Des résections de jusqu'à 70 % du foie sont suivies d'une séquence d'événements qui résultent généralement en la restitution complète de la masse et de la fonction hépatiques. L'hypertrophie des hépatocytes se manifeste dans un délai de quelques heures, de même que l'accumulation d'acides aminés et de triglycérides et que l'activation des enzymes associées à l'activité proliférative. Une synthèse accrue de l'ADN est reliée, au départ, à l'hyperplasie des hépatocytes, puis à celle d'autres cellules, qui se manifeste dans la région périportale et se propage par vagues à la région péricentrale du lobule. Les hépatocytes quiescents sont poussés à pénétrer dans cycle cellulaire, puis à traverser les points de restriction G<sub>1</sub>/S et G<sub>2</sub>/M, sous l'influence de diverses protéines, de divers facteurs de croissance (surtout le facteur de croissance des hépatocytes) et des kinases dépendantes du cycle. À chaque phase, une interaction s'installe entre les promoteurs et les inhibiteurs de croissance, y compris le facteur de croissance transformant bêta et le GABA. Les facteurs qui initient la régénération hépatique demeurent inconnus, mais peuvent inclure une dépolarisation hépatique, un accroissement du débit sanguin, une destruction de la matrice hépatique (avec libération des facteurs de croissance) et une augmentation de la production ou de l'expression des promoteurs de croissance par rapport aux inhibiteurs. L'activité régénératrice augmente avec l'importance de la résection jusqu'à un certain point, puis elle décline relativement. La prolifération incontrôlée du tissu hépatique après une résection ou une lésion n'est pas nécessairement bénéfique, car elle peut entraîner une diversion des ressources destinées au maintien de la fonction hépatique et une augmentation du risque de néoplasie. Par conséquent, on ne sait pas vraiment si les cliniciens devraient tenter de favoriser la régénération hépatocytaire. Puisque tant la régénération hépatique que la fonction métabolique exigent de l'énergie tirée de nucléotides triphosphates à haute énergie, et surtout de l'adénosine triphosphate (ATP), une stratégie raisonnable pourrait consister à augmenter la livraison d'énergie et la production d'ATP. Le taux de mortalité (inférieur à 70 %) après une résection limitée et une lésion bénigne ou modérée de foies auparavant sains sont faibles, et des soins d'entretien suffisent souvent. Le pronostic est incertain. Cependant, en cas de résection plus massive, de résections au foyer d'une maladie hépatique sous-jacente ou d'une cirrhose ou d'insuffisance hépatique fulminante, la greffe du foie demeure une option.

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## HISTORICAL PERSPECTIVE

The story of Prometheus is often considered compulsory reading for students and investigators of hepatic regeneration. A common version of the tale has Prometheus and his brother Epimethius (possessor of foresight and hindsight) fearful of the emerging powers of the gods who are intent on eliminating the two brothers because they represent the last surviving Titans and hence, challengers to their authority. Thus, in an effort to placate the gods, Prometheus decides to create humans and have them burn sacrifices as offerings to the gods. To obtain the fire required, Prometheus travels to Olympus where he steals a flame from Hephestus' forge. However, he is caught in the act and the gods (Zeus in particular) decide to punish both brothers. In the case of Epimethius, the punishment consists of having to mate with Pandora and forcing their offspring to live and intermarry amongst humans (a lower form of life according to the gods). Prometheus' punishment consists of being chained to a rock where a vulture (although some believe a raven) would come daily to feast on his liver (Figure 1). Fortunately, after 30 years, Hercules takes pity on Prometheus and frees him from the rock and his sentence of perpetual torture.

The 'take home' message from this myth (beyond that crime does not pay) is that the liver has an unlimited ability to regenerate. Somewhat more subtle, yet equally important, messages include the following: the liver does not require infusions of growth promoters to restitute liver mass following partial hepatectomy; signs and symptoms of hepatic decompensation do not occur following limited hepatic resections; and hepatocellular carcinoma may be an adverse outcome in regenerating livers (1).

The purpose of the present review is to elaborate on each of these messages and thereby introduce some caution to clinicians and scientists as they strive to identify means of enhancing hepatic regeneration (often irrespective of the clinical setting and/or indications).

### Disclaimer

Before describing hepatic regeneration in detail it should be noted that much of what follows stems from studies of partial hepatectomy in experimental animals and, therefore, may not be relevant to humans recovering from surgical resections, or, even less likely, to those with acute or chronic liver disease. Nonetheless, with the exception of interspecies differences in hepatocyte ploidy (largely 4N in rodents compared with 2N in humans) and the time scale involved (days in rodents versus months in humans), hepatocyte regeneration in humans appears to resemble the process in rodents (2).

## LIVER REGENERATION

The three tissues in the human body capable of regenerating themselves are skin, bone marrow and liver (3). Of these, only the liver can regenerate in the absence of progenitor or stem cell activity. Liver regeneration is a carefully orchestrated process that occurs following even minimal (approximately 10% or less) loss of hepatic mass. Regeneration is complete when 100% of the original liver mass has been restituted. The shape of the regenerated liver is influenced by adjacent structures so that the end result somewhat resembles a normal liver despite the lack of actual regrowth of resected lobes (4). In the case of a liver too large for the space available, the liver involutes by apoptosis and adopts a size that is appropriate for the site and volume of available hepatic blood flow (5).



**Figure 1)** Prometheus was chained to a rock where a vulture returned daily to feast on his liver

In general, hepatic regeneration is divided into two distinct phases: cellular hypertrophy (increase in cell volume or mass) and hyperplasia (increase in cell number).

### HYPERTROPHY

Although hepatocytes represent only 60% to 70% of liver cell numbers, their size is such that they constitute over 95% of total liver mass (6). Thus, changes in hepatocyte dimensions largely dictate changes in liver volume.

Within hours of the loss of liver mass, a series of metabolic changes occur in the remaining hepatocytes that result in their enlargement. One of the first events is an increase in sodium-coupled amino acid uptake by hepatocytes. This results in a minor increase in cell swelling because water follows an osmotic gradient. It is mediated by specific transporters of the alpha-methylamino-isobutyric acid/alanine-serine-cystein-amino acid transporter systems and is considered essential to the regenerative process (7). Shortly thereafter, intracellular triglyceride levels increase and histological evidence of fatty infiltration becomes apparent (6). Increased delivery of triglycerides to the liver rather than *de novo* hepatic synthesis is largely responsible for the fatty infiltration (8). Before and concomitant with these changes is a loss of intracellular glycogen, particularly within pericentral hepatocytes (6).

At an organelle level, mitochondria initially become depleted but rebound within days to become more numerous than at baseline (9). In the remainder of the cell, enzymes associated with proliferative activity (ornithine decarboxylase, thymidine kinase, sodium/potassium-ATPase, etc) are upregulated, whereas those responsible for hepatic and extrahepatic functional activity (albumin synthesis, bilirubin metabolism, etc) and 'housekeeping' are downregulated (2). Of note, enzymes required for oxidative demethylation of and binding to carcinogenic amines are also downregulated during the regenerative period, which renders hepatocytes more prone to malignant transformation (10).

At the lobular level, the distance between portal tracts and central veins increases approximately three-fold immediately

after partial resection, only to recede to a 1.5- to two-fold increase once regeneration is complete (2,11).

Thus, much of the initial restitution of liver mass following partial hepatectomy reflects hypertrophy of hepatocytes and increased lobular dimensions rather than increases in the numbers of hepatocytes or lobules.

### HYPERPLASIA

While hepatocytes are increasing in size and mass, the process of hyperplasia (increase in cell numbers by cell division) is already underway. DNA synthesis begins 10 to 12 h after resection in rats and peaks at 24 h, with a second smaller peak occurring at 36 to 48 h (2,12). Each hepatocyte replicates an average of 1.7 times (4). Proliferation begins with hepatocytes in the periportal area but, after 36 to 48 h, has progressed in a wave-like fashion to the pericentral region (13). Other liver cells proliferate approximately 24 h after hepatocytes, which likely reflects their dependency on the release of hepatocyte-derived growth factors (14). As a result of this somewhat asynchronous (vis-à-vis cell populations) proliferative activity, clumps of cells consisting only of hepatocytes appear initially. These clumps become infiltrated by hepatic stellate cells to form plates and then by endothelial cells to form capillaries (with basement membranes). Eventually, the basement membranes become disrupted and the mature sinusoids are infiltrated by Kupffer cells, which completes the complement of cells present in a mature liver lobule. The final step in lobular development is a change from an immature laminin matrix to one containing fibronectin, collagen IV and I, protein and glycosaminoglycans (15).

### CELL CYCLE

In the quiescent liver, mature, nonproliferating hepatocytes exist in the resting or G<sub>0</sub> phase of the cell cycle (Figure 2). In regenerating livers, hepatocytes become 'primed' to proliferate and enter the cell cycle. This is known as the G<sub>0</sub>/G<sub>1</sub> transition. Initially, under the influence of immediate early proteins (c-fos, c-jun, c-myc, etc) and, subsequently, growth factors, cells proceed through G<sub>1</sub>, a process that takes six to eight hours, until a critical stage, referred to as the G<sub>1</sub>/S restriction point, has been reached (16). Progression beyond this point depends on the relative contributions of growth promoters and inhibitors and cyclin activity.

Of the growth promoters involved, hepatocyte growth factor (HGF) appears to play a pivotal role (4). Also known as scatter factor, HGF is a complete mitogen, capable of stimulating hepatocyte proliferation in vitro in the absence of other mitogens or comitogens (17). It acts by binding to its receptor c-Met (17). Mutations of either the HGF or C-Met genes result in intrauterine death from hepatic agenesis. Following partial hepatectomy, the concentration of HGF increases 20-fold within one hour before returning to normal by 72 h (18). In addition to facilitating immediate early proto-oncogene expression, HGF also enhances insulin-like growth factor binding protein-1 and liver regeneration factor-1, growth promoters that may play a special role in hepatic regeneration (19). Of note, the important growth-promoting cytokine interleukin-6 (IL-6), increases the expression of HGF and c-Met gene promoters (20).

Epidermal growth factor (EGF) is another important growth factor and primary mitogen involved in the progression of cells through the G<sub>1</sub>/S restriction point; however, the con-

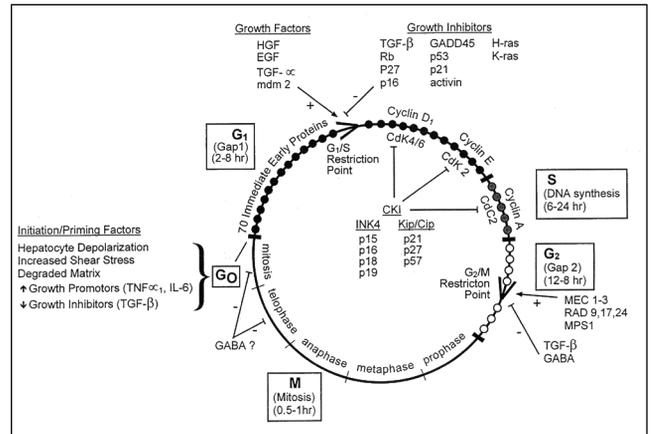


Figure 2) The cell cycle and factors that initiate, promote and inhibit progression of hepatocytes from a resting G<sub>0</sub> state to mitosis. EGF Epidermal growth factor; HGF Hepatocyte growth factor; GABA Gamma aminobutyric acid; TGF Transforming growth factor

centration of EGF increases by only 30% after partial hepatectomy (21). Although this limited increase is often used as an argument against EGF playing an important role in cell cycle progression, it may yet be sufficient because there is less liver tissue to influence following partial hepatectomy. Moreover, EGF is largely released from the Brunner's glands of the duodenum and, therefore, portal venous concentrations may be significantly higher than those measured in the systemic circulation (22).

The other important growth factor and primary mitogen is transforming growth factor-alpha (TGF-α). Although likely contributing to progression through the G<sub>1</sub>/S restriction point, TGF-α appears to play a more important role at later stages in the cell cycle. Increases in TGF-α mRNA expression are delayed by two to three hours following partial hepatectomy and do not peak until 12 to 24 h, before returning to baseline by 48 h (23). This delay raises the possibility that TGF-α might also contribute to non-hepatocyte proliferation, such as that of endothelial cells (along with acid fibroblast growth factor and vascular endothelial growth factor). That TGF-α-deficient mice are able to undergo normal hepatic regeneration suggests that EGF and presumably other growth factors are able to compensate for its absence (24).

The growth inhibitors most often cited as influencing priming and/or G<sub>1</sub>/S transition are TGF-β, Rb, p27, p16, GADD45, p53, p21 and activin (25). If cellular or DNA abnormalities are detected, these regulatory factors, and p53 in particular, exert sufficient inhibitory effects to prevent cell progression through the restriction point. If, on the other hand, no such abnormalities exist, growth promoters predominate and progression ensues.

The actual progression of cells through the cell cycle occurs as a result of activation of cycle dependent kinases (CDKs). CDKs are enzymes consisting of a labile, structural protein and a more stable kinase (25). A series of CDKs (CDK4/6, CDK2, and CDC2) are activated as the cell progresses through different stages of the cell cycle and each binds to a succession of cyclins (D, E, A, and B) to form the complex required for the next step in the cycle (26). Once again, if a defect in DNA synthesis or structure is recognized, the CDK of the CDK/cyclin complex does not become phosphorylated, the

enzyme remains inactive, and CDK/cyclin-induced activation of additional transcription factors (TFs) and genes required for further progression (including the CDKs and cyclins themselves) does not occur.

Another important regulatory step in the cell cycle occurs during the second gap phase ( $G_2$ ), which follows DNA synthesis (S phase) and precedes mitosis (M phase). Here too, progression through  $G_2$  to M is determined by the influence of various growth promoters and inhibitors on a restriction point referred to as  $G_2/M$ . The precise regulation of this site is less well understood but appears to involve MEC1-3, RAD 9, 17, 24, and perhaps metalloproteinase-1 proteins (26). Gamma aminobutyric acid (GABA) exerts its major inhibitory effect on hepatic regeneration at this point in the cell cycle, although spindle formation and chromosomal segregation of the M phase also appear to be adversely influenced by GABA-induced changes in cell membrane potentials (27).

### PRIMING FACTORS

What remains the Holy Grail of hepatic regeneration is the identity of the factor(s) responsible for priming quiescent hepatocytes to enter the cell cycle (28). Leading candidates include: changes in the electrical charges or potential differences across hepatocyte membranes; increases in remnant liver blood flow; destruction of the liver matrix; release (or increased expression) of growth promoting cytokines/factors; and loss (or decreased expression) of growth-inhibiting cytokines/factors.

#### Hepatocyte depolarization

The resting hepatocyte membrane potential *in situ* ranges between  $-30$  and  $-40$  mV (29). Immediately following partial hepatectomy, hepatocytes depolarize to levels approaching  $-25$  mV (30). It has been suggested that this depolarization, which occurs as a result of GABA- $\beta$ 3 receptor downregulation, results in the translocation of positively charged growth promoters such as polyamines to the nucleus. Where these growth promoters bind to the important 'TATA' sequence element, involved in the initiation of transcription, and to negatively charged nucleosomes, may cause unraveling of the DNA helix and, thereby, the initiation and promotion of transcriptional activity by other transcription factors and regulatory elements (31). Although prevention of hepatocyte depolarization by augmenting GABAergic expression results in attenuated regenerative activity, activation of immediately early genes, an early step in the proliferative process, remains unaffected (32,33). Thus, changes in hepatocyte membrane potentials are more likely to play a role in the progression of cells through the cell cycle rather than initiating the process *per se*.

#### Increased hepatic blood flow

Increases in hepatic flow cause an increase in endothelial cell shear stress, which in turn results in increased nitric oxide synthesis (34). High nitric oxide levels activate specific cytokines and transcription factors known to play an early role in the regenerative process (35). While there are many proponents of this theory, the principle arguments against it stem from early studies wherein it was demonstrated that: proliferative activity can occur without increases in and, indeed, in the absence of portal venous blood flow (36); the extent of proliferative activity in the liver is significantly less than would be predicted fol-

lowing arterialization of the portal venous system (37); non-parenchymal cells tend not to proliferate following experiments designed to enhance hepatic blood flow (2); and in carbon tetrachloride-induced liver injury, regeneration occurs but hepatic blood flow remains largely unchanged (36). However, these arguments do not preclude an important role for hepatic blood flow in the regenerative process, since extra-hepatic hepatocytes proliferate following partial hepatectomy and the livers of parabiotic rats also regenerate when portions of their partner's liver are resected, indicating the presence of a humoral contribution to the regenerative process (38).

#### Degradation of the liver matrix

Within five minutes of partial hepatectomy, urokinase receptors appear on the surface of hepatocytes and hepatic urokinase activity significantly increases (39). Urokinase converts plasminogen to plasmin. Plasmin in turn activates metalloproteinases, which cause matrix degradation (39). Because HGF is contained within the liver matrix, matrix degradation results in its release, and urokinase, its activation (40). In addition, collagenase, which is also activated following partial hepatectomy, directly stimulates hepatocyte proliferation (4).

#### Increase in growth-promoting cytokines/factors

The growth-promoting cytokines that have attracted the most interest in explaining hepatocyte entry into the cell cycle are tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and IL-6 (41). TNF- $\alpha$  is released by non-parenchymal cells in response to inflammatory activity and the generation of reactive oxygen species (ROS). That TNF- $\alpha$  might play an important role in initiating the regenerative process is suggested by data indicating that TNF- antibodies inhibit DNA synthesis following partial hepatectomy and abrogate early TF activation (41). Moreover, mice with TNF- $\alpha$  receptor deficiencies do not regenerate their livers following partial hepatectomy (42). Increased TNF- $\alpha$  activity also induces nonparenchymal cells to release the other important regenerative cytokine, IL-6. IL-6 is a downstream mediator of TNF- $\alpha$ , as injections of IL-6 in TNF- $\alpha$  receptor-deficient mice restore hepatic regenerative activity to normal (42).

The precise mechanisms whereby TNF- $\alpha$  and/or IL-6 prime hepatocytes enter the cell cycle are only just now being elucidated. Key to the process is the activation of four important TFs: nuclear factor for kappa chain on B cells (NF- $\kappa$ B), signal transducer and activator of transcription-3, c-Fos- and c-Jun-derived AP1 and CCAAT/enhancer-binding protein alpha (4,12). Of these, TNF- $\alpha$  is largely responsible for NF- $\kappa$ B activation while IL-6 tends to activate signal transducer and activator of transcription-3 (41). Once activated, these and perhaps other less well characterized TFs, such as hepatocyte nuclear factor 1 (HNF1), HNF3, and HNF4 translocate to the nucleus, where they bind to immediate early promoter genes, resulting in the upregulation of approximately 70 immediate early genes (c-fos, c-jun, c-myc, etc). These in turn increase the production of additional TFs in the presence of adequate amounts of HGF, EGF, and TGF- $\alpha$  (4).

In addition to stimulating TNF- $\alpha$  and IL-6 release from nonparenchymal cells, ROS also directly activate TFs and increase the amounts of growth factors. For example, in the case of NF- $\kappa$ B, ROS dissociate the inhibitor of NF- $\kappa$ B from the P65 subunit of the p65/p50 NF- $\kappa$ B heterodimer, thereby permitting activated NF- $\kappa$ B to translocate to the nucleus (12,42).

### Decrease in growth factor inhibitory cytokines/factors

If loss of an inhibitory cytokine is responsible for priming hepatocytes to enter the cell cycle, the cytokine most likely to be involved is TGF- $\beta$ 1. TGF- $\beta$ 1 is a member of the multifunctional TGF- $\beta$  superfamily that is synthesized by hepatic stellate cells, macrophages, lymphocytes, platelets and bile duct epithelial cells. It produces changes in transcriptional activity by activating the Smad intracellular signaling cascade and TFs, SP1, and p53 (43). In vitro studies have demonstrated that the addition of TGF- $\beta$ 1 to primary hepatocyte cultures results in cessation of hepatocyte proliferation (44). In vivo, TGF- $\beta$ 1 expression is attenuated first in the periportal region of the lobule and then in a wave-like pattern towards the pericentral region (preceding hepatocyte mitosis) (45). TGF- $\beta$ 1 receptors are also downregulated during the early regenerative period, rendering them refractory to the effects of exogenous TGF- $\beta$  (46). However, as with other growth regulators, compensatory inhibitory pathways must exist, because TGF- $\beta$ 1 transgenic mice with increased TGF- $\beta$ 1 expression undergo normal hepatic regeneration following partial hepatectomy (47). Some of these compensatory pathways include IL-1 and cyclin/CDK inhibitors such as p21, p19 and p27.

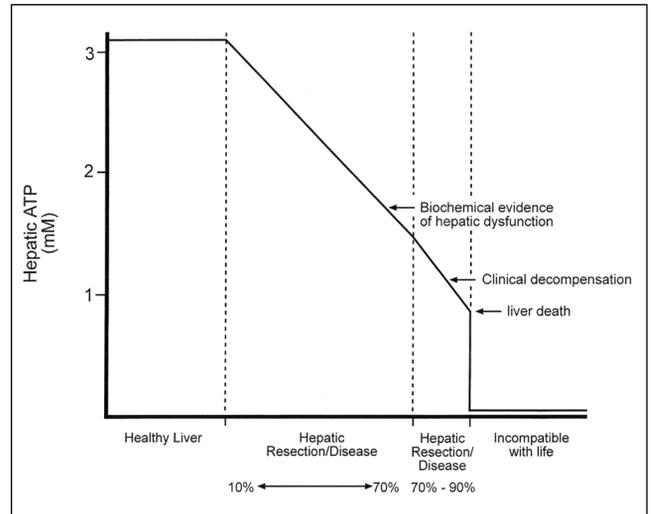
Other factors may contribute to the cessation of regenerative activity. For example, the hepatocyte depolarization that results in translocation of TFs, growth factors and other growth regulators from the cytoplasm to the nucleus resolves within five to seven days after partial hepatectomy. Furthermore, the shear stress associated with increased hepatic blood flow would also diminish as liver mass and blood flow return to normal.

### CLINICAL IMPLICATIONS

Hepatic regeneration following surgical resections or injury involving less than 70% of total liver mass proceeds uneventfully until restitution of the original liver mass is complete, often within three to six months in an otherwise healthy human liver (48). Rates of regenerative activity are proportional to the regenerative stimulus until 70% of the liver has been resected or destroyed (49). Thereafter, there is a precipitous decline (but not to baseline levels) in regenerative activity despite increased stimulation (49).

In the postresection period or convalescent phase following liver injury, serum alkaline phosphatase levels tend to rise (peaking approximately two to four weeks after the initial surgery or injury) (2). This rise reflects bile ductular proliferation and reductions in bile flow. At a functional level, serum albumin, bilirubin and clotting times remain intact with limited resections and/or injury, but become transiently abnormal when 70% or more of the liver mass has been removed or damaged (2,4,50). Mortality rates with up to 70% partial hepatectomy (and presumably liver injury) are less than 5%, and more often reflect complications of the anesthetic or the surgical procedure (for example, pulmonary embolism) than liver failure per se. In view of these good outcomes, the only requirement for hepatic regeneration in patients undergoing limited resections (70%) or recovering from mild or moderate hepatitis is supportive care and patience.

More clinically relevant are cases of extensive hepatic resections in healthy patients, more limited resections in patients with cirrhosis, and fulminant hepatic failure. In these cases, more than 70% (and often 90%) of the liver has been resected or destroyed. Here, the key question is whether the attenuated hepatic regenerative activity that exists in these



**Figure 3)** Hepatic ATP levels decline as the amount of healthy liver tissue becomes limited. The decline is most acute when biochemical followed by clinical signs of decompensation appear. Eventually, hepatic ATP content falls to levels that are no longer compatible with organ survival

settings is protective or harmful. To properly address this question, it must be noted that hepatocytes require energy for the maintenance of hepatic and systemic homeostasis. Some of the more energy consuming activities in the liver include: carbohydrate, fat and protein metabolism; clearance and metabolism of hormones, neurotransmitters and toxins; and excretion of endogenous and exogenous products and metabolites. For the most part, these energy requirements are met by using high-energy nucleotide triphosphates, such as ATP, GTP and UTP. In a quiescent liver, ATP stores are abundant ( $3.2 \pm 0.15$  mmol/L) and more than adequate to meet the functional requirements of the liver (Figure 3). Following hepatic resection or injury, however, new structural proteins and membranes must be synthesized and, as a result, demands for cellular ATP increase (51). When less than 70% of the liver has been resected or injured, hepatic ATP stores fall by approximately 20% but are still sufficient to cover both functional and regenerative requirements. When larger amounts of the liver are removed or damaged, ATP levels fall by 60% and preferential shunting of ATP towards proliferative activity occurs at the expense of hepatic function (51). In addition, actively proliferating hepatocytes are de-differentiated and, as mentioned earlier, down-regulate the enzymes required for functional and housekeeping needs.

Thus, attenuated or submaximal hepatic regenerative activity may represent an inherent attempt to limit the amount of ATP being applied to proliferative activity to maintain an essential amount of hepatic function. Were that the case, what therapeutic options should be considered beyond supportive measures and, for those who are candidates, liver transplantation? The intervention that makes most theoretical sense is to increase energy delivery and/or availability to the liver, thereby allowing both proliferative activity and metabolic function to proceed as required. That might be achieved by: optimizing oxygen delivery to the liver (maintaining high partial pressures of oxygen, adequate hemoglobin levels, and hepatic blood flow); providing adenosine, S-adenosyl-methionine or other

precursors required for ATP synthesis; direct infusions of ATP into the systemic circulation; and in the future, gene therapy with the ATP-encoding creatinine kinase gene (52,53). Bioartificial hepatic support systems and auxiliary or hepatocyte transplantation might also permit failing livers to regenerate without further compromising essential hepatic functions. Finally, recent data indicate that some of the b-hydroxy-b-methylglutaryl-coenzyme A reduction inhibitors, such as lovastatin, alter TFs and signal transduction pathways so that functional activity is enhanced with only limited inhibition of regeneration. This raises the possibility that such agents might be of value in the setting of liver failure (54).

On the other hand, it is also possible that attenuated hepatic regeneration following extensive resections and/or advanced liver disease is potentially harmful and should be corrected to improve survival. This might be particularly true if the critical mass of viable hepatocytes required for survival no longer exists. Were this the case, the therapeutic options to be considered include: augmenting growth promoters; negating the effects of growth inhibitors; and suppressing hepatic functional demands. To date, the value of growth factor and cytokine administration has not been clearly established in the setting of liver failure. This is not surprising because high concentrations of growth factors have already been documented in experimental animals and humans with advanced liver disease (55). Moreover, the biological half-lives of these agents tend to be short (minutes rather than hours or days) and their costs are high. With respect to neutralizing the effects of growth inhibitors, for reasons outlined previously, the principle target of this strategy is TGF- $\beta$ . Pentoxifylline is a phosphodiesterase inhibitor commonly used to improve the circulation of patients with peripheral vascular disease. In addition to inhibiting platelet-derived growth factors, pentoxifylline also inhibits the expression and function of TGF- $\beta$  (56). Of interest, pentoxifylline has recently been reported to improve survival in acute liver failure secondary to alcohol abuse (57). In addition to TGF- $\beta$ , other inhibitors of hepatic regeneration that may be suitable therapeutic targets include ammonia, octanoic acid, mercaptans and GABA (58-60). Each of these compounds is present in increased amounts in patients with advanced liver disease and has been implicated in the pathogenesis of hepatic encephalopathy. Their levels fall with treatment of encephalopathy with neomycin or lactulose, raising the possibility that the clinical improvement achieved with these agents might in part reflect improved hepatic regenera-

tion rather than the elimination of neural inhibition. Less common clinical settings in which suppression of hepatic regeneration is detrimental include protein depletion or fasting in young adults, and patients with adrenal, parathyroid, or perhaps pituitary (or at least prolactin) deficiency (12). Excessive intake of alcohol, certain drugs and local radiation therapy can also decrease and/or delay hepatic regenerative activity (12,60). Implicated medications include actinomycin, cycloheximide, tamoxifen, colchicine and indomethacin. Thus, the correction of the above factors might potentially enhance hepatic regeneration, were that the therapeutic objective. The third option, limiting hepatic functional activities and, thereby, increasing the amount of ATP available for proliferative activity, represents a high risk proposition that must be carefully studied in animal models before being attempted in humans. Nevertheless, the use of an antimetabolite medication, such as propylthiouracil, might be beneficial (61).

Finally, whether by directly stimulating hepatic regeneration or permitting more regenerative activity through interventions that either maintain or limit hepatic function, there remains the theoretical concern that artificially driving cells through the cell cycle, particularly when DNA damage rates are high, could result in: a tendency to overwhelm protective cellular mechanisms; extensive shortening of telomeres; genomic instability; mutagenesis; and, eventually, unregulated cell growth and development of hepatocellular carcinoma. Concerns about carcinogenesis are increased by the fact that proliferating hepatocytes downregulate the enzymes required for demethylation of and binding to carcinogenic amines.

In conclusion, the capacity of the liver to regenerate has been appreciated for centuries. For limited (fewer than 70%) resections and mild or moderate liver injury, the extent and rate of regenerative activity is sufficient to result in the complete restitution of liver mass and function. Whether the same can be said about more extensive resections and fulminant hepatic failure is unclear. Until this question can be answered, it seems prudent to continue to offer transplantation (where indicated) while supporting both hepatic regenerative and functional activity by maximizing ATP synthesis and/or delivery to the failing liver.

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