HEPATOCYTE PROLIFERATION AND DNA SYNTHESIS AFTER PARTIAL ORTHOTOPIC LIVER TRANSPLANTATION.

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ABSTRACT.

Knowledge of liver regeneration following reduced liver transplantation is limited. In order to fully examine the regenerative process, each component of the transplant procedure was examined in isolation. The effects of warm hepatic ischaemia, portal venous occlusion, hepatic sympathetic denervation, graft storage and flushing of the liver with preservation solutions at different temperatures were studied. Finally these factors were combined, and the regenerative capacity of the partial orthotopic liver graft in allogeneic combination studied.

In the first part, partial hepatectomy (ph) was performed on fasted male Long-Evans rats, with animals then divided into groups: group A had ph only; group B had ph + 40 minutes ischaemia; group C had ph + 40 mins ischaemia + temporary portocaval shunt; group D had ph + orthotopic autograft; group E had ph plus orthotopic allograft after 4 hrs cold storage; Group F were sham-operated. Animals were sacrificed after periods of 4, 24, 48, 72 and 96 hrs and the livers weighed, examined histologically, and assayed for thymidine kinase (TK) and ornithine decarboxylase (ODC) activity by standard methods. Serum aspartate aminotransferase (AST) was recorded. A liver mass index (LMI) was derived for each animal as an index of liver to mass ratio. Mitotic figures per 1000 cells were counted (mitotic index or MI). In group A (ph), peak levels of TK activity (dpm per mg protein) (36 021+-8060) and MI (25+-7) were measured at 24 hrs, with

restitution of the liver mass to that of shams (group F) by 96 hrs. In groups B, C, D and E maximal TK and MI was observed at 48 hrs. The magnitude of the peak response in these groups appeared to correlate with the duration of portal venous occlusion, with greatest increases occurring in those groups where portal stasis was most prolonged. The increase in liver mass for these groups was also delayed with respect to controls. The anticipated peak in ODC was seen at 4 hrs in group A. The ODC response in the other groups was disorganized. The effect of hepatic dearterialization on liver regeneration was examined in group K. In these animals TK activity (36 308+-6 063) and LMI recorded at 24 hours (0.476+-0.33) was no different to that of controls, although the MI (5+-3) was diminished at this time. This suggested a comparable rate of regenerative growth to partially hepatectomized controls. The effect of *in situ* liver flushing on hepatic regeneration was studied by dividing animals into 6 groups: group A ph only (controls); group G ph + Ringers lactate flush at 37°C; group H ph + Ringers lactate flush at 4°C; group I ph + University of Wisconsin solution flush at 4°C; group J ph + Euro-Collins solution flush at 4°C. In groups G (4789+-618), H (10531+-3572), I (11790+-=5278) and J (7130+-2060) TK activity was diminished at 24 hrs ($p<0.05$) with respect to controls. The MI was similarly diminished: group G (1+-0), group H (1+-1), group I (1+-0) and group J (1+-0)($p<0.01$). Instead, peak TK activity was recorded at 48 hrs: groups H (24916+-6434), I (33655+-6697) and J (26676+-3932). The MI demonstrated a similar pattern, and was greater than the MI of group A (9+-3) at this time: groups H (18+-4)($p<0.01$), I (40+-6)($p<0.01$) and J (17+-3)($p<0.05$). The LMI showed restitution to that of shams (group F) by 96 hrs in groups H, I and J. ODC activity was disorganised in all but group A.

The results of these studies suggest that hepatic ischaemia of 40 minutes duration delays the onset of liver regeneration following partial hepatectomy. This delayed

response may reflect the effects stimulation of cytokine release or diversion of hepatocyte synthetic function towards essential protein synthesis. Portal venous stasis appears to enhance the proliferative response. Hepatic denervation by surgical means has no apparent effect on the early regenerative response. *In situ* liver flushing has a similar effect on the initiation of DNA synthesis to that observed in ischaemia. That is, the process is delayed by 24 hrs but is nevertheless complete by 96 hrs. There is no advantage of any one flushing solution over another in this regard. The observed effect cannot be altered by normothermic flushing. The technical procedure of liver transplantation has no direct implications in its own right on liver regeneration. The effects of ischaemia and liver flushing on liver regeneration are not additive when in combination, that is in the stored liver allograft. Despite an early delay in the initiation of the process, the regenerative response of the partial liver graft is largely unaffected by the multiple insults to which it is exposed during experimental transplantation.

I declare that this dissertation is my own unaided work, except for the assistance acknowledged. It is being submitted to the University of Cape Town for the degree of Doctor of Philosophy in Surgery. It has not been submitted before for any other degree or examination at any other University.

Douglas Glynn Bolitho

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To my family for their unfailing support.

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1. INTRODUCTION.

Among the most important problems facing patients with end-stage liver disease who qualify for liver transplantation is the shortage of donor organs.

This has led to the usage of split and reduced liver transplantation in the paediatric age group as a means of improving the utilization of donor material. In this procedure, the intention is generally to transplant a mass of liver parenchyma that is appropriate to the size of the recipient.

Despite the knowledge that the liver has an unsurpassed ability to regenerate (78), and the finding that immunosuppressive drugs enhance this process (149), little attempt has yet been made in clinical practise to transplant a small-for-size segment of liver into an adult donor (71). This would enable, for example, the matching of a single adult donor to two adult recipients (split liver transplant) or a related living transplant into an adult recipient.

Little is known about the regenerative response of reduced liver grafts. The regenerative response of full-size liver grafts is delayed in laboratory animals (73,290). Knowledge of the proliferative kinetics of reduced liver transplants is of cardinal importance to the clinical practice of reduced liver transplantation in adult recipients. Furthermore, ischaemia has been noted to be a particular problem in reduced liver grafting (21), contributing to greater graft complications.

In order to examine more closely the regenerative process, we elected to dissect the transplant procedure into its various components:

1. Warm hepatic ischaemia.

2. Portal venous occlusion.

3. Hepatocyte and endothelial damage imposed by flushing with preservation solutions.

4. Storage of the liver.

5. Technical factors associated with the procedure itself.

The purpose of the study therefore, was to study whether a normal regenerative response occurs in the most abnormal circumstance of liver transplantation. Additional insults would take the form of drugs commonly administered to the transplant recipient, and rejection of the allograft. The effects of these two factors were excluded from the current study. The problem of rejection was avoided by utilizing a reduced liver autograft model.

It seemed that if smaller-than-normal liver transplants regenerated in a near normal fashion, then the first major obstacle towards a new era in clinical practise in which reduced and split liver grafting can be applied to adults would have been overcome. This is already being achieved in renal transplantation, where reliance is placed on the ability of the kidney to undergo compensatory hypertrophy in operations using small-for-size donor kidneys on adult recipients. The additional advantage of this practise in liver transplantation would be the greatly improved utilization of donor material.

A review of current literature in the fields of liver regeneration, ischaemia and reduced liver transplantation is presented in the next three chapters to provide a background for the development of the hypothesis.

2. REVIEW OF HEPATIC REGENERATION.

An attribute of the liver that continues to fascinate investigators is its latent capacity for growth. The hepatocyte is a highly differentiated cell that rarely divides in adult humans or animals (approximately 1 mitosis is seen in 10 000 to 20 000 hepatocytes). Mature hepatocytes are long-lived and may survive for the adult life of the animal. The simple technique of partial hepatectomy, a well tolerated and highly reproducible operation, sets in motion a burst of astonishingly rapid growth. This growth is precisely regulated, and ceases when the deficit is restored (31).

Most of the knowledge on hepatocyte growth *in vivo* derives from studies of the regenerating liver after partial hepatectomy in rats. The term *regeneration* is not biologically correct however, in that the growth response induced by the removal of liver mass is not truly regenerative. It entails enlargement of the residual lobes rather than regrowth of the excised lobes. Restoration of the liver mass occurs by the compensatory hyperplasia and hypertrophy of the cells in the remaining lobes (32). However, it is a term that is entrenched in the literature and thus it will be used throughout the study.

In addition to pathological types, three types of hepatic growth are generally recognized (31). That occurring (a) during normal development, (b) in response to altered physiological demands such as pregnancy, and (c) in compensation for lost

or damaged tissue. They may be termed (a) developmental, (b) additive and (c) reparative respectively (31). Additive and reparative growth are relevant to liver regeneration.

The technique used for partial hepatectomy in rats was described by Higgins and Anderson in 1931 (116), and consists of excision of the two main lobes of the liver (median and left lateral). It has been shown that in most strains of rats these lobes bear a constant relationship to the mass of the whole organ; that is 68% (+-2%) (30). Unless otherwise stated in this thesis therefore, partial hepatectomy (ph) will mean resection of this amount of liver tissue.

Despite intensive study for a period of over 60 years (116), our knowledge of the key factors governing regeneration is still incomplete. The topic of liver regeneration has been extensively reviewed (78,194,259). In this review emphasis is given to those aspects of liver regeneration that are of relevance to this study.

2.1 MORPHOLOGIC ASPECTS OF REGENERATION.

The liver mass of partially hepatectomized animals is restored with extraordinary rapidity, the residual lobes in normal adult rats nearly doubling in size by 48 hours and approaching the original liver mass by seven days (32). After a 24 hour lag during which synthesis of new protoplasmic constituents and replication of DNA are initiated, mitosis of hepatocytes begins (25). The most pronounced change in gross appearance is caused by an extensive temporary infiltration of neutral fat, which results in the enlarging remnant taking on a yellowish hue (32).

Parenchymal liver cells constitute 90 to 95% of the total hepatic cellular volume, and 60 to 65% of the cellular population (28). The first morphologic changes to

occur after partial hepatectomy take place in this cell population. Within an hour after partial hepatectomy, the so-called basophilic bodies of the cytoplasm start to disintegrate, beginning in the hepatocytes at the periphery and spreading to the centre of the lobule during the next 8 hours. By 2 to 3 hours cytoplasmic inclusion bodies or fat vacuoles have begun to accumulate and become increasingly conspicuous (45). This accumulation of neutral fats is usually attributed to the metabolic overload imposed by the circulating lipids upon a reduced cell population (45). Meanwhile, glycogen stores are rapidly depleted, falling to very low levels by 10 hours and thereafter reappearing gradually. These changes have prompted observers to compare the histologic changes in regenerating liver to those of starvation (31). Cells, nuclei and nucleoli start enlarging by 6 to 12 hours, more than doubling in size by 24 hours (281). During this period, the sinusoidal spaces become progressively diminished as the enlarged fatty hepatocytes crowd in, distorting the normal lobular pattern. Littoral, ductular and connective-tissue cells remain fairly unchanged (32). These histologic changes have been examined in detail under the electron microscope (278), but discussion of these is beyond the scope of this review.

After partial hepatectomy, the first clear-cut morphologic evidence that cellular proliferation is imminent is the incorporation of nucleic acid precursors into DNA. This has been shown by the incorporation of radioactive precursors into DNA by autoradiography (105). This results in doubling of the DNA pool, a prerequisite for mitosis. After a lag period of about 12 hours, there is a sudden burst of activity, reaching a sharp peak at about 20 hours, and followed by a more gradual decline (32). The pattern and timing of this response are dependent on the age of the animals studied (29), with weanling rats demonstrating an early biphasic response, whereas in older rats the initial rise is more gradual and shows a broader and slightly later peak (29).

Initially, DNA synthesis occurs only in the cells of the outer two-thirds of the lobule, although later on this becomes more random (105). This is the area surrounding the terminal portal venules and the site of entrance of portal blood into the parenchymal unit. Whether this reflects the presence of hepatotrophic substances in the portal blood or simply the greater availability of labelled precursors is not known (31).

Peak mitotic activity follows DNA synthetic activity by about 6 to 8 hours (32). However, there is not complete synchrony in their division. This is evident from the finding that whilst mitosis in any given cell lasts less than 60 minutes (67), the mitotic peak for the liver as a whole is spread over 12 hours (32). About 20 to 30% of normal adult hepatocytes are binucleate, with a high rate of polyploidy. During regeneration the proportion of cells with two nuclei drops to 10% and the ploidy increases correspondingly (31).

The nonparenchymal cells, unlike the hepatocytes, are uniformly diploid. In their response to partial hepatectomy, they lag approximately one day behind the hepatocytes with their mitotic peak occurring near the beginning of the third day (105). Despite this initial lag, by the eighth day their numbers have increased by the same factor (3.37 fold) as the parenchymal cells (31). Of interest is that in the regenerating liver the Kupffer cells (littoral cells that exhibit phagocytic activity) are doubled in number and demonstrate enhanced phagocytic activity for several months (28). The proliferative response however, appears to be more active in the hepatocytes than in the nonparenchymal cells.

Connective tissue is restored slowly, with the collagen content remaining low for more than 6 months (31). The cells of the bile ducts do not contribute to the growth in the parenchymal cell population during regeneration, nor is there evidence for

the existence of a reservoir of "stem cells" from which new hepatocytes could arise following partial hepatectomy (105). As regeneration approaches completion, the normal appearance of the liver lobule is restored, although some changes such as polyploidy (28) persist.

2.2 BIOCHEMICAL EVENTS AFTER HEPATECTOMY.

2.2.1 Protein and nucleic acid synthesis.

Biochemical alterations in the stimulated hepatocyte occur prior to, and simultaneous with the abovementioned morphologic changes. These biochemical events concern primarily the macromolecules at the core of the regenerative scheme - proteins and nucleic acids. Important alterations in protein and RNA formation precede the increase in DNA synthesis that occurs 12 to 14 hours after partial hepatectomy (31).

Prior to mitosis, DNA replication occurs in order that each daughter cell receives an exact copy of the parental genome. By the process of transcription, information contained in the DNA is transferred to several types of RNA: transfer RNA (tRNA), messenger RNA (mRNA) and ribosomal RNA (rRNA). These in turn participate in translation of the genetic code by providing a template for the synthesis of the cellular proteins responsible for cell structure and function. The precise details of the process of protein synthesis are beyond the scope of this review and can be found in standard biochemical texts (283).

Instead, this discussion will concentrate upon the timing of the protein synthetic response following partial hepatectomy. Deoxyribonucleotides, normally present in minute amounts in the liver, increase during the period of 12 to 24 hours after

partial hepatectomy (28). Of these, deoxythymidine triphosphate (dTTP) has the distinction of utilizing enzymatic pathways that are unique to its synthesis ie. not shared in the synthesis of the other 3 deoxyribonucleotides. During regeneration the activities of these enzymes increase (variously from several to 25 fold or more), starting at the time that DNA synthesis increases (17) and reaching a maximum at 10 to 20 hours after the DNA synthetic peak (28,79). It has also been found that X-irradiation before, or shortly after partial hepatectomy, not only blocks DNA synthesis but the thymidine kinases do not appear at the expected time (17). These results were the first to suggest that the enzymes that phosphorylate thymidine may play an important role in the initiation and control of DNA synthesis (12). Confirmation of this was provided by the finding that the elevation in thymidine kinase corresponded with the incorporation of [$_{14}$C]thymidine into DNA of regenerating rat and pig liver (133). The increase noted in thymidine kinase and other deoxyribonucleotide kinases during liver regeneration reflects enhanced production of new enzyme molecules, probably via the mechanism of derepression (31). The synthesis of thymidine kinase at this time is governed by negative feedback control mechanisms (125), in addition to other control mechanisms (194) which will be discussed later in detail. There would appear to be many safeguards against untimely or inappropriate DNA synthesis.

DNA polymerase is another enzyme which increases with DNA synthesis (79), although differing in that it is active in normal liver. Whilst DNA replication cannot occur in its absence, cessation of DNA replication is not related to the decline in DNA polymerase activity. This would suggest an accessory, rather than primary role in the control of DNA synthesis in regenerating liver (31).

Puromycin, a protein synthesis inhibitor, will suppress DNA replication in regenerating liver, despite the presence of adequate amounts of DNA polymerase and the various deoxyribonucleotide kinases (103). It would appear that in addition to these enzymes, a necessary prerequisite for DNA synthesis is the synthesis of some protein.

2.2.2 Polyamine metabolism.

Another metabolic pathway to be stimulated by the regenerative stimulus of partial hepatectomy is that concerned with polyamine metabolism. The polyamines putrescine, spermidine and spermine have been shown to play vital roles in hepatocellular growth and differentiation (127). The first step in the synthetic pathway to the polyamines is the decarboxylation of ornithine, a reaction catalyzed by ornithine decarboxylase (ODC). After partial hepatectomy a marked change in ornithine decarboxylase activity occurs during the prereplicative phase of the regenerative process (80,250). By the use of competitive inhibitors of ODC (141), and the specific irreversible inhibitor alpha-difluoromethylornithine (DFMO) (230), investigators demonstrated that ODC inhibition prevented accumulation of polyamines and reduced DNA synthesis during the early phase of liver regeneration. Luk (172) confirmed these findings and further demonstrated that liver weight gain, the ultimate variable of the regeneration process, is inhibited by DFMO. Furthermore, the administration of putrescine resulted in a marked reversal of the inhibitory effect of DFMO on hepatic DNA synthesis and weight gain (172). A later study demonstrated the correlation between hepatic putrescine concentrations and restitution of liver mass after hepatectomy (195). Against this however, McGowan and Fausto (186) using protein deprived and hypophysectomized rats showed that the time course of ODC activity was independent of the onset of DNA replication in

experimental situations in which the timing of regeneration had been altered. Taken together, these studies would suggest that polyamine metabolism although important, may not be pivotal to the regenerative process.

2.3 REGULATORY MECHANISMS IN HEPATIC REGENERATION.

In the adult animal the liver is normally in a state of growth arrest. It can however be stimulated to grow by a number of means, including exposure to certain toxic agents and chemicals, surgical ablation and during the increased metabolic demands of pregnancy.

The regulation of the regenerative process following partial hepatectomy has been the focus of much study, and the subject of several excellent reviews (78,166). This process must be initiated, and to some degree, regulated by humoral factors for at least two reasons (264). Firstly, cell division is seen throughout the entire liver remnant and not merely the cut edge, as would occur in simple wound healing. Secondly, the process is terminated once the liver remnant has restored itself to the size of liver normally present in the animal. The latter would suggest that the control mechanism is governed by functional demands.

Some, but not all of these putative hepatotrophic factors derive from the portal splanchnic organs and are therefore more concentrated in portal than peripheral blood (26). Much research has been devoted to the study of these substance, as reviewed by Starzl and Terblanche (275), whilst more recent study has focused on non-portal substances and of hepatocytes in culture (194).

2.3.1 Portal hepatotrophic factors.

The regeneration of mammalian liver is largely governed by humoral factors (26). The term hepatotrophic has been loosely used to describe substances that result in both hyperplasia and hypertrophy of hepatocytes, although liver regeneration is largely a hyperplastic process (26).

Following the observation that portal vein occlusion or diversion led to a decrease in the size of the liver deprived of this blood supply, it was postulated that regeneration was mediated by portal blood flow through the hepatic remnant following partial hepatectomy (177). This was the basis of the vascular theory and was based on studies in dogs using the so-called Eck fistula (55,177). An Eck fistula consists of a side to side portocaval shunt with ligation of the distal portal vein, so converting it into a functional end to side fistula.

However, despite the atrophy of the liver consequent to portal venous diversion in the Eck fistula model, there appeared to be surprisingly little impairment of regenerative capacity, although the process was somewhat delayed (299). This provoked the question of whether the observed atrophy was due to deprivation of the constituents of portal blood, or merely a non-specific effect of diminished blood supply.

This dilemma prompted Child *et al* (54) to deprive the hepatic remnant of portal blood and in its place to substitute vena caval blood. This was achieved by portocaval transposition in a series of experiments on dogs. Under these circumstances the liver enlarged. From this it was concluded that portal blood was probably not specifically required for liver regeneration. This was later confirmed by Weinbren in a further study involving portal diversion and partial hepatectomy

(298). In this study the liver remnant, though deprived of portal blood and undergoing atrophy, exhibited an increase in mitoses. These findings have been confirmed in the orthotopic liver transplant by Sumimoto *et al*, who showed no evidence of hepatic atrophy following portocaval transposition in rat liver allografts (287).

It appears then that the liver can regenerate in the presence of diminished blood flow, and that portal diversion produces superimposed atrophy. This would suggest no more than a permissive role for bloodflow in liver regeneration.

2.3.1.1 Insulin and glucagon.

The role of insulin in liver growth was first demonstrated by Younger *et al* (305), who showed liver growth following insulin administration in rats rendered diabetic by the administration of alloxan. Subsequently in a series of experiments involving so-called 'splanchnic division', Starzl (272,273,274) demonstrated the beneficial role of insulin to the regenerating liver. In these experiments, the pancreatic venous effluent was routed to the right lobes whereas the nutrient-rich venous blood from the intestine drained into the left lobes of the liver. The response to partial hepatectomy was assessed on both sides and found to be greater on the side receiving pancreatic blood. This advantage was lost following the administration of alloxan. This not only demonstrated the hepatotrophic effect of portal venous blood in a model in which differences in bloodflow had been excluded, but also implicated insulin as a responsible factor. It was however stated that other potentially hepatotrophic factors in portal venous blood may have been present.

This led Bucher and Swaffield to study hepatic DNA synthesis in eviscerated, partially hepatectomized rats maintained on a nutrient infusion (33,34). They found

that regenerative activity was both delayed and diminished, and did not respond to the infusion of insulin or glucagon alone. When both of these hormones were infused however, a synergistic effect was noted with DNA synthetic activity being restored to that of partially hepatectomized controls.

Following partial hepatectomy, portal and systemic levels of insulin are diminished, whilst glucagon levels are elevated (153,205). The exact role for these two hormones has probably not been adequately explained, but powerful evidence for a direct action comes from the more recent finding that the stimulation of DNA synthesis in hepatocyte culture by epidermal growth factor is enhanced by the addition of insulin and to a lesser extent glucagon (245). However, insulin does not stimulate hepatocyte proliferation in culture despite potent trophic effects (194).

In summary, there is convincing evidence that insulin and glucagon play a permissive role in hepatocyte DNA synthesis and liver regeneration. There is no evidence however that these substances have any direct mitogenic effects on the liver.

2.3.1.2 Nutritional factors.

Apart from insulin, glucagon and possible other hormones in the portal circulation, the other constituents of portal blood that are of importance in hepatic regeneration are the nutrients absorbed from the intestine.

Starvation for 48 hours delays and diminishes the regenerative response, but does not completely abolish it (200,280). The effect of pure protein deprivation on the other hand has been variously reported to have either no effect on DNA synthesis (200), or to result in a decrease (265). DNA synthesis in partially hepatectomized rats fed amino acids and branched-chain amino acids is diminished (152). The

infusion of glucose has been demonstrated to inhibit the onset of hepatic regeneration (117).

Absolute clarity on the effects of nutritional factors in hepatic regeneration has therefore not been obtained, however it is unlikely that they play a central role in the regulation of the regenerative process.

2.3.2 Non-portal factors.

While portal factors have been shown to influence regeneration, they may not initiate this process but merely play a permissive role.

The finding that injection of regenerating liver homogenate resulted in the increase in hepatic mitotic activity in a second animal (188,291,296), suggested that the initiation of the regenerative process may begin in the liver itself. This concept has been more recently examined by Levi (168) and Kahn (137).

Evidence for the implication of circulating non-portal factors in regeneration came from cross-circulation experiments. Regenerative activity was increased in the intact liver of the unresected partner of a pair of parabiotic rats after partial hepatectomy in the parabiotic twin (27). Also, mitotic activity was increased in small heterotopic liver autografts in partially hepatectomized animals (167,264). The same phenomenon occured when isolated hepatocytes were transplanted into a partially hepatectomized animal (128).

The stimulatory effect of serum from animals with a regenerating liver was first demonstrated in hepatocyte culture in 1952 (100). This heralded the onset of the new era of *in vitro* investigation of the regenerative process.

Important progress in defining the key regulatory factors has been achieved by using hepatocyte cultures in serum-free media. This is achieved by the perfusion of rat liver with collagenase (258). The technical details of this method are beyond the scope of this review, however. It is nevertheless of interest to note that an essential prerequisite for hepatocyte cell culture is insulin. Despite having strong trophic effects, it does not itself stimulate DNA synthesis in chemically defined media (194). Using hepatocyte culture, it was possible to show that a few specific polypeptide growth hormones could stimulate DNA synthesis. These factors can be classified as follows (194):

1. Complete hepatocyte mitogens.
2. Growth inhibitors.
3. Incomplete mitogens or comitogenic substances.

2.3.2.1 Complete hepatocyte mitogens.

These are defined as substances that are able to initiate DNA synthesis in serum-free hepatocyte culture (194). Substances capable of this action include the following:

Epidermal growth factor (EGF).

Epidermal growth factor is a polypeptide of 53 amino acids (284) that stimulates the proliferation of hepatocytes *in vitro* (187) and *in vivo* (35). It exerts its effects via a specific cell membrane receptor which it shares with transforming growth factor alpha (TGF alpha) (284). After binding of EGF or TGF alpha to the cell membrane receptor rapid autophosphorylation and dimerization occurs (284). EGF is the prototype mitotic stimulator which stimulates division of hepatocytes amongst other epithelial cells (187). It was the first substance shown to have this effect on

hepatocytes in culture, inducing thymidine labelling indices of 60-80% (194). Insulin is not essential for EGF-stimulated mitogenesis, but is necessary if the full magnitude of the response is to be produced (86). The peak of EGF-mediated DNA synthesis does not occur until 48 to 72 hours (297), in contrast to the 24 hour peak normally witnessed during liver regeneration (29). This delay has not been explained, although it may represent repair processes following the action of collagenase used to prepare cells for tissue culture, and cellular adaptation to the *in vitro* environment (194). EGF mediated stimulation of DNA synthesis in hepatocyte culture usually results in 2 to 3 cycles of DNA synthesis and cell division after which the process stops (194).

EGF receptors decrease in primary hepatocyte culture (129), and the affinity of remaining receptors for EGF diminishes (303). There are two types of EGF receptor: high affinity (ha) and low affinity (la) (284). The ha receptors are lost following hepatocyte isolation, whereas the la receptors persist. The la receptors are the only receptors present when the mitogenic response is stimulated. This has led to the hypothesis that the la receptor is the true mitogenic EGF receptor and the ha receptor merely serves to deny access to the mitogenic receptors at a time when mitogenesis is not appropriate (303).

The fate of the EGF receptor complex has been studied *in vivo* (242). One fraction is taken up by the nucleus and the other secreted into the bile (242). The significance of the translocation of this nuclear fraction is unknown.

EGF stimulates other hepatocyte functions such as protein synthesis and amino acid transport (284). TGF beta inhibits the EGF-mediated DNA synthesis but not protein synthesis (119).

Although EGF has been used as the prototype mitogen for hepatocytes its role in regeneration is still not clear (194). Although no changes in serum levels of EGF have been shown following partial hepatectomy, there is a decline in the number of EGF receptors (249). Saturation of the remaining binding sites may account for the decreased expression of EGF receptors after partial hepatectomy, since binding sites are reduced by 2/3 whilst serum levels of EGF are constant. EGF is produced in the Brünners glands in the duodenum (284), and hence is not affected by liver resection. An alternate explanation may lie in the fact that transforming growth factor alpha (TGF alpha) and EGF share the same receptor on the hepatocyte membrane (190). Increased production of TGF alpha by hepatocytes, such as occurs during liver regeneration, might therefore account for this effect. A decline in EGF receptor mRNA production occurs, which would account for diminished receptor synthesis and resultant decreased EGF receptor binding (129).

The mitogenic effects of EGF are inhibited by TGF beta (20). This finding has led to the hypothesis that TGF beta forms part of a paracrine control mechanism that limits cell proliferation.

Both *in vivo* and *in vitro*, noradrenaline has been shown to down-regulate the EGF receptor in a heterologous manner (64). It is via the alpha-1 receptor that this effect is mediated (62). Catecholamine levels are elevated after partial hepatectomy in the rat (62). Surgical hepatic denervation reduced incorporation of [3H] thymidine into liver DNA during the first 24 hours in partially hepatectomized rat livers (62). This would implicate catecholamines, acting via the alpha-1 receptor, as playing an important role in the early regenerative response (64). This factor may be of cardinal importance in the current study.

Many questions relating to whether EGF is in fact the naturally occurring mitogen that induces the changes associated with regeneration have yet to be answered. However, it is the only factor which after injection into laboratory animals with intact livers results in increased DNA synthesis (36).

Transforming growth factor-alpha (TGF alpha).

Recent work has shown regenerating hepatocytes produce TGF alpha (190). TGF alpha shares the same receptor as EGF (190) and has been found to be a more powerful mitogen than EGF in hepatocyte culture (190). An increase in TGF alpha concentration has been shown in regenerating liver (190). Being a complete hepatocyte mitogen, TGF alpha may constitute an autocrine mediator whereby hepatocytes initiate DNA synthesis. The mitogenic effects of TGF alpha are (like EGF) inhibited by TGF beta (20). TGF beta is derived from nonparenchymal liver cells, and its inhibitory effects may therefore form part of a paracrine mechanism of growth inhibition (20).

Gene expression changes may represent a priming state in which cells are prepared for division but are not yet committed to it (194) (see page 42). Subsequent TGF alpha synthesis may then be the critical step prior to initiation of DNA synthesis. Increased levels of TGF alpha occur after 8 hrs and peak after 24 hrs with a subsequent smaller peak at 72 hrs (194). The kinetics of TGF alpha synthesis therefore parallel those of normal DNA synthesis.

Hepatocyte growth factor (HGF) or Hepatopoeitin A (HPTA).

This substance of 100 000 kDa was found in the serum of hepatectomized rats and described as hepatopoeitin A by Michaelopoulos *et al* in 1983 (192,193). In 1984 Nakamura isolated a similar substance and called it hepatotropin (206). In 1986

Gohda *et al* (101) identified a factor in patients with fulminant hepatic failure and termed it human HGF. A substance with a heterodimer structure was identified by Nakamura in 1987 from rat platelets and called hepatocyte growth factor (HGF) (207). Most recent work suggests these (HPTA and HGF) are the identical molecule, with small differences due to species variation (199,306).

The molecular structure of HPTA/HGF has recently been described (307). It is a heterodimer consisting of two chains, one of 70 000 kDa and another of 35 000 kDa, held together by 2 disulphide bonds. It is 10 times more potent than EGF in terms of nuclear labelling index in hepatocyte culture. Furthermore, its effects are additive to, and distinct from, those of EGF (209).

Like EGF, the mitogenic effect of HPTA/HGF is inhibited by TGF beta (194), although it does not share the same receptor as EGF (307). The major tissue sources of HPTA/HGF are the pancreas, brain, the interfollicular cells of the thyroid and the Brünners glands in the duodenum (194). Secretion of HPTA/HGF by the pancreas and duodenum suggest its presence in the portal circulation where it would be continually available to the liver (194).

Heparin binding growth factor (HBGF-1).

This heparin-binding growth factor, like HPTA/HGF, stimulates hepatocyte DNA synthesis. Unlike HPTA/HGF it is totally inactive in the absence of heparin, and by definition therefore not a complete mitogen. Hepatic gene expression of HBGF-1 precedes the expression of the TGF alpha gene (145). This suggests that HBGF-1 may provide the autocrine stimulus that initiates regeneration, prior or simultaneous to the EGF/TGF alpha mediated stimulus. It is synthesized by regenerating

hepatocytes, with the peak of secretion occurring at the time of peak DNA synthesis (145).

Hepatic stimulatory substance (HSS).

Previous descriptions of this substance have referred to crude cytoplasmic extracts obtained from regenerating liver (137,168,188,291,296). More recently however, a hepatic stimulator substance has been characterized independently by Francavilla (87), LaBreque (157) and Fleig (83). The substance is described as a factor of approximately 16 000 kDa molecular weight which results in enhanced DNA synthesis in rat hepatocytes *in vivo*. It is extracted from neonatal and regenerating livers. Like HBGF-1, HSS is not a complete mitogen for hepatocytes in culture, as it also requires heparin for activity.

Hepatopoeitin B (HPTB).

This is the name assigned to the other identified factor isolated from the serum of hepatectomized rats, other than HPTA (194). A complete hepatocyte mitogen, it acts synergistically with both EGF and HPTA. Little more is known about it.

2.3.2.2 Growth inhibitors.

These substances have been defined in primary culture, based on their ability to inhibit EGF-mediated mitogenesis. Three have been identified.

Transforming growth factor beta (TGF beta).

This is a group of three cytokines, with reference usually being made to TGF beta 1. This is a molecule of 26 000 kDa, which is elaborated by activated Kupffer cells or macrophages. TGF beta has been associated with a number of *in vivo* functions

including wound healing and the inhibition of mesenchyme-derived cell proliferation (271).

TGF beta inhibits the mitogenic effects of EGF and TGF alpha on hepatocytes (48). The addition of TGF beta to hepatocyte culture inhibited the peak of EGF-mediated DNA synthesis (at 72 hrs) (48). The mechanism whereby TGF beta exerts this effect is poorly understood. TGF beta has no effect on EGF receptor binding or EGF receptor autophosphorylation (252). *In vivo*, TGF beta mRNA production is first detected in nonparenchymal cells 4 hours after partial hepatectomy, thereafter peaking sharply at 72 hours. Levels remain high for more than 96 hrs (20,49). Russell (253) demonstrated an inhibition of DNA synthesis following partial hepatectomy by the administration of TGF beta at various times before and shortly after the procedure. However, repeated and stronger doses of TGF beta failed to obliterate the peak which occurred at 72 hours after partial hepatectomy.

Interleukin 1 beta.

It was recently shown that interleukin 1 beta inhibits hepatocyte proliferation, although the degree of inhibition is not as great as with TGF beta (208). Similar but lesser effects have been observed with Interleukin 6. Interleukin 1 beta is known for its effects in redirecting protein synthesis in liver cells toward acute phase reactants (208). Its inhibitory effect on DNA synthesis might reflect a reprogramming of hepatocyte gene expression and DNA synthesis toward acute phase protein synthesis in preference to synthetic processes leading to hepatocyte proliferation. This is a postulated mechanism for the delayed DNA synthesis in partially hepatectomized animals in which a simultaneous inflammatory stimulus is produced (11).

Hepatocyte proliferation inhibitor.

This protein, isolated from normal rat liver, was found to inhibit DNA synthesis specifically and reversibly in normal hepatocytes. It is distinct from TGF beta as no inhibition was produced in hepatocyte culture with the addition of anti-TGF beta antibodies (121).

2.3.2.3 Hormones (comitogens).

These have been defined by Michalopoulos (194) as substances that affect growth positively in an indirect manner. They all have the following properties:

a) enhancement of the mitogenic effects of growth stimulators (EGF,TGF alpha).

b) decrease the inhibitory effect of growth inhibitors.

c) no direct mitogenic effects of their own in culture (194).

The nett effect of these substances is to tilt the balance in favour of those factors stimulating proliferation.

Noradrenaline.

A role for the sympathetic nervous system, noradrenaline and the alpha-1 adrenergic receptor in liver regeneration has been described (62). Blockade of the alpha-1 receptor by prazosin abolished the 24 hour DNA synthetic peak in rats *in vivo* (62). Similar effects were seen with hepatic denervation. The alpha-1 adrenergic receptor has been shown to mediate the catecholamine effect, by downregulation of the EGF receptor and enhanced EGF mitogenic effects (64). A similar decrease in EGF receptors and enhanced responsiveness to EGF is seen

during liver regeneration (86). Noradrenaline also decreases the inhibitory effect that TGF beta has on hepatocyte mitogenesis in culture (119). If hepatocytes from liver 12-16 hours after partial hepatectomy are cultured in the presence of EGF and TGF beta, then noradrenaline greatly enhances their effect on mitogenesis (194). There is thus clear evidence that the alpha-1 adrenergic receptor is essential during the early regenerative response.

It appears that the catecholamine effect is mediated by a receptor distinct from both the EGF/TGF alpha and the TGF beta receptor. The alpha-1 receptor is linked to a recently described G protein (111) which stimulates increased activity of phosphatidylinositol diphosphate (PIP2) phosphodiesterase. Stimulation of the alpha-1 receptor results in greater breakdown of PIP2 with a resultant accumulation of mediators which trigger a cascade of intracellular events, including the activation of protein kinase C and release of intracellular calcium stores (77). Phenomena described as occurring very early after partial hepatectomy include membrane hyperpolarization, glycogenolysis, and an increase in diacylglycerols (119). All of these are classically mediated by receptors that result in mobilization of intracellular calcium reserves.

Catecholamines increase sharply in the serum following partial hepatectomy, perhaps due to the removal of 68% of monoamine oxidase activity (with the resected liver) (86). These catecholamines are a potential source of stimulation to the alpha-1 receptor (86).

Vasopressin and angiotensin.

Liver regeneration is impaired in strains of rats that are congenitally deficient in vasopressin production (251). Both vasopressin and angiotensin act via receptors

that enhance PIP2 turnover and stimulate glycogenolysis (77). Vasopressin is secreted by the sympathetic nerves of the liver along with noradrenaline (90). Both noradrenaline and vasopressin may therefore be involved in the previously observed effects of the sympathetic nervous system on liver regeneration. Vasopressin and angiotensin 2 have a similar intracellular cascade following the activation of their respective receptors.

Oestrogens.

Substantial evidence exists for the role of oestrogens in liver regeneration (85,90). Oestrogen levels increase after partial hepatectomy, peaking at 24 to 48 hours. Testosterone levels show a corresponding decrease. Similarly, oestrogen receptors increase after partial hepatectomy, whilst androgen receptors decrease (85). The evidence for the direct involvement of oestrogen in regeneration comes from the finding that when added to primary hepatocyte cultures with serum or EGF, oestrogen enhances the mitogenic effect of EGF (262). Oestrogen is also known to promote hepatic tumorigenesis (194).

Corticosteroids.

Both adrenocorticotrophic hormone (ACTH) and glucocorticoids have been shown to induce liver enlargement. This is almost entirely due to cellular hypertrophy (26). However, glucocorticoids in pharmacologic doses actually tend to retard or delay the normal proliferation of liver that occurs following partial hepatectomy (26). Surgical stress significantly accelerates the rate of DNA synthesis following partial hepatectomy (201). It would appear that the effect of these hormones on liver regeneration cannot be seen in isolation.

The clue to the role of corticosteroids in liver regeneration may lie in the regulation of circadian rhythm. The mitotic activity of regenerating rat liver is maximal during the day (309). Barbason *et al* have shown that in rats hepatectomized in the late afternoon or evening the peak mitotic activity occurs both later and is of greater magnitude than after partial hepatectomy in the morning (310). Furthermore, hepatocellular proliferation may be mutually exclusive of normal hepatocellular function in the regenerating rat liver (309). Barbason and Van Cantfort (309) have postulated the derepression of genes responsible for cellular proliferation following partial hepatectomy, with simultaneous repression of genes governing normal hepatic synthetic and degradative function. Corticosteroid hormones, themselves secreted in a circadian rhythm, may therefore form part of the mechanism controlling expression of enzymes and proteins involved in hepatocellular proliferation.

2.3.3 Proto-oncogene expression.

Proto-oncogenes can be assumed to be normal genes which are expressed during periods of normal and neoplastic growth. Proto-oncogene expression is a phenomenon occurring as part of the normal regenerative response (78). There is sufficient evidence to suggest that they appear to be essential for normal growth. The expression of these genes and their products after partial hepatectomy is specific, sequential and highly regulated, with each oncogene being expressed at specific times after partial hepatectomy (132). Changes have been shown in the *c-fos*, *c-myc*, *p53*, and the *ras* family of proto-oncogenes (132). These genes may be primary regulators of key events in the cell cycle (78).

Notwithstanding the existing uncertainties in the current understanding of the regenerative process, it is possible to use proto-oncogene expression to identify

different stages of regeneration (78). In the first stage, identified by the expression of *c-fos* and *c-myc*, the cell becomes capable of proliferating if the appropriate inducer is present. The 'primed' hepatocytes then progress to the second stage which is characterized by the expression of the *p53* and *c-ras* proto-oncogenes. It is proposed that the autocrine and paracrine mechanisms previously discussed are responsible for regulation of this stage (78). This would imply that the regenerating hepatocytes are largely responsible for their own growth. The rapidity of the expression of the *c-fos* proto-oncogene (0 to 4 hours) suggests that priming starts almost immediately after partial hepatectomy.

2.3.4 Endotoxin and hepatic regeneration.

Endotoxin is the lipopolysaccharide (LPS) outer wall of gram-negative bacteria and is present in large quantities in the gut from death of bacteria and release from intact organisms. The normal transit of this macromolecule from the gut into the portal circulation in small quantities has been demonstrated (126). The mechanism of this absorption has been demonstrated in intestinal ischaemia to be due lymphatic absorption from the peritoneal cavity (216).

The administration of exogenous endotoxin has been shown to significantly enhance DNA synthesis (266). Cornell (58) showed that pretreatment with endotoxin 24 hours prior to partial hepatectomy accelerated DNA synthesis. Systemic hyperglucagonaemia and hyperinsulinaemia as well as an elevation in EGF were associated with this response. Systemic gut-derived endotoxaemia has been demonstrated following partial hepatectomy in rats (58). Moreover, restriction of gut-derived endotoxin by decontamination of the gut diminished DNA synthesis and

the alterations in plasma insulin and glucagon levels following partial hepatectomy (60).

There is thus a striking similarity between the release of putative hepatotrophic factors following systemic endotoxaemia caused by exogenous endotoxin administration, and by endogenous endotoxin release by the gut after partial hepatectomy (61). This would suggest a direct role for endotoxin in normal hepatocyte proliferation following partial hepatectomy. Endotoxin is one of the activators of the monocyte-macrophage effector system which by the release of potent cytokines cause a variety of tissue effects. The range of cytokines released by endotoxin-activated Kupffer cells includes prostaglandin-E2, which has been implicated in the regulation of the regenerative process (43).

It would appear that in the case of regeneration that endotoxin may be inextricably linked to normal physiological processes. Perhaps the role of gut-derived endotoxin in normal physiology is to stimulate the secretion of hepatotrophic factors for liver regeneration through as yet undefined pathways. By the activation of Kupffer cells, endotoxin may provide a paracrine trigger for the initiation of the regenerative response.

2.4 THE EVALUATION OF THE REGENERATIVE RESPONSE.

Despite the simplicity of the model by which regeneration is initiated by partial hepatectomy, accurate measurement of the ensuing process has proven to be evasive. Most investigators have relied on liver weight, the estimation of mitotic activity by histologic or autoradiographic methods, measurement of DNA synthesis and other indirect markers of the regenerative process (68). Unfortunately each of

these parameters is subject to recognized errors and inconsistencies. The best measure of hepatic regeneration would be some metabolite, cytokine or hormone which is centrally involved in the regulation of liver growth. Until the components of the hypothetical regulatory cycle have been comprehensively described, this will not be forthcoming. Parameters in current use reflecting cell growth and division can serve as an assay of the regenerative process, provided their limitations are borne in mind. Such parameters should be easily and reproducibly assayed and should not be influenced artefactually by an experimental manipulation.

We have elected to utilize four independent parameters as indices of liver regeneration and DNA synthesis. These parameters are:

a) Mitotic index.

b) Thymidine kinase.

c) Ornithine decarboxylase.

d) Liver to body mass ratio.

For details of the techniques involved, the reader is referred to page 77.

2.5 SUMMARY.

1. The liver has the inherent capacity to regulate its own size.

2. Regulation of liver growth is dependent on both intrahepatic and humoral factors.

3. The exact role of circulating hormonal factors remains unknown, mainly due to the complexity of changes occurring *in vivo*.

4. From studies in hepatocyte culture, TGF alpha and TGF beta and HGF have emerged as growth factors of major importance. TGF alpha is an autocrine factor produced by hepatocytes that results in enhanced DNA synthesis. TGF beta is produced by nonparenchymal cells and functions as a paracrine signal to terminate hepatocyte proliferation.

5. The triggering of hepatocyte proliferation involves the priming of hepatocytes, a process closely related to the expression of proto-oncogenes.

6. Endotoxin is a possible mediator of the normal regenerative process, by a paracrine mechanism.

3. REVIEW OF HEPATIC ISCHAEMIA.

The temporary interruption of hepatic bloodflow is obligatory in hepatic transplantation, and is sometimes desirable in the management of acute hepatic trauma and hepatic malignancy. The purpose of this review is to summarize the existing knowledge on the effects of warm hepatic ischaemia.

3.1 HEPATIC ISCHAEMIA AND REGENERATION.

The question of hepatic regeneration following an ischaemic insult has been addressed by MacKenzie *et al* (173). In this study, total hepatic vascular occlusion with portal decompression and subsequent partial hepatectomy were performed in the canine model. The liver mass was studied at the time of sacrifice 6 weeks postoperatively, at which time a 98% return to normal weight was demonstrated. No other parameters of liver regeneration were however studied, and at no other time periods. More recently, the effect of warm ischaemia on liver regeneration has been examined in rats (96), with depression of hepatocyte regeneration noted upon exposure to ischaemia of up to 25 mins duration.

Given the burgeoning interest in reduced and split liver transplantation, and in hepatic resection using total hepatic vascular isolation, a clear need has arisen to examine more closely the effects of hepatic ischaemia on liver regeneration.

3.2 HISTORICAL OVERVIEW.

Consideration of the tolerance of the liver to ischaemia has been the subject of study for those whose interest has been in the fields of hepatic resection and liver transplantation for almost a century (233).

Early studies addressed the question of mortality associated with hepatic ischaemia, and the maximum duration of occlusion of the porta hepatis. Studies on dogs showed a high mortality when the duration of hepatic ischaemia was extended beyond 20 minutes (240). Occlusion of the porta hepatis and the superior mesenteric artery for 60 minutes' duration resulted in 100% mortality. The same manoeuvre lasting for 30 minutes caused the death of 7 out of 9 dogs, whilst there was no mortality associated with 20 minutes' hepatic ischaemia. The classical maximum duration of hepatic ischaemia was derived from these observations. The demonstration of transient hyperkalaemia following periods of inflow occlusion served to strengthen the argument of those who believed that further prolongation of hepatic ischaemia was possible (110, 311).

Jolly and Foster later showed that extension of the ischaemic period to 49 minutes was possible in dogs provided adequate splanchnic decompression was employed (131). This was provided by means of a side-side portocaval shunt. In a subsequent study, improved mortality (60%) following 75 minutes of ischaemia and partial hepatectomy was demonstrated in dogs in which adequate splanchnic decompression was employed (173). These studies appeared to provide strong evidence that the mortality associated with hepatic inflow stasis in dogs was more a function of the adequacy of splanchnic decompression than hepatic ischaemia *per se.*

It was perhaps bad fortune that dogs were used for the initial studies of hepatic ischaemia. The canine hepatic veins have well-developed sphincters which when constricted contribute to "outflow block" in response to acidosis, hypothermia or excessive handling (202). This phenomenon has not been reported in man or in the pig. Secondly, it was shown that the canine liver tends to be colonized by Clostridial organisms following ischaemia (52). The validity of the canine model and the applicability of these observations to the clinical situation was therefore challenged.

Subsequent studies in pigs showed tolerance of longer periods of ischaemia (8,81,112,135,214) The maximum safe period was therefore progressively extended to 35 minutes (8), and subsequently to 120 minutes (214).

Subsequent work even challenged the belief that hepatic ischaemia was deleterious at all (81), arguing that deprivation of portal venous blood has the concomitant advantage of reducing the metabolic requirements associated with the passage of portal blood. Furthermore, the liver has a high concentration of antioxidants and free radical scavengers, a high oxygen extraction ratio, and the ability to autoregulate its own bloodflow (81). These factors all contribute to an extraordinary hepatic resistance to ischaemic insult. An additional protective factor is the inadvertent hypothermia that occurs during laparotomy. This would serve to diminish the hepatic metabolic rate and improve the ischaemic tolerance of the liver.

Certainly it would appear that the liver is uniquely equipped to deal with deprivation of its blood supply. Furthermore, the tolerance of the liver to ischaemia is largely dependent on the method of splanchnic decompression employed as well as on the species studied. In this regard it is unfortunate that few satisfactory studies have been performed on non-human primates (118). Importantly, the human liver

tolerates ischaemia well due to the presence of well-developed portosystemic collateral channels. From clinical studies (122) and subsequent experimental studies (112,214) it has been suggested that the classical maximum time for porta hepatis occlusion be extended to 1 hour. The liver's tolerance to ischaemia is again being tested in the clinical practise of hepatic inflow occlusion at the time of hepatic resection (292).

3.3 EFFECTS OF PORTAL VEIN OCCLUSION.

The effect of acute ligation of the portal vein is a topic of recurring interest. It has been demonstrated to be fatal in a number of animals (7,130). The cause of this mortality has been an area of some controversy. Claude Bernard in 1877 postulated as follows: "The mechanism of death is easy to understand. All the blood tends to accumulate in the vessels of the intestine. The brain and other organs become exsanguinated and the animal dies, in fact, of anaemia" (10). Schiff, by tying the portal vein of the cat, claimed to suppress biliary secretion, and he considered that death was due to a toxic substance in the portal blood which the liver destroyed to form the elements of bile (257).

Thus it appeared that two main theories were popularized to explain the high mortality associated with portal venous occlusion: the first, (of Bernard) suggested it to be due to splanchnic pooling of blood with death ensuing from hypovolaemic shock, whilst the second (of Schiff) proposed that an undesignated toxin produced in the gut resulted in the death of the animal. Several studies set out to prove or disprove these two theories, using a variety of animal models and with conflicting results. These have been reviewed by Johnstone (130).

A much more recent study demonstrated impaired Kupffer cell function following portal vein occlusion (118), and further suggested that failure of the reticuloendothelial system to neutralize endotoxin released from the gut may account for increased mortality. Portal venous occlusion for 30 minutes resulted in the appearance of endotoxin in the portal and systemic circulation (215). The presence of gram-negative bacteria has been shown in both the portal and systemic blood in the calf following occlusion of its portal vein (7). It was noted in the study of Olcay *et al* that all animals in which portal endotoxaemia was demonstrated following portal venous occlusion, also had systemic endotoxaemia (215). This suggested a decreased clearance of endotoxin by the liver. This diminution in phagocytic activity by Kupffer cells was documented in portal vein-occluded rats (215) as had been demonstrated in non-human primates (118). An element of this post-occlusive depression was accounted for by the known reticuloendothelial depressive properties of endotoxin (260). However, it is unlikely to play a major role as phagocytic depression has also been shown in gnotobiotic rats (215). It would appear that in addition to the venous pooling with resultant hypotension that characterizes portal venous occlusion, an added insult to the host occurs in the form of portal and systemic endotoxaemia, as well as reticuloendothelial depression. These composite events may well be contributory to the effects of acute temporary portal venous occlusion. The role of endotoxin in the hepatic regenerative response has been previously discussed on page 43.

3.4 HAEMODYNAMIC EFFECTS OF HEPATIC ISCHAEMIA.

Hypotension is a universal phenomenon following hepatic inflow occlusion. The pathophysiology of this hypotension has been the focus of much study. From an early stage a biphasic response to hepatic inflow occlusion has been noted (4,131).

Early hypotension.

Immediately following the application of clamps to the portal vein and hepatic artery of dogs, there is a profound fall in blood pressure (4,131). A simple haemodynamic explanation for this, as originally proposed by Bernard (10) has gained universal acceptance. Following occlusion of the portal vein and the concomitant splanchnic pooling of blood, there is a diminution in venous return. This results in decreased right atrial filling with the decline in cardiac output manifesting as systemic hypotension. Following the initial depression, the blood pressure tends to remain constant during the ischaemic period due to circulatory compensatory mechanisms (131).

Late hypotension.

Jolly (131) reported a profound fall in blood pressure on release of the portal vein and inferior vena cava (IVC) clamps after a 49 minute period of clamping, despite employing a side-to-side portocaval shunt for portal venous decompression. This followed a brief period of normotension. The observed hypotension was initially sensitive to vasopressors, but subsequently became increasingly resistant to their action. This biphasic blood pressure response was also observed by Backlund who noted the hypotension to be associated with bradycardia (4). The inappropriate

bradycardia suggested that a purely haemodynamic aetiology for the hypotension may be an oversimplification.

Buckberg attributed the hypotension to sequestration of blood in the ischaemic liver and the elaboration of the so-called vasodepressor material (VDM) (37). This substance was thought to be produced by the anoxic liver at the time of reperfusion. This proposal was based on the finding that aliquots of hepatic venous blood collected from ischaemic dog livers following revascularization resulted in profound shock when infused into dogs with hepatic inflow occlusion (37). Hypotension did not result when this blood was infused into dogs without hepatic inflow occlusion. This prompted the conclusion that normal liver function was necessary to remove the vasodepressor material from the circulation.

Other possible aetiologic factors for the unexplained hypotension were reported as hyperkalaemia (110,279), acidosis (37) and bacterial factors (52). However, no reversal of the hypotension was observed in subsequent studies in which pH and potassium levels were corrected (4,131). It was however shown that acidosis does increase hepatic vascular resistance in dogs with resultant hepatic congestion (37). Hepatic congestion would therefore appear to be an epi-phenomenon rather than a cause of hypotension.

The possibility of bacterial factors having a role to play was brought into question by the finding by Drapanas (65) that cultures taken from ischaemic dog livers were sterile. This did not however exclude the possibility that the products of bacteria were responsible for the observed effect.

In summary, no conclusive evidence was forthcoming which directly implicated acidosis, hyperkalaemia or bacterial overgrowth in the postischaemic hypotensive response.

The evidence in favour of VDM or an as-yet-unknown humoral factor was strengthened by the finding that hypotension could be avoided if the initial hepatic venous effluent following reperfusion was run to waste (37). Moreover, shock could be induced in other dogs with inflow stasis by the infusion of the wasted blood. Further suggestion that the aetiology of the hypotension involved some other factor was provided by the finding that pigs tolerated occlusion of the IVC better than occlusion of the portal vein (8). This was suggestive of a contribution by a component of portal venous blood, perhaps acting in concert with the ischaemic liver.

The question of whether the hypotension resulted from warm ischaemia *per se* or whether it resulted from the reperfusion of the liver with pooled portal blood was indirectly addressed by Nitta (211). Hepatic inflow stasis was induced in two groups of dogs, with splanchnic decompression by portocaval shunt provided in the first but not the second group. No significant depression in blood pressure was noted in the first group but in the group without splanchnic decompression marked hypotension occurred and portal venous pressures approached normal systemic levels. Importantly, systemic levels of endotoxin were elevated in the non-shunted group at the time of portal venous occlusion.

Clearly the haemodynamic tolerance to hepatic inflow occlusion parallels the quality of splanchnic decompression. This has been shown in pigs exposed to 2 hours of normothermic ischaemia (214). Furthermore, a correlation between splanchnic stasis, endotoxaemia and hypotension appears to exist (215,222).

More recent studies have demonstrated the release of potent vasodepressor cytokines by macrophages following their activation by endotoxin (228).

3.5 ISCHAEMIA-REPERFUSION INJURY.

Reversible ischaemia of the liver is a double insult: damage is incurred both during the ischaemic period and during the subsequent period of reperfusion. During the past decade, much attention has been devoted to the study of the latter phenomenon. Oxygen free radicals have been proposed as mediators of the microcirculatory and parenchymal cell injury associated with reperfusion injury (1,185,221).

Much study has been devoted to the identification of the source of these free oxygen radicals. Granger, Rutili and McCord proposed that the enzyme xanthine oxidase was a major source of the oxidants produced following reperfusion of the feline small intestine (104). Their hypothesis was that the enzyme xanthine dehydrogenase (XDH) was converted to xanthine oxidase (XO) in ischaemic tissue. Simultaneous with this conversion, cellular ATP was catabolized to hypoxanthine during the ischaemic period. Upon reperfusion and reoxygenation, molecular oxygen would react with hypoxanthine and xanthine oxidase to form the toxic oxygen metabolites, superoxide (O_2-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (.OH). This theory has been adapted from the feline intestine in which it was first described to explain the reperfusion injury in various tissues including the liver.

Much of the evidence implicating oxidative stress in reperfusion injury has come from the finding that antioxidants protect against the phenomenon (180). Administration of the antioxidants superoxide dismutase (SOD) or catalase

attenuate the release of enzymatic markers of hepatocellular injury (1) and improve the function of liver grafts exposed to ischaemia (3).

Treatment with alpha-tocopherol (182) and coenzyme Q_{10}; (180) has been shown to improve survival, to accelerate the resynthesis of ATP, to lower the consumption of both water-soluble and lipid-soluble antioxidants, and to inhibit the lipid peroxidation associated with reperfusion injury.

Glutathione is a major endogenous antioxidant in liver tissue. The concentration of reduced glutathione (GSH) decreases following the onset of ischaemia, whilst the concentration of glutathione disulphide (GSSH) correspondingly increases (182). The ratio of GSSH to GSH therefore increases following ischaemia, so reflecting the prevailing oxidative stress. Exogenous administration of GSH results in the decreased formation of lipid peroxides during hepatic reperfusion (181).

Hepatic ischaemia is associated with decreased adenine nucleotides (182), decreased superoxide dismutase activity (1), and depletion of water and lipid-soluble antioxidants (182). A reduction in endogenous antioxidants would render the hepatocytes more susceptible to free radical actions and resultant lipid peroxidation.

There is therefore a growing body of evidence that oxygen free radicals mediate a portion of the structural and functional damage associated with reperfusion of ischaemic tissues.

The origin of the oxygen radicals includes activated leukocytes, soluble enzymes and proteins, and membrane-bound enzymes of the electron transport chain (95). Of these, there has been suggestion that xanthine oxidase is the major source (1).

Allopurinol is a competitive inhibitor of xanthine oxidase. The implication of xanthine oxidase as a major source of reactive oxygen species is based on the observation that allopurinol is as effective as oxygen radical scavengers in attenuating the injury associated with hepatic ischaemia (221). This has not however been a universal finding. Metzger *et al* (191) were unable to demonstrate a beneficial effect of allopurinol following oxidant stress induced by reperfusion of the ischaemic rat liver. A number of unexpected actions has been attributed to allopurinol, including the induction of enzymes responsible for the synthesis of free radical scavengers and direct scavenging of free radicals. A metabolite, oxypurinol has also been implicated in the latter action. Peterson (223) provided evidence that allopurinol acts as an electron transfer agent and in so doing facilitates electron transport during reperfusion. Metzger, using the standard allopurinol administration regimen found no evidence that allopurinol was protective against reperfusion injury in the rat liver (191). At much higher concentrations, allopurinol has been shown to have a protective effect (178). It appears likely that the protective effect of allopurinol in high doses is related to direct scavenging of free radicals rather than inhibition of xanthine oxidase.

Given the current uncertainty regarding the specificity of allopurinol action, it appears that an alternate means of studying xanthine oxidase inhibition should be sought. Possible modalities include folate, methotrexate and a tungsten-supplemented, molybdenum-deficient diet (221).

The duration of ischaemia is vital since it has been shown that significant xanthine dehydrogenase to xanthine oxidase conversion occurs only after 2 hours of ischaemia (75). Therefore if xanthine oxidase conversion had a significant part to play in the genesis of reperfusion injury, it would only occur where the ischaemic

period was of sufficient duration. Any benefit attributable to xanthine oxidase inhibition would then be unlikely in ischaemia of shorter duration. An understanding of the kinetics of the xanthine dehydrogenase to xanthine oxidase conversion is important therefore in the interpretation of studies of free radical production (221).

Finally, there are a several other possible sources of oxygen free radicals other than xanthine oxidase. Recent work by Grisham *et al* (107) suggests that neutrophils are the source of free radicals and the ultimate mediators of reperfusion injury. In their view, xanthine oxidase-derived oxidants appear to play a more important role as initiators of neutrophil infiltration rather than mediating microvascular injury (107). The role of neutrophils in reperfusion injury of the liver remains undefined. The finding that reperfusion injury in the liver is an oxidative stress that is not modified by allopurinol (191) clearly outlines the need for further work in this field.

3.6 PARAMETERS OF HEPATOCELLULAR INJURY.

Another difficulty in the interpretation of results is created by the use of different indices of liver damage in different studies. The commonly used indices generally fall into 4 groups:

a) those relating to changes in cellular permeability or cytolysis,

b) altered hepatocyte function,

c) impaired circulatory function,

d) resulting from oxidative stress (221).

Release of endogenous aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) are the commonest indicators of cellular injury (93). There is evidence however to suggest that the release of cytosolic enzymes may not be adequate indicators of early cellular damage (56). The phenomenon of potocytosis, by which apical blebs are lost from ischaemic, but not necrotic cells contributes to the elevation of transaminase levels following ischaemia (102). The presence of blebs on hepatocytes has been reported following storage of rat liver allografts (40,189). The mitochondrial enzymes ornithine carbamyl transferase (94) and mitochondrial AST (210) have been utilized as indices of hepatocellular necrosis in an attempt to circumvent this problem.

Apart from estimations of mitochondrial integrity, many different techniques have been utilized to assess the impairment in hepatic function associated with ischaemia and reperfusion. These include oxygen consumption, bile flow, bile acid production, dye recovery, ATP clearance, protein and DNA synthesis (221). In a study in isolated perfused rat livers, bile flow ceased after 15 minutes of warm ischaemia (19). In the transplantation setting this may be of great clinical usefulness.

Few investigators have attempted to quantitate the degree of oxidative stress following ischaemia/reperfusion. Lipid peroxidation is a complex process whereby unsaturated lipid material undergoes reaction with molecular oxygen to yield lipid hydroperoxides. Methods that have been utilized to quantitate this include measurement of the loss of unsaturated fatty acids and phospholipids and production of alkanes, chemiluminescence, diene conjugation, thiobarbituric acid-reacting substances and protein carbonyls (221).

Clearly no consensus has been reached as to which index of hepatocellular injury is most appropriate.

3.8 THE HEAT SHOCK RESPONSE.

When a cell is exposed to stressful situations, such as increased temperature or ischaemia, it abruptly begins to synthesize large quantities of several specific proteins to the relative exclusion of others (99). These are termed heat-shock proteins and the response is the heat-shock response. This phenomenon was first described in *Drosophila* by Ritossa (246), but subsequent studies have demonstrated heat-shock proteins in a number of species including man. Heat-shock proteins are identified by their molecular weights, being referred to for example as hsp84, hsp70 or hsp22. Thus hsp70 has a molecular weight of 70kD. In the original description in *Drosophila* puffing of the DNA in specific chromosomal regions was noted and these regions accounted for the sites of heat-shock protein synthesis (246). Approximately 30% of protein synthesis occurring after heat shock was accounted for by 6 of these proteins (294). It has been postulated (159) from bacterial models that in response to oxidative stress, adenylated nucleotides (alarmones) are synthesized which serve to trigger the synthesis of heat-shock proteins (159). One of these nucleotides is adenosine tetraphosphate (ApppA).

Warm ischaemia and the heat-shock response.

Most metabolic activities, including protein synthesis are impaired shortly after the onset of ischaemia (39). Protein synthesis decays progressively with time, and returns to normal only if ischaemia does not exceed 60 minutes (39). It would appear unlikely that proteins of any description are synthesized during the period of ischaemia. Synthesis of heat shock proteins has been demonstrated in the period following warm ischaemia in the rat liver (38). Postischaemic recovery of rat liver results in the accumulation of polysomes with resultant increased expression of

genes coding for the hsp89 and hsp70 heat-shock proteins (99). This is facilitated by the structural features of heat-shock protein mRNA that confer on them a high intrinsic translational efficiency (38).

Like acute phase proteins, heat-shock proteins are produced in response to a variety of noxious stimuli. This suggests that they may have some protective effect. The phenomenon of thermotolerance, in which animals demonstrate enhanced heat tolerance on second exposure supports this hypothesis. According to the adenylated nucleotide alarmone theory, alarmones would induce the heat-shock response as protection against oxidative stress (159).

The association between the heat-shock response and the acute phase reactants such as C-reactive protein is suggestive that they may constitute part of the same universal response to stress (294). Differential molecular weights, and the finding that acute phase proteins are synthesized at a later time during the postischaemic recovery, provide conclusive evidence of two distinct phenomenae (6).

Liver regeneration and the heat-shock response.

Carr (46) studied the expression of heat-shock proteins following the administration of hepatocarcinogens and during hepatic regeneration. Increased levels of both hsp83 and hsp70 were demonstrated following administration of hepatocarcinogens and during hepatic regeneration. This suggested their expression during both normal and abnormal hepatocyte proliferation. These results are similar to those observed for expression of *c-H-ras* amd *c-myc* in rat liver during regeneration (175), and suggest a coordinate expression for these genes during liver regeneration.

The alteration of rat hepatocytes, with the resultant heat shock response that occurs following the administration of hepatocarcinogens or during liver regeneration is capable of protecting against the cytocidal effects of toxins such as adriamycin (47). The *c-H-ras* and the *c-myc* oncogenes, like the heat-shock proteins, are elevated following administration of hepatocarcinogens and during liver regeneration (46). The biologic significance of the increased expression of these genes during both normal and abnormal growth is not clear. Resistance of hepatocytes to toxins occurs likewise during both normal and abnormal growth. It is possible therefore that the heat-shock proteins participate in an alteration in hepatocyte function that mediates a resistance to a variety of toxins. Lee *et al* (159) postulated that all agents capable of causing oxidative stress induce the heat-shock response. The products of this response would then mediate protection against subsequent oxidative attack.

In summary, the biological significance of the heat-shock phenomenon remains obscure. The expression of these proteins during both normal liver regeneration and following ischaemic injury to the liver suggest they may be of importance in the interpretation of this study.

4. PARTIAL ORTHOTOPIC LIVER TRANSPLANTATION.

Orthotopic liver transplantation has become the standard treatment for adults and children with irreversible liver disease (84,276). The earliest efforts in liver transplantation were limited by the concept that removal of the liver was a prohibitive surgical undertaking, and that the animal was unlikely to survive the anhepatic phase. Recent refinements in surgical technique, including novel haemostatic modalities and advances in liver preservation (139) have greatly facilitated the procedure, to the extent that the single greatest limitation to the use of liver transplantation in clinical practise is the availability of donor organs.

Partial orthotopic liver transplantation (POLT), in which the donor liver is surgically reduced by *ex vivo* hepatic resection, was first developed to overcome the problem of space experienced in models of auxiliary liver transplantation (84). This technique found its first clinical usage in 1984 when Bismuth reported the transplantation of a reduced-size liver graft (14). The technique has been increasingly used to overcome the size disparity between donor and recipient in paediatric liver transplantation. The obvious wastage of hepatic parenchyma associated with this procedure has prompted further innovation, in the form of the split liver graft. In this procedure the liver is divided into two, so providing donor material for two patients.

4.1 THE NEED FOR NOVEL TRANSPLANTATION TECHNIQUES.

A dramatic improvement in the results of orthotopic liver transplantation (OLT) has been reported over the last decade (276). With an overall 4 year survival rate of 81% (23), liver transplantation is having an impact on mortality in paediatric liver disease. In contrast, the mortality of children with biliary atresia is over 50% without transplantation (169). Moreover, the success rate for the Kasai procedure for biliary atresia is in the order of 30% (169).

Pretransplant mortality is a major problem in paediatric liver transplantation, particularly in the under 2 age group where scarcity of donors is even greater. Lilly and Hall (169) reported that pretransplant mortality of their patients with biliary atresia exceeded transplant mortality, with most deaths occurring in infants. In Pittsburgh, one quarter of children accepted as candidates for transplantation died prior to a liver becoming available (176).

Part of the explanation for this is the delayed referral of potential transplant recipients, due to decreased awareness of the benefits of transplantation. Furthermore, there appears to be a "marked disparity between the optimism of the transplant centres and the pessimism of the referring paediatricians" (71).

Paediatric liver disease primarily affects infants and small children. In the USA 55% of paediatric deaths due to liver disease occur in the age group 0 to 2 years (69). In contrast, children under 1 year of age made up only 10% of a large reported series of paediatric liver transplants. Technical difficulty is usually cited as the major reason for OLT not being performed in infants.

The shortage of paediatric liver donors was analyzed by Emond *et al* (69). Only 4% of livers were of suitable size for infant recipients. In addition, only 32% of adult and school-aged renal donors yielded a liver for donation (69). Therefore, not only was there an absolute shortage of small livers, but the available livers from older donors were under-utilized. A more recent study demonstrated an improved yield, with livers being harvested from 71% of renal donors (70).

Although it could be argued that using larger donors for small recipients places larger recipients at risk for not receiving a transplant, the patterns of organ donation suggest that older children still benefit from the more abundant supply of grafts in their age group.

The usage of RLT to improve liver utilization by overcoming size disparity has had a positive effect on waiting-list mortality in many centres since its inception (69,217). Simple graft reduction does not increase the total number of available livers. The technology of RLT has therefore been extended to include split liver transplantation, in which a liver is divided to create two grafts, and live donor hepatic transplantation.

4.2 SURGICAL ASPECTS.

The external morphology of the liver does not reflect its internal 'functional' architecture. The segmental anatomy of the liver is based on the branches of the portal vein as described by Bismuth (13,14). This knowledge has provided the anatomical basis for the advances in surgery of hepatic resection and reduced liver transplantation. Preparation of a reduced graft is accomplished by *ex vivo* hepatectomy based on the anatomic planes between functionally independent

portions of the liver. In practice, three separate grafts can be prepared for overcoming various degrees of size disparity (21). The right lobe is used when the size disparity between donor and recipient does not exceed 2:1, whilst usage of the left lobe is possible with a size disparity of up to 4:1 (21). A graft composed of segments 2 and 3 can be used to make up a disparity of up to 10:1. The donor vena cava is used for full right and left grafts, whilst the left hepatic vein is used for left lateral lobe grafts. Roux-en-Y hepaticojejunostomy is used for biliary reconstruction of all types of reduced liver grafts (71).

4.3 NOVEL TRANSPLANTATION TECHNIQUES.

Response to the increasing pressure of donor shortages has resulted in the advent of novel transplantation modalities. These have endeavoured to extend the utilization of donor material in different ways, and include reduced-size liver transplantation (RLT), split liver transplantation (SLT) and liver transplantation using a living donor (LRT).

4.3.1 Reduced-size liver transplantation (RLT).

This procedure was first performed by Bismuth and Houssin (14). The initial experience of Broelsch *et al* (22) with RLT was in a group of patients who were critically ill and not expected to survive the time to perform a conventional organ search. Although the survival was poor, the experience obtained demonstrated that the procedure was feasible.

An added advantage was that RLT reduced the mortality of patients on the transplantation waiting list, by improving donor organ utilization. A reduction of pre-transplant mortality to 7% was reported by Broelsch *et al* as a consequence of

the initiation of a RLT program (22). This compared favourably to centres in which intact OLT only is performed, where between 25 to 50% of potential paediatric recipients do not survive to transplant (22). Ethical studies demonstrated that even with the increased mortality associated with RLT, a reduction in global mortality was accomplished (267). With increasing experience, technical complications diminished and survival improved. In addition, the advent of UW solution (139) made extended preservation of the liver possible and has permitted extensive *ex vivo* surgery on the transplanted liver to become routine. A recent report from Chicago gives the survival of paediatric recipients of RLT at 78% against 81% for full-size grafts (71). The corresponding data from Brussels indicate a 77% survival rate for RLT against 85% for full-size OLT (218). These rates were not statistically significantly different.

The complication rates for the two procedures were similar. Of interest was that primary non-function was less common in RLT (70). This may reflect less stringent donor selection being employed for paediatric size-matched organs, which again reflects the paucity of donor livers in the paediatric age group. Furthermore, arterial thrombosis was more common in OLT than in RLT (70). This may reflect the larger calibre of vessels in the larger donors. Biliary complications were not more common after RLT (71).

A further most useful application of the technique may be for retransplantation, due to the emergent nature of this indication and the greater availability of reducible adult donor material (70).

4.3.2 Split liver transplantation (SLT).

Simple reduction of the liver graft, while improving the utilization of available donors, does not expand the available pool of donor livers. The practice of split liver grafting is based on the success of an initial operation performed by Pichlmayr (225), who had previously introduced the concept of *ex situ* liver resection and re-implantation (224).

Susequently, Emond *et al* (70) have reported a series of 30 patients in which two grafts are prepared from a single donor. In contrast to simple graft reduction, this adds significant complexity to the transplantation procedure. Over and above increased technical demands, it requires the performance of two concurrent recipient procedures, utilizing two surgical teams.

Although children receiving RLT have done as well as those receiving OLT, this has not been shown in adults. Implantation of a SLT into an adult would require knowledge that firstly, the regenerative capacity of the transplant is intact and secondly, that the graft is capable of normal liver function. These uncertainties have resulted in ethical questions being raised as regards the usage of SLT in adults. Innovative approaches in children have been supported in concept due to the lack of paediatric donors. The same support for innovative surgery may be lacking in adults where the shortages are not as acute. Nevertheless, improved results in paediatric recipients of SLT may bring increasing acceptance of SLT as a treatment modality in adults with end-stage liver disease.

There is little in the reported literature on the usage of this technique (SLT). Broelsch *et al* report an overall survival rate of 67% and graft survival of 55% in a series of 21 children (23). Complications were however more frequent in SLT than

in full size OLT. Haemoperitoneum occurred in 33% of SLT against 14% in full size grafts. Biliary leakage complicated 27% of SLT against 4% in full size OLT. Arterial thrombosis and primary non-function occurred with equal frequency in both groups (70).

The results of SLT in adult patients are less encouraging, with a reported graft and patient survival of 25% in a group of 4 adults receiving SLT (23). However, all of the adult recipients received their transplants exclusively in emergency circumstances. Biliary complications occurred in more than a quarter of patients. Arterial thrombosis was no more common in SLT than in other grafts, even though many SLT utilize interposition grafts. Necrosis of segment 4 was reported in 2 cases. Bismuth *et al* (15) have however reported on two cases in which SLT was performed on two adult recipients in emergency circumstances. Both died from causes not specifically related to operative technique, one from multiple organ failure on the 20th day and the other from systemic cytomegalovirus infection on the 45th day.

In the opinion of Emond *et al* (71), optimal use of SLT is currently restricted to the treatment of 2 children with a single paediatric donor.

4.3.3 Liver transplantation using a living related donor (LRT).

Statistics obtained from kidney transplantation centres have shown that tissue compatibility with the recipient is the most important factor deciding the fate of a transplanted organ. Kidneys from living related donors have the best graft survival, whilst those obtained from unmatched cadavers have the worst survival statistics. These observations led to the proposal of living related liver transplantation by Smith in 1969 (269). Only recently, due to the advances in liver transplantation technology has it been considered technically feasible.

An absolute imperative for the clinical usage of this technique is negligible donor mortality. A further prerequisite is acceptable survival rates for graft and recipient. The technique of hepatic inflow occlusion has reduced the complications of liver resection to a point where mortality for the procedure is negligible and the need for blood transfusions diminished (292). Usage of this technique should likewise diminish morbidity in the LRT donor.

The procedure has been tested in a laboratory animal model (53). The results of the latter study in dogs suggested that a viable graft could be obtained, and that the donor procedure was safe (53). However, it was not possible to achieve prolonged survival in the recipients.

The first LRT was performed in two patients in Brazil by Raia in 1989 (241). Unfortunately both recipients died despite technically successful transplants. Subsequently, Strong has successfully performed a RLT for a patient with biliary atresia, with good results for both donor and recipient at 6 months follow-up (282).

The preliminary results of Broelsch *et al* have been reported in a series of 5 cases (23). In this series, complications were not experienced in those donors in which a left lateral segment only was transplanted (23). In those in which total left hepatectomy is performed, all three developed complications. These included bile leakage from the transected parenchyma, a subhepatic fluid collection and an intraoperative splenic tear requiring splenectomy. It appears that donor morbidity is significant where total left hepatectomy is performed (23). This would suggest left lateral hepatectomy as the routine donor operation, although this adds significantly to the technical difficulty of the recipient procedure. This was performed in the latter 2 cases in this series. This procedure obviates the need to dissect and discard segment 4, as in the case of the total left hepatectomy (23).

In the same five patients reported from Chicago, patient and graft survival is reported as 100% for donors and recipients with follow-up from 2 to 6 months (23). These results, although very encouraging, should probably not be judged until several years of accumulated experience are available.

The main benefit of RLT is a reduced risk of pretransplantation mortality. At most liver transplant centres, because of the shortage of donors, up to 50% of potential recipients die awaiting transplantation (69,169,176). Additional benefits include a totally elective transplant procedure, potentially improved graft quality since it is harvested from a live donor, and the possible immunologic advantage conferred by a related donor.

The ethical considerations of RLT require a careful decision-making process in order to assess correctly the relative risks to both donor and recipient. Donor selection is critical and requires an elaborate procedure for the assurance of informed consent.

In conclusion, the techniques of reduced, split and living donor liver transplantation are making it possible to treat all children needing liver transplantation despite the limitations in supply of cadaveric donors. Liver transplantation has already had a clear impact on global mortality in children with liver disease (276). As therapy for the future, orthotopic liver transplantation may not be exclusively based on cadaver organ donation.

4.4 LIVER REGENERATION AFTER INTACT AND REDUCED TRANSPLANTATION.

The inherent capability of the liver to regenerate is well known. Hepatocyte proliferation following transplantation is less well documented. An attenuated pattern of hepatocyte proliferation has been demonstrated in pigs (134) and rats (73,290) following intact liver transplantation. This diminished response may however reflect the absence of a true regenerative stimulus, since the mass of liver transplanted in these studies was similar in size to the native liver, and therefore represented a similar liver-body mass ratio. A similarly diminished regenerative response, albeit measured only by increase in volume of the transplanted liver, has been shown in dogs by Kam *et al* (142). Where the transplanted liver was of a similar size to the donor liver, a negligible increase occurred, whereas an increase in graft volume was noted where the transplanted liver was small-for-size. This study introduced the concept that recipient size determines, at least in part, liver graft size after transplantation.

Enlargement of small-for-size intact liver grafts has been reported in two transplant patients by Van Thiel *et al* (295). Despite graft enlargement, no evidence of an increased rate of DNA synthesis or hepatocyte proliferation was shown. Furthermore, the absence of a relationship between liver enlargement and serum levels of putative hepatotrophic substances in this study served to illustrate a clear need for a specific marker of hepatocyte proliferation following clinical transplantation.

The regenerative response has been studied following partial orthotopic liver transplantation in dogs (9) and in pigs (248). In the former study, the liver weights

were studied at 24 weeks postoperatively and found to be similar in mass to the native liver. In the latter study, the liver weight at the time of sacrifice was noted to be greater than at the time of operation. In neither of these studies was the question of hepatocyte proliferation or DNA synthesis specifically addressed however, nor was the time course of liver regeneration following transplantation studied.

The regenerative capacity of the liver is often been assumed to be constant, whether it be following reduced-size transplantion or hepatic resection (53). This may not be the case.

Given the discrepancy between the numbers of organs transplanted and the number of patients requiring transplantation, there is an ever increasing need for these innovative transplantation modalities. Concomitant to the successfull usage of small-for-size liver transplantation is knowledge of the proliferative kinetics of the transplanted hepatic parenchyma. In addition, there is a need for the development of noninvasive and direct measurements of hepatocyte DNA synthesis and mitosis in the transplanted liver. A further question is whether regeneration is indeed desirable in view of the attenuation of critical liver function known to occur at this time (154).

The current study of hepatocyte proliferation attempts to answer some of these questions.

5. STATEMENT OF OBJECTIVE.

Having reviewed the literature pertaining to liver regeneration, the effects of hepatic ischaemia, and partial orthotopic liver transplantation, it is apparent that the regenerative response following partial liver transplantation has not been fully documented. There has been enormous recent progress in the development of new transplantation modalities, one of which may utilize reduced donor livers for adult recipients. This procedure has numerous potential complications, only one of which may relate to the often assumed capacity of the transplanted liver segment to regenerate. Although it is likely that this will occur, the magnitude and time course of this proliferative response is unknown. The overall purpose of this study therefore is to examine hepatocyte proliferation and DNA synthesis after partial orthotopic liver transplantation in the rat model.

6. GENERAL METHODS.

Materials and methods used in all experiments will be discussed in this chapter, whilst procedures unique to the particular experiment will be discussed in the relevant section.

6.1 Animals.

Male Long-Evans rats weighing 250-350 grams were used in the study. The animals were maintained on standard rat feed *ad libitum* until 24 hours prior to surgery. They were allowed free access to water throughout. Animals were kept in cages at 22°C with fixed daylight cycling, with cages lit between 07:00 and 19:00. They had been trained in the feeding and lighting schedule from birth.

6.2 Anaesthesia.

All surgical procedures were performed under ether anaesthesia. A bell jar was used for induction, and a nose cone for the delivery of maintenance anaesthesia. Preference was given to an inhalational agent, due to the need to rapidly alter the level of anaesthesia in some of the experiments.

6.3 Surgery.

Clean, but not sterile technique was used throughout. Microsurgical technique was employed where necessary. All procedures were performed between 09:00 and 12:00 in order to avoid variations in hepatic regeneration due to circadian rhythm (32). Times of sacrifice were adjusted accordingly. A midline laparotomy extending from the xiphisternum posteriorly for +- 6 cm was performed in all cases.

Details of specific procedures are mentioned in the relevant chapters. In all cases the laparotomy wound was closed in two layers by continuous suture using 3/0 chromic catgut. Following all procedures, animals were allowed to recuperate in a warmed environment until fully awake after anaesthesia. They were then returned to their holding cages and allowed free access to water and feed. Non-survivors were examined at autopsy whenever this was feasible, and the cause of death noted.

The programme was accepted by the Animal Ethics Committee, University of Cape Town.

6.4 Sampling.

Groups of rats (n=4-9 per group according to survival) were exsanguinated by aortic puncture under ether anaesthesia after periods of 4, 24, 48, 72 and 96 hours. A 5 ml blood sample was taken for measurement of aspartate aminotransferase. The liver was removed and the mass determined. The liver was divided into four: two sections of at least 1 gram for thymidine kinase and ornithine decarboxylase assay, a section for histological examination and a section for the determination of tissue water. The latter section was weighed before and after being dried in an oven at 70° for 48 hrs

in order to determine tissue water. Sections of liver for thymidine kinase and ornithine decarboxylase assay were immediately placed in liquid nitrogen for storage.

6.5 Parameters of liver regeneration.

The following parameters were used as indicators of regenerative activity:

6.5.1 Restitution of liver weight.

The mass of the remnant liver was determined at the time of sacrifice. A section of this liver was weighed, then dried in an oven for 48 hrs, and weighed again in order to determine tissue water. The gross mass of the liver was corrected for differences in tissue water and expressed as an index (liver mass index or LMI) of the animal's mass at the time of sacrifice.

Tissue water was calculated by the formula:

tissue water = $(M_w - M_d) 100 / M_w$.

Liver mass index was calculated by the formula:

$LMI = (M_l \times M_d / M_w) / (M_a \times 10^2)$.

Where LMI = liver mass index

M_l = mass of liver remnant

M_w = mass of fresh liver sample

M_d = mass of dried liver sample

M_a = gross mass of animal at sacrifice.

6.5.2 Thymidine kinase activity.

Thymidine kinase activity was assayed in the (1 gram) sample of liver tissue by standard methods (133). Briefly, weighed portions of frozen pulverized liver were homogenized in 10 vol of buffer (0.1 M Tris-HCl, pH 8.0) using a Ultra-Turrax homogenizer. The homogenate was centrifuged at 105 000 $g_{av.}$ for 60 min at 4°C in a Beckman L5.65 ultracentrifuge. The supernatant, which was carefully aspirated avoiding the top layer containing fat, served as an enzyme source.
Samples for background activity were prepared by terminating the reaction as soon as all components were mixed together, and these zero time values were then subtracted from the values obtained at the end of the incubation period. In each assay a standard sample containing a known amount of radioactive substrate was prepared.
The reaction mixture contained in a final volume of 1.0 ml: 0.05 M Tris-HCl buffer (pH 8.0), 5 mM ATP, 3.6 mM $MgCl_2$, 0.01 mM [3H]thymidine, and 0.2 ml of supernatant. The reaction mixture was incubated for 10 min at 37°C, and the reaction was stopped by immersing the assay tubes in boiling water for 2 minutes. The tubes were cooled on ice, and the denatured protein was removed by centrifuging at 100 g for 10 min. Duplicate aliquots of 0.1 ml each were removed from each assay tube and spotted onto DEAE-cellulose discs. The discs were washed in 1 mM ammonium formate (25 ml) for 5 min, distilled water (25 ml) for 3 min, ammonium formate for 5 min, and finally rinsed in distilled water again. The discs were allowed to drain and were then placed in scintillation vials. To each vial was added 1.0 ml of 0.1 M HCl/0.2 M KCl solution. The vials were gently shaken for 15 min to allow the labelled nucleotides to be eluted from the exchange paper. After elution was complete, 10 ml of "Instagel" (Packard Instrument Co., Downers Grove, Ill.) was added to each vial, and the vials were shaken until the emulsion was

clarified and the discs were fully permeated with solvents. The discs were counted for radioactivity in a Beckman 3-channel scintillation counter.

Results were expressed as disintegrations per minute per milligram protein (dis.min-1.mg protein -1).

6.5.3 Ornithine decarboxylase activity.

Ornithine decarboxylase activity was assayed in the (1 gram) sample of liver tissue by standard methods (186). Briefly, the enzyme was assayed *in vitro* by measuring the release of $_{14}CO_2$ from DL-[1-$_{14}$C]ornithine (50mCi/mmol; Amersham, England). Excised livers were homogenized in 0.25 M-sucrose containing 0.5mM EDTA, 10mM-mercaptoethanol and 10mM-Tris/HCl buffer, pH 7.4. The homogenates were centrifuged for 60 min at 36 000 $g_{av.}$ in a Beckman L5.65 ultracentrifuge and samples of the cytosol were assayed for enzyme activity. The incubation mixture (1ml total) consisted of 0.4 ml of cytosol, 0.2 mol of pyridoxyl phosphate, 5mM-dithioreitol, 10mM-Tris/HCl buffer, pH 8.0, and 0.8 Ci of [1-$_{14}$C]ornithine and unlabelled L-ornithine to 2mM. After pre-incubation for 5 min at 37°C, labelled ornithine was added and the tubes were capped and incubated for 60 min. The $_{14}$C evolved during the reaction was trapped in KOH spotted onto the filter paper. For radioactivity determination the filter paper was placed into a vial containing scintillation fluid.

6.5.4 Histology.

A segment of the liver was stored in formol saline at the time of sacrifice. Sections of liver tissue were prepared by paraffin section and stained with haematoxylin and eosin. Sections were examined for fatty infiltration and evidence of hepatocyte

proliferation. Mitotic figures were counted and expressed as mitoses per 1000 cells (mitotic index).

6.6 Aspartate aminotransferase (AST).

Blood samples for AST were collected into heparinised tubes. The 5 ml blood sample was centrifuged at 30000 rpm for 10 minutes. Plasma was decanted and frozen for subsequent AST assay by standard methods.

6.7 Data analysis.

Data were expressed as the means +- standard error. Data were compared using one-way ANOVA at each point, and two-way ANOVA where appropriate. The level of significance was at least $p < 0.05$ for all experiments.

7. PARTIAL HEPATECTOMY.

This component of the study aimed to establish a universal control against which other experimental groups could be compared. Although the regenerative response to partial hepatectomy has been previously documented in this laboratory (133), it remained nevertheless important to establish a standard pattern of hepatocyte proliferation under constant conditions to be applied in each experiment.

7.1 Methods.

7.1.1 Sham operation.

Sham operation comprised a midline laparotomy and liver dissection to free all attachments and collateral blood vessels. These animals were immediately sacrificed under anaesthesia. Sampling was performed as previously described.

7.1.2 Partial hepatectomy.

Laparotomy was performed as for sham animals. Full hepatic dissection as described for subsequent groups was not performed (see Chapters 8-12). Standardized 68% partial hepatectomy was employed as described by Higgins and Anderson (116). This was modified by the application of a vascular clamp to the porta hepatis prior to partial hepatectomy, in order to secure inflow occlusion and

minimize blood loss. The liver was then extruded through the abdominal incision and the median and left lateral lobes ligated at their base using a 5/0 silk tie, and resected. Care was taken in the placement of the suture so as not to include the vena cava. The clamp was then removed. This manouevre markedly minimized blood loss at the time of partial hepatectomy. The duration of hepatic ischaemia associated with this procedure was less than 60 seconds. Animals were sacrificed after periods of 4, 24, 48, 72 and 96 hours, and sampling performed as described. Sampling could potentially have been performed at intervals of 2 to 4 hours to define each peak of activity as closely as possible. However since this may not have provided greater clarity on the definition of the peak, and considering the implications in terms of animal usage, this was not considered justifiable. The term 'peak' as used in all subsequent discussion therefore indicates the highest recorded activity.

7.2 Results.

7.2.1 Survival.

Of the 52 rats in this group 50 survived until the time of sacrifice (96.1%). There was no mortality in sham-operated animals (n=6).

7.2.2 AST.

Plasma AST reached a maximum of 390+-31 at 24 hrs after an initial elevation of 328+-40 at 4 hrs. Thereafter there was a gradual decline to 192+-32 at 72 hrs and 111+-9 at 96 hrs. In shams AST levels were 93+-5.

7.2.3 Thymidine kinase.

Thymidine kinase levels reached a recorded maximum at 24 hrs (36021+-8060). The elevation persisted at 48 hrs (26042+-3957) and showed a smaller peak at 72 hrs (28378+-5545). By 96 hrs levels had declined to 10179+-982 (Table 7.1). The activity in sham-operated animals was 8071+-979 at the time of operation.

7.2.4 Mitotic index.

The mitotic index reached a recorded maximum of 25+-8 at 24 hrs. This corresponded to the peak of thymidine kinase activity. Therafter the mitotic activity diminished to levels of 9+-3 at 48 hrs, 14+-3 at 72 hrs and 9+-2 at 96 hrs. A second wave of mitotic activity was apparent however, with the mitotic index at 72 hrs greater than that at 48 hrs (Table 7.1). In sham-operated animals the mitotic index was 1+-0.3.

Table 7.1 Thymidine kinase activity (dpm per mg protein) and mitotic index (mitotic figures per 1000 cells) after partial hepatectomy in fasted rats.

Time	Thymidine kinase	Mitotic index
Shams	8071+-979	1+-0.3
4 hrs	4637+-796	1+-1
24 hrs	36021+-8061	25+-8
48 hrs	26042+-3957	9+-3
72 hrs	28378+-5545	14+-3
96 hrs	10179+-983	9+-2

7.2.5 Liver mass index and tissue water.

The liver mass index of sham-operated animals gives an indication of the normal liver to body mass ratio in fasted Long-Evans rats. The liver mass index showed a progressive increase from 4 hrs through to 72 hrs, and subsequently declined. No significant differences were noted in tissue water in the 96 hours following partial hepatectomy (Table 7.2).

Table 7.2 Liver mass index (LMI) and tissue water after partial hepatectomy in fasted rats.

Time	Liver mass index	Tissue water
Shams	0.942+-0.07	68.2%+-0.5%
4 hrs	0.353+-0.028	71.6%+-1.4%
24 hrs	0.517+-0.021	70.2%+-0.3%
48 hrs	0.755+-0.03	71.2%+-0.5%
72 hrs	0.835+-0.043	71.4%+-0.4%
96 hrs	0.732+-0.053	71.2%+-0.4%

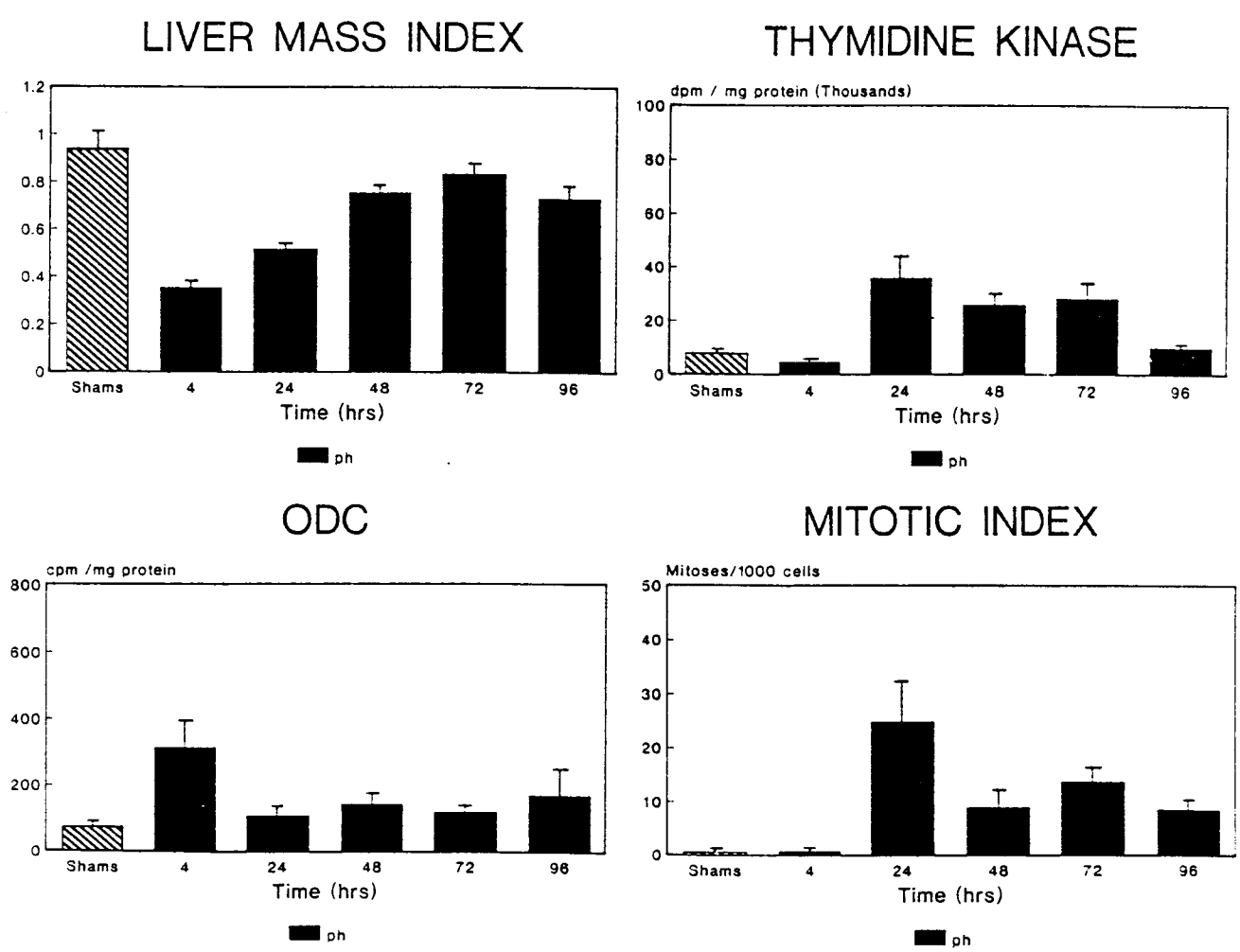
7.2.6 Ornithine decarboxylase.

Ornithine decarboxylase levels reached a recorded maximum of 313+-80 at 4 hrs. Thereafter ODC activity declined progressively (Table 7.3). ODC activity in sham-operated animals was 76+-13.

Table 7.3 Ornithine decarboxylase activity (cpm per mg protein) after partial hepatectomy in fasted rats.

TIME	ODC
Shams	76+-13
4 hrs	313+-80
24 hrs	109+-26
48 hrs	144+-32
72 hrs	121+-19
96 hrs	171+-77

Figure 7.1 *Liver regeneration following 68% partial hepatectomy. Parameters of liver regeneration measured were the liver mass index (LMI), thymidine kinase (dpm per mg protein), mitotic index (mitotic figures per 1000 cells) and ornithine decarboxylase (ODC) (cpm per mg protein). Animals were sacrificed 4, 24, 48, 72 and 96 hrs after partial hepatectomy. Results were expressed as means +- SE.*



7.3 Discussion.

This group served as a universal control against which other experimental groups were compared. The response of the rat liver to partial hepatectomy has been studied for over 60 years (116). This simple technique is highly reproducible and well tolerated, and provides the stimulus for the regenerative response. Not all rats respond to partial hepatectomy in an identical fashion, however. The rate of DNA synthesis in weanling rats follows a biphasic curve, with peaks at approximately 20 to 22 hours and 33 to 35 hours after partial hepatectomy. In young adult Holtzman rats a single broad peak appears somewhat later, at 23 to 25 hours (4 months old, averaging 233+-22 gram) (29). The span of this peak is approximately 12 hours as compared to the very narrow peak in weanling rats. Peak mitotic activity follows maximal DNA synthetic activity by about 6 to 8 hours (32). It was desirable in the present study to utilize rats in which the hepatocellular proliferation kinetics had been previously studied and were well-known. Furthermore, from previous work on liver transplantation in rats done in this laboratory (72), it has been our experience that optimal survival occurs in rats of mass greater than 300 grams. Given these restrictions, Long-Evans rats of 3 months age with an approximate weight of 300 grams were used (range +-50 grams). The broad peak of DNA synthesis previously observed in these animals following partial hepatectomy (29) would serve to obviate the need to perform closely spaced observations to detect a narrow peak of DNA synthesis. The slightly older animals could also be expected to survive the transplantation procedure in a more predictable manner. A time of 24 hours after partial hepatectomy was chosen therefore as the most likely to show peak DNA synthesis in animals in the chosen weight range.

Marked changes in polyamine metabolism occur in regenerating rat liver (186). Ornithine decarboxylase is the rate-limiting enzyme in polyamine synthesis in mammalian tissues. Ornithine decarboxylase activity following partial hepatectomy follows a biphasic curve, with maxima occurring at 4 to 6 hours and at 16 hours (186). In order to study polyamine metabolism therefore, a time interval of 4 hours after partial hepatectomy was chosen for sampling.

Times of 4 and 24 hours were therefore chosen to represent the likely maxima of ODC and thymidine kinase activity respectively. The remaining time periods, namely 48 hours, 72 hours and 96 hours were arbitrarily chosen.

The peak in thymidine kinase activity in this group was recorded at 24 hours, with activity remaining significantly elevated until 72 hours. Kahn *et al* (133) have previously demonstrated the accurate correlation between thymidine kinase activity and [$_{14}$C]thymidine incorporation as measures of DNA synthesis in rat hepatocytes. This data therefore confirms previous findings that peak [$_{14}$C]thymidine incorporation occurs at 24 hours in animals of this approximate age (29). Elevated thymidine kinase activity at 72 hours probably represents a second wave of DNA synthesis.

The pattern of mitotic activity observed in this group corresponded well with DNA synthesis, as it did in other experimental groups (see Chapters 8,9 and 12). It is possible that the true mitotic peak was somewhat greater than that recorded, since peak mitotic activity follows DNA synthetic activity by about 6 to 8 hours (32). The ODC peak at 4 hours confirmed the finding of McGowan and Fausto (186) that maximal ODC activity occurs at this time. Restoration of liver mass to that of sham-operated animals occurred by 96 hours after partial hepatectomy, with the majority of growth occurring in the first 48 hours (see Table 7.2). A 3 fold elevation in

aspartate aminotransferase (AST) was demonstrated following partial hepatectomy, reaching a peak at 24 hours. This probably resulted from mechanical damage at the time of surgery.

Having thus established the pattern of hepatic regeneration occurring after partial hepatectomy under constant conditions in our laboratory, we could proceed to compare the kinetics and magnitude of this response with changes in each of the experimental groups in turn.

8. HEPATIC ISCHAEMIA.

To our knowledge, the question of hepatic ischaemia and its influence on the early regenerative process has not previously been examined. A single study in dogs (173) examined the liver mass 6 weeks after hepatic vascular occlusion and partial hepatectomy. In this component of the study we aimed to investigate the effect of warm hepatic ischaemia on hepatocyte proliferation and DNA synthesis in the first 96 hours after partial hepatectomy.

8.1 Methods.

Midline laparotomy and partial hepatectomy (with modification as discussed on page 81) were performed. The bowel was retracted to the left and covered with a saline-soaked gauze swab. Great care was exercised throughout the procedure to keep the liver and intestines moist, and to minimize their handling. Liver dissection was then performed. Briefly, this was as follows: the falciform, left lateral and gastrohepatic ligaments were divided. The pyloric vein was ligated and divided, and the hepatic artery dissected free from the portal vein. Both left and right phrenic veins were divided and ligated. The oesophageal branches of the left gastric artery were diathermized and divided. The suprarenal inferior vena cava was dissected free from adjacent fatty connective tissue. The right adrenal vein was ligated and

divided. Remaining posterior attachments were freed. This dissection was performed in order to simulate the dissection of the recipient in the transplant procedure, and to ensure that no collateral blood supply reached the liver.

Transient total hepatic ischaemia was then induced by clamping the hepatic artery, portal vein and bile duct for 40 minutes. The bile duct was clamped to ensure that no blood reached the liver via the biliary collateral vessels. During the period of ischaemia the liver appeared pale whilst signs of portal stasis became apparent. These included distension of the portal vein and its tributaries and cyanotic discolouration of the bowel. In all cases reperfusion was noted upon release of the clamps, with the liver and gut regaining their normal colour.

India Ink test. The completeness of ischaemia was tested in a separate sample group in which 0.5 ml of Indian ink (diluted 50/50 in water) was injected into the dorsal vein of the penis following liver dissection and application of the clamps. Animals were sacrificed immediately thereafter and the liver examined histologically for evidence of carbon particle deposition in the Kupffer cells. Failure to demonstrate carbon particle accumulation would confirm firstly the adequacy of dissection and clamping, and secondly that retrograde filling via the hepatic veins had not occurred.

8.2 Results.

See also Figure 8.1.

8.2.1 Survival.

In this group 32 out of 76 animals survived until sacrifice (42%). The adequacy of reperfusion was examined at autopsy. In none of the animals was failure of reperfusion apparent. Mortality was highest in the first 24 postoperative hours.

8.2.2 AST.

Plasma AST reached a maximum of 470+-17 at 4 hours. Thereafter there was a sustained elevation persisting from 24 hrs (405+-102) through to 48 hrs (443+-45). This was followed by a gradual decrease to 301+-45 at 72 hrs and 133+-36 at 96 hrs.

8.2.3 Thymidine kinase.

Thymidine kinase levels were depressed at 4 hrs (2395+-477) and at 24 hrs (6115+-2129). The recorded peak was demonstrated at 48 hrs (76804+-12340). Thereafter levels fell to 25047+-4178 at 72 hrs and 8535+-1701 at 96 hrs (Table 8.1).

8.2.4 Mitotic index.

Mitotic activity was depressed at 4 hrs (1+-0) and at 24 hrs (1+-0). Therafter a dramatic increase was noted to reach a level of 28+-14 at 48 hrs. The mitotic index then declined to 11+-3 at 72 hrs and 8+-1 at 96 hrs (Table 8.1).

Table 8.1 Thymidine kinase activity (dpm per mg protein) and mitotic index (mitotic figures per 1000 cells) in fasted rats after partial hepatectomy and 40 mins hepatic ischaemia.

TIME	THYMIDINE KINASE	MITOTIC INDEX
4 hrs	2395+-477	1+-0
24 hrs	6115+-2129	1+-0
48 hrs	76804+-12340	28+-14
72 hrs	25047+-4178	11+-3
96 hrs	8535+-1701	8+-1

8.2.5 Liver mass index and tissue water.

The liver mass index demonstrated a progressive increase with the greatest increase shown in the time period 48-96 hrs. Tissue water tended to be greater in the ischaemic livers than those subjected to partial hepatectomy only, although statistical significance was not reached (Table 8.2 and Table 7.2).

Table 8.2 Liver mass index (LMI) and tissue water after partial hepatectomy and 40 mins hepatic ischaemia in fasted rats.

TIME	LIVER MASS INDEX	TISSUE WATER
4 hrs	0.399+-0.054	74.3%+-2.3%
24 hrs	0.514+-0.033	71.6%+-0.7%
48 hrs	0.577+-0.029	73.1%+-0.6%
72 hrs	0.615+-0.030	72.5%+-0.5%
96 hrs	0.852+-0.053	70.8%+-0.3%

8.2.6 Ornithine decarboxylase.

Ornithine decarboxylase levels are demonstrated in Table 8.3. A gradual increase was shown with the recorded maximum (438+-306) recorded at 72 hrs.

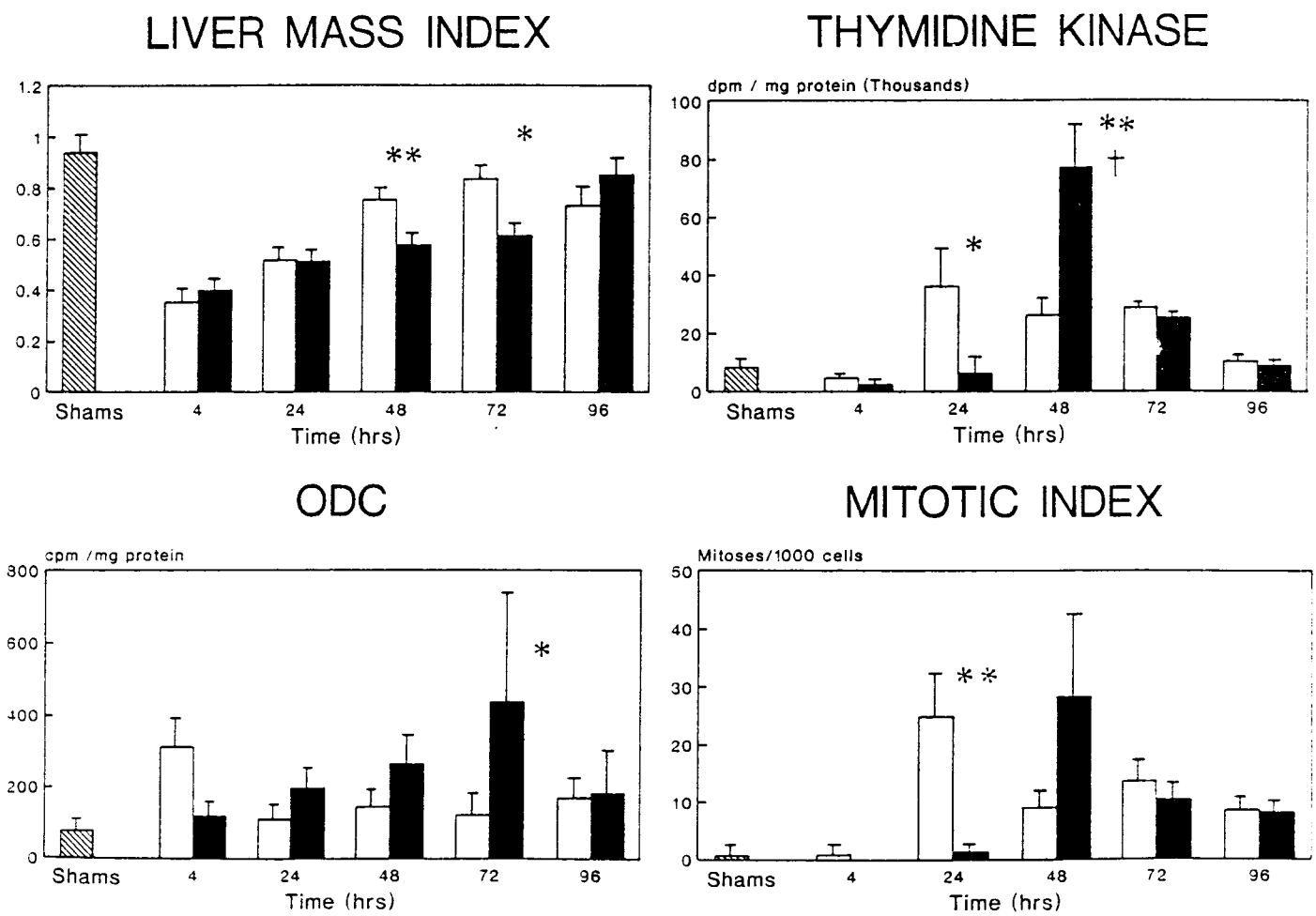
Table 8.3 Ornithine decarboxylase (ODC) activity (cpm per mg protein) after partial hepatectomy and 40 mins ischaemia in fasted rats.

TIME	ODC
4 hrs	117+-15
24 hrs	197+-51
48 hrs	266+-86
72 hrs	438+-306
96 hrs	183+-116

8.2.7 Histology.

No hepatic carbon deposition was observed in the animals in which the India ink test was performed. In none of the sections studied was there evidence of massive hepatocyte necrosis. Intracellular accumulation of neutral fat, cellular hyperplasia and clumping of cytoplasmic basophilic material was noted. There was no consistent difference in the degree or timing of fatty infiltration.

Figure 8.1 *Influence of 40 mins hepatic ischaemia without portal decompression on hepatic regeneration in fasted rats. Partial hepatectomy was performed as a control (clear bars) and compared to partial hepatectomy plus 40 mins hepatic ischaemia (solid bars). Parameters of liver regeneration measured were the liver mass index (LMI), thymidine kinase (dpm per mg protein), mitotic index (mitotic figures per 1000 cells) and ornithine decarboxylase (ODC) (cpm per mg protein). Animals were sacrificed 4, 24, 48, 72 and 96 hrs after partial hepatectomy. Results were expressed as means +- SE. * $p<0.05$ and ** $p<0.01$ by ANOVA. † indicates TK for solid bars at 48 hrs greater than TK for clear bars at 24 hrs ($p<0.05$ by ANOVA).*



8.3 Discussion.

A delay in the time to reach a recorded peak in regenerative parameters was characteristic of the results for this group. The recorded maxima for the mitotic index and thymidine kinase activity were reached at 48 hours. The liver mass index, although reaching parity with partially hepatectomized controls by 96 hours, showed the greatest increment in the time period from 48 to 96 hours. By contrast, in partially hepatectomized controls, the peak thymidine kinase and mitotic activity was recorded at 24 hrs, and the LMI demonstrated greatest increases between 4 and 48 hours.

The cause of the delay in the initiation of the regenerative process merits further discussion. Briefly, this may be related either to nonspecific phenomena associated with the metabolic response to the surgical trauma of partial hepatectomy and ischaemia, or due to interference by ischaemia with specific hepatocyte proliferative control mechanisms.

The first factor in regard to delay which requires consideration is the possibility that it is a false observation resulting from the sampling schedule, that is that the peak occurred somewhere between 24 and 48 hours. In an attempt to solve this, a small number of animals (6) was prepared in a side-arm of the study for sacrifice at 28 and 32 hours. These samples also showed depressed levels of thymidine kinase and mitotic indices (data not presented). Further extension of sampling which could be almost infinite, was deemed to be impractical.

Another potential source of spurious results was investigated in a separate limb of the study (data not presented): partially hepatectomized rats were exposed to 20 mins of inflow occlusion. In these animals, the liver had been fully mobilized. No

differences in the time course, or magnitude of liver regeneration could be demonstrated when these were compared to partially hepatectomized control animals (with no liver mobilization). These animals therefore served as a further control group to validate the differences observed between partially hepatectomized controls and animals exposed to 40 mins of inflow occlusion.

Recently, a role for the sympathetic nervous system, noradrenaline and the alpha-1 adrenergic receptor in the control of liver regeneration have been described (62). Sympathetic denervation by microsurgical dissection of the hepatic artery, or administration of the alpha-1 blocker prazosin, results in abolition of the 24 hour DNA synthetic peak in rats *in vivo* (62). The alpha-1 adrenoceptor has been shown to mediate the catecholamine effect, by downregulation of the EGF receptor and enhanced EGF mitogenic effects in primary hepatocyte culture (64). The delay in initiation of DNA synthesis seen in this group may therefore represent the results of hepatic sympathetic denervation, incurred at the time of the liver dissection, rather than any effect attributable to hepatic ischaemia or portal venous stasis. Cognizance should be taken of the fact that full hepatic dissection was not performed in partially hepatectomized controls.

The initial depression of regenerative activity confirms the observations of Garcio-Alonzo *et al* in rats exposed to ischaemia of up to 25 mins duration (96). A delay in the onset of the regenerative process following partial hepatectomy has also been observed in animals subjected to a simultaneous local inflammatory stimulus (11). In a study in rats, a focus of acute inflammation was induced by the injection of turpentine simultaneous to partial hepatectomy (11). This resulted in significant inhibition of the first peaks of hepatocyte DNA synthesis and mitosis (11).

In the current study, the hepatic ischaemia-reperfusion sequence may provide a comparable inflammatory stimulus. An invariable concomitant of local inflammation is an increase in tissue water in the form of oedema. The liver tissue water in those animals subjected to ischaemia tended to be higher than that of animals that had partial hepatectomy only. If this observation is valid, the increase in tissue water may represent a sequel to a local inflammatory response. The cause of the delay may be due to competition at the transcriptional or translational level for the synthesis of various proteins, with acute-phase proteins being synthesized in preference to those involved in cell proliferation.

The imposition of an inflammatory stimulus upon the body usually results in the production of a broad range of acute phase proteins by the hepatocyte (11). This is a well documented phenomenon and part of the body's response to trauma. Removal of a segment of the liver sets into motion a burst of regenerative liver growth. If the animal is subject to a stressful stimulus simultaneous to the resection of part of the liver, then a reprogramming of function may occur which sees preference given to the production of acute phase proteins, later followed by a period in which regenerative proteins are synthesized. This could account for the delay in the initiation of the process in this and other groups.

Acute phase proteins apart, the liver may be responsible for the synthesis of additional proteins at this time. When a cell is exposed to stressful situations, such as increased temperature or ischaemia, it begins to synthesize so-called heat-shock proteins to the relative exclusion of others (99).

Like the acute phase proteins, heat-shock proteins are produced in response to a variety of noxious stimuli. This is suggestive of a protective effect. Despite obvious

similarities, these two phenomena appear to be distinct entities (see previous discussion).

The expression of heat-shock proteins during hepatic regeneration has been demonstrated (46), with increased synthesis of both hsp83 and hsp70. A similar pattern occurs following the administration of hepatocarcinogens (46), which suggests their expression during both normal and abnormal hepatocyte proliferation. The circumstances of heat-shock protein synthesis are therefore similar to those observed for the expression of the c-H-ras amd c-myc oncogenes (46). This would suggest a coordinate expression for these genes during liver regeneration.

Apart from oncogenes, there are remarkable similarities in the expression of heat-shock proteins and other proteins known to be associated with fetal and neoplastic growth, such as alpha-fetoprotein. The association of heat-shock proteins, c-H-ras and c-myc and alpha-fetoprotein with the regenerative response suggests that they might serve as useful clinical indicators of regeneration. The latter has been suggested as a noninvasive clinical test of regenerative activity in the clinical setting after transplantation (154).

The biological significance of the heat-shock phenomenon is obscure. The expression of these proteins both during normal liver regeneration and following ischaemic injury to the liver suggest they may be of importance in the interpretation of the results of this study. An extension of this study could include the measurement of some of these proteins in the transplantation context.

Further explanations for the observed delay in initiation of the regenerative process should be sought. Elevated corticosteroid levels have been observed following an

inflammatory stimulus (156). There is evidence for an inhibitory role for corticosteroids in liver regeneration (234). This would suggest that increased circulating corticosteroid hormones may account for the observed inhibitory effect. This explanation would however conflict with earlier studies in female rats in which prior surgical stress was shown to accelerate DNA synthesis (200). This apparent disparity in turn may be due to the sexual differences that occur in the response to irritative substances (140). Resolution of this question may only be feasible in primary hepatocyte culture, due to the multiplicity of factors complicating hormonal measurements *in vivo*.

Fasting has also been shown to depress increases in DNA synthesis and mitotic activity following partial hepatectomy (200). However in this study all animals were fasted for 24 hours.

ODC levels in this group were disorganized, with a peak being recorded at 72 hours. In a study of partially hepatectomized and hypophysectomized rats, McGowan and Fausto (186) demonstrated that although DNA synthesis was delayed by this procedure, the kinetics of polyamine metabolism were unaltered. This suggested that increased polyamine concentrations in partially hepatectomized liver may not be the trigger for the initiation of DNA replication. Our findings are strongly supportive of this contention, since the peak ODC activity in this group occurs well after maximal DNA replication has taken place. It is likely therefore that increased levels of polyamines are essential for cellular functions such as DNA synthesis, but that they do not constitute initiators of DNA synthesis in the prereplicative phase of cell division. This conclusion is however merely derived from the finding that the time course of ODC activity is not directly co-ordinated with the onset of DNA synthesis.

In addition to the delay in initiation of the regenerative process, apparent enhancement of hepatocyte proliferation was observed in animals subjected to hepatic ischaemia. Maximal DNA synthesis and mitotic activity were demonstrated at 48 hrs in this group. This was significantly greater than the peak response seen in the partially hepatectomized group at 24 hours. Although delayed, the proliferative response in this group was therefore enhanced.

In the production of hepatic ischaemia by acute ligation of the porta hepatis, there is inevitable splanchnic stasis due to portal venous occlusion. This raises the question of whether the enhanced proliferative response is truly due to hepatic ischaemia or perhaps due to the sequelae of portal venous occlusion. This dilemma made necessary the creation of a model of hepatic ischaemia that obviated splanchnic stasis. This will be further discussed on page 109.

DNA synthesis is enhanced after 90% partial hepatectomy (98). Hepatic ischaemia may result in ischaemic necrosis of hepatocytes. The possibility exists therefore that hepatic ischaemia results in an additional loss of functional hepatic parenchyma, with consequent enhancement of the regenerative stimulus. However in these animals which were subject to 40 minutes ischaemia, AST levels did not indicate significantly greater hepatocyte damage than in the animals with partial hepatectomy only. Furthermore, DNA synthesis was relatively diminished in those animals subject to hepatic ischaemia with portal venous decompression (see page 105). This would counter the contention that ischaemia resulted in additional hepatocyte necrosis, and a greater regenerative stimulus.

Portal venous occlusion of 30 minutes duration results in the appearance of endotoxin in the portal and systemic circulation (215). This may be accounted for by the translocation of endotoxin across the damaged intestinal mucosa (216). The

administration of exogenous endotoxin has been shown to enhance DNA synthesis significantly (266). Pretreatment with endotoxin 24 hours prior to partial hepatectomy accelerated DNA synthesis (61), and restriction of gut-derived endotoxin inhibited DNA synthesis following partial hepatectomy (60). The possibility exists therefore that the observed apparent enhancement in DNA synthesis and mitosis following portal venous occlusion may be accounted for by portal endotoxaemia. This effect may be mediated by cytokine release from endotoxin-activated Kupffer cells (43). Stimulation of DNA synthesis in hepatocytes by Kupffer cells has been demonstrated *in vitro* (146). Recently, Callery *et al* have demonstrated that Kupffer cell production of prostaglandin E2 is amplified during liver regeneration, whilst production of tumor necrosis factor-alpha is suppressed (44). These observations serve to strengthen the functional relationship between activated Kupffer cells and the initiation of DNA synthesis in hepatocytes.

In summary, the results from this group demonstrate that warm ischaemia significantly delays the first wave of DNA synthesis and mitosis in the partially hepatectomised rat liver. Furthermore, the magnitude of the regenerative response in this group is enhanced. A possible reason for this would be the activation of hepatic or gut macrophages by endotoxin, with resultant release of mitogenic cytokines. We have previously alluded to the necessity for a model of hepatic ischaemia that allows portal venous decompression. This is the subject of the following chapter.

9. HEPATIC ISCHAEMIA WITH PORTAL DECOMPRESSION.

This component of the study aimed to investigate the effect of warm hepatic ischaemia, with portal venous decompression, on hepatocyte proliferation and DNA synthesis following partial hepatectomy. This was necessary in order to differentiate the effects of hepatic ischaemia *per se* from those of portal venous occlusion.

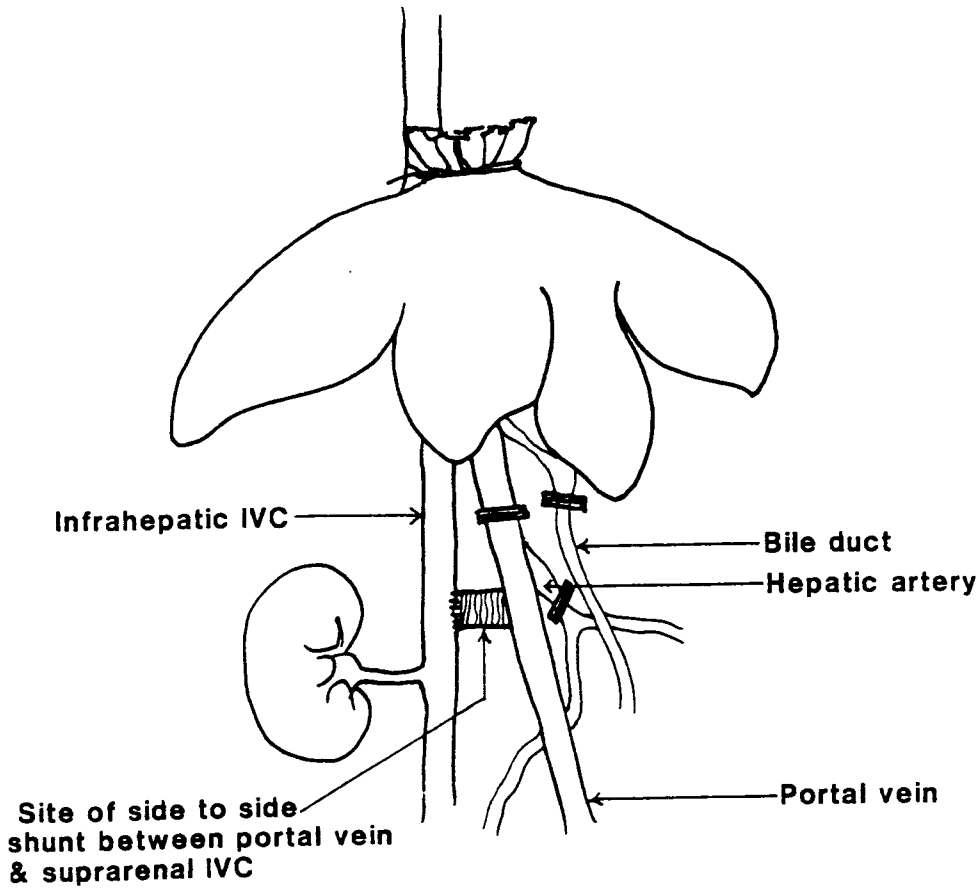
9.1 Methods.

Midline laparotomy and partial hepatectomy (as modified, see page 81) were performed. The bowel was retracted to the left and covered with a saline-soaked gauze swab. Great care was exercised to keep the liver and bowel moist, and to minimize their handling. Liver dissection was then completed as described on page 90.

Hepatic inflow occlusion was effected by application of vascular clamps to the hepatic artery, portal vein and bile duct. Further vascular clamps were applied to the suprahepatic IVC, infrahepatic IVC and distal portal vein. Using microvascular technique, a side-side portocaval shunt was made from the portal vein immediately distal to its confluence with the splenic vein to the infrahepatic vena cava. For this, 10/0 nylon-70 m needle was used. A vascular clamp on the portal vein distal to the shunt converted this into a functional end-side shunt for the duration of the

ischaemic period. Time taken to perform the shunt was recorded in all cases. The mean duration of portal vein occlusion was 14 min +- 1 min, timed from the onset of inflow occlusion. The portocaval shunt was graded in terms of function (0 to 3/3). Parameters utilized in the grading were the absence of stenosis at the anastomotic site (1) and the visible admixture of portal and systemic blood (2). The normal appearance of the bowel was noted as an additional index of shunt patency (3). Only animals with shunts assessed as performing adequately (grade 3/3) were included in the study. On completion of the 40 minute ischaemic period the clamps were removed, and the portocaval shunt reversed by the application of a small-size "liga clip" (Ethicon) across the suture line.

Figure 9.1 *Diagram depicting the partially hepatectomized liver and the site of the portocaval shunt.*



9.2 Results.

See Figure 9.2 for reference to partially hepatectomized controls.

9.2.1 Survival.

In this group 31 out of 40 animals survived until sacrifice (77%). This compared favourably to the high mortality observed in those animals subject to partial hepatectomy and 40 mins hepatic inflow occlusion without portal venous decompression (42%).

9.2.2 AST.

Plasma AST reached a maximum of 534+-47 at 24 hrs after an initial elevation of 424+-162 at 4 hrs. The elevation was sustained at 48 hrs (429+-50), but fell thereafter to reach levels of 199+-16 at 72 hrs and 100+-12 at 96 hrs respectively. This pattern was similar to that observed in partially hepatectomized animals and animals following partial hepatectomy and inflow occlusion, with no significant differences occurring at any sampling time.

9.2.3 Thymidine kinase.

Thymidine kinase levels were depressed at 4 hrs (12374+-9873) and at 24 hrs (9569+-3713). A modest peak was demonstrated at 48 hrs (22873+-6865). Thereafter levels gradually fell to 8804+-2907 at 72 hrs and 9305+-2536 at 96 hrs (Table 9.1).

9.2.4 Mitotic index.

No significant mitotic activity was recorded until 48 hrs in this group, with mitotic indices prior to this being diminished. The maximum recorded value occurred at 48

hrs (17+-3), with mitotic activity persisting until 72 hrs (16+-5). By 96 hrs levels had fallen to 8+-1 (Table 9.1).

Table 9.1 Thymidine kinase activity (dpm per mg protein) and mitotic index (mitotic figures per 1000 cells) in fasted rats after partial hepatectomy and 40 mins hepatic ischaemia with portal decompression.

TIME	THYMIDINE KINASE	MITOTIC INDEX
4 hrs	12 374+-9 873	1+-0
24 hrs	9 569+-3 713	2+-2
48 hrs	22 873+-6 865	17+-2
72 hrs	8 804+-2 907	16+-5
96 hrs	9 305+-2 536	8+-1

9.2.5 Liver mass index and tissue water.

The liver mass index is shown in Table 9.2. There was a progressive increase in liver mass, with the greatest increase shown in the second 48 hour period. Tissue water increased in the period from 24 hrs to 72 hrs.

Table 9.2 Liver mass index (LMI) and tissue water after partial hepatectomy and 40 mins hepatic ischaemia with portal decompression in fasted rats.

TIME	LIVER MASS INDEX	TISSUE WATER
4 hrs	0.434+-0.025	70.5%+-0.7%
24 hrs	0.439+-0.037	72.8%+-1.7%
48 hrs	0.581+-0.029	73.1%+-0.4%
72 hrs	0.734+-0.031	72.2%+-0.2%
96 hrs	0.912+-0.029	70.7%+-0.5%

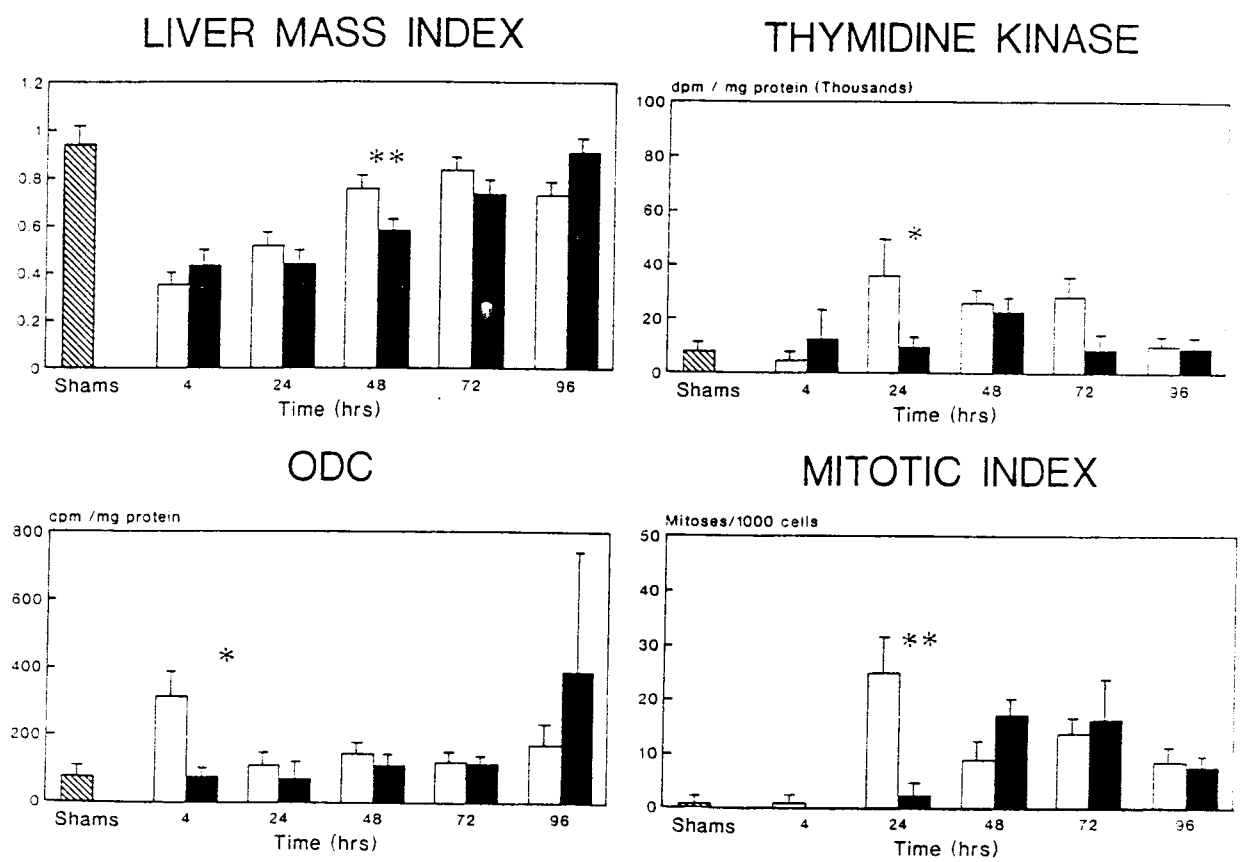
9.2.6 Ornithine decarboxylase.

Ornithine decarboxylase activity was disorganised, with maximal activity shown at 96 hrs (Table 9.3).

Table 9.3 Ornithine decarboxylase (ODC) activity (cpm per mg protein) after partial hepatectomy and 40 mins ischaemia with portal decompression in fasted rats.

TIME	ODC
4 hrs	77+-11
24 hrs	70+-17
48 hrs	111+-21
72 hrs	117+-20
96 hrs	389+-324

Figure 9.2 *Influence of 40 mins hepatic ischaemia with portal decompression on hepatic regeneration in fasted rats. Partial hepatectomy was performed as a control (clear bars) and compared to partial hepatectomy plus 40 mins hepatic ischaemia (solid bars). Parameters of liver regeneration measured were the liver mass index (LMI), thymidine kinase (dpm per mg protein), mitotic index (mitotic figures per 1000 cells) and ornithine decarboxylase (ODC) (cpm per mg protein). Animals were sacrificed 4, 24, 48, 72 and 96 hrs after partial hepatectomy. Results were expressed as means +- SE. * $p<0.05$ and ** $p<0.01$ by ANOVA.*



9.3 Discussion.

Rationale for the use of the portocaval shunt.

In the production of hepatic ischaemia by acute ligation of the porta hepatis, there is inevitable splanchnic stasis due to portal venous occlusion. It is necessary therefore to differentiate between hepatic ischaemia and splanchnic stasis as possible causes for the observed effects. Hence the need for a model of hepatic ischaemia that obviates splanchnic stasis.

In previous studies, this question has been resolved by use of the model described by Baker (5). This involves the temporary ligation of the hilar pedicle of the left lateral and median lobes, with the remaining portal tributaries left intact to allow drainage of the portal venous system (38,191). The perfused posterior lobes are then excised, with the result that ischaemia is produced in the left lateral and median lobes without the occurrence of portal stasis. For direct comparison to other groups in this study, it would have been necessary to perform this technique in reverse, in order to preserve the posterior lobes and resect the left lateral and median lobes as in 68% partial hepatectomy (116). This was not a feasible consideration, due to technical difficulty associated with the accurate dissection of the posterior lobar branches of the hepatic artery. The importance of the latter dissection is in the adequacy of sympathetic denervation at this time. This is discussed in detail on page 119.

A further possibility is the construction of a portafemoral shunt using polyethylene tubing (179). However, extracorporeal shunts have been found to be cumbersome and associated with numerous technical problems (161). These include shunt occlusion, increased bloodloss and increased operative time (161). Although extracorporeal portosystemic shunting would have closely matched the clinical

situation, we thought it impractical in view of the problems encountered in its previous use.

Discussion of results.

Unlike the group in which hepatic ischaemia was associated with portal stasis, in these animals the proliferative response was delayed only. At 48 hours a modest peak in thymidine kinase activity as well as mitotic index was demonstrated. This was significantly less than the corresponding peak for animals with ischaemia and portal stasis. However, it is important to note that it was not statistically different from the peak in partially hepatectomized animals at 24 hours. Hence the nett effect was one of delay only, with no attenuation of the proliferative response as compared to partially hepatectomized controls.

Since the delay in the initiation of DNA synthesis and cytokinesis was observed in all animals in which hepatic ischaemia occurred, irrespective of portal stasis, it is likely that the cause of this is unrelated to portal venous congestion or its sequelae.

Two thirds partial hepatectomy in the rat results in a highly reproducible regenerative response if the animal and the liver are normal (31). Hepatocyte growth appears to be controlled by complex interacting mechanisms to which changes in the cell cycle (239), tissue and plasma enzymes (229), and plasma hormones (91) all contribute. In general, manipulations have resulted in stimulation of the response but inhibition has also been described and in a few cases, delay has been noted. To date, only the effects of warm ischaemia upon the regenerative response have been studied (96), but not in the context of transplantation of reduced livers. Depression of the regenerative response at 24 hours was reported

after ischaemia of 25 minutes' duration but the subsequent pattern was not mentioned (96).

Although the increase in liver mass was delayed by 24 hours in this group, by the end of the experimental period (96 hours) there was no difference in liver mass of these rats as compared to partially hepatectomized controls. Also of note was that there was no difference in plasma levels of aspartate aminotransferase (AST).

There are several descriptions in the literature of manipulations which result in delay of the regenerative response, as distinct from inhibition. The most likely of these is the denervation associated with dissection. Denervation or blockade of alpha-1 adrenergic receptors has been shown to attenuate the 24 hour peak of DNA synthesis, although this demonstrates a return to within normal limits by 72 hours (62). This possibility made necessary the extension of this study to include the examination of hepatic dearterialization in a separate group of animals. The results of this are discussed at length in Chapter 10.

Another possibility is that DNA synthesis is inhibited by high circulating levels of glucagon which have been shown to prolong the transition from the G1 to S phase (256). The immediate effects of portal occlusion upon plasma levels of glucagon do not appear to have been studied but certainly, plasma glucagon levels are elevated significantly within thirty minutes of portacaval shunting (115). This may account for the delay in this group of animals in which temporary portocaval shunting is performed. Other substances which interfere with the activity of ornithine decarboxylase, such as gamma-amino-isobutyric acid (195) do not appear to have been measured after portal occlusion.

In his original studies, Rappaport showed the subdivision of the liver into functional units around the branches of the portal vein and the hepatic artery (243). Subsequently, Rabes *et al* suggested that location with respect to microvascular and actual functional state appear to be the decisive factors in the initiation of DNA synthesis in individual hepatocytes (235). It has been shown that the zone of maximal proliferation proceeds through the unit to the perivenous area; a second wave occurs at 56 hours (235,263). It is speculated that the first wave begins in the periportal area because of hepatotrophic factors in portal blood. While such hepatotrophic factors have not been positively identified, it is possible that levels of some substance may be depressed after portal vein occlusion or that periportal hepatocytes may be rendered temporarily unresponsive to such substances. There are several possibilities in this regard. Significant changes in the hepatic microcirculation after portal venous clamping have recently been described, including activation of hepatic macrophages and increased leucocyte adhesion (184). It has been stated that "portal venous clamping during transplantation surgery may have an impact on the hepatic microcirculation and cell-to-cell interaction due to endotoxin mediated activation of various inflammatory cascades and cell populations" (184). During portal venous occlusion there may be depressed synthesis of epidermal growth factor in the Brünner's glands which would impair DNA synthesis (155); also disturbance of other cytokines including transforming growth factor beta (253) or interleukin 1 beta (208) would lead to disorganisation of the replicative response. This pattern seems to involve reprogramming of gene expression with competition at the transcriptional or translational level which allows synthesis of acute phase proteins first and then replication. Animals subjected to partial hepatectomy with a simultaneous local inflammatory stimulus also show a

delay which is attributed to the same mechanism (11) and in the present studies, the ischaemia-reperfusion sequence may provide such an inflammatory response.

The response of liver cells to damage or removal appears to be proportional to the amount of mass removed and it has been noted that removal of 80% of the liver mass results in delayed DNA synthesis (300). Also, administration of allyl formate to animals which have been subjected to partial hepatectomy causes cells nearer the hepatic veins to replicate (238). The addition of ischaemia to partial hepatectomy may have resulted in similar increased damage but this was not evident from the plasma levels of AST.

The apparent increase in liver mass without the associated increase in cell number may be the result of hypertrophy of cells. Infusion of hydroxyurea prevents liver cells moving from the G1 to the S phase of the cell cycle and causes cells to accumulate in that part of the cycle. Since they cannot respond with hyperplasia, cells appear to undergo hypertrophy (236). Confirmation that this is the cause of increased liver mass would be obtained from cell morphometry. Indeed, detailed analysis of changes in the events of the cell cycle would probably clarify the precise mechanisms for the observed delay.

Tissue levels of ornithine decarboxylase were measured as an additional index of cell replication. The peak of ODC activity was at 96 hours in this group, but was not significantly greater than controls, and demonstrated a wide standard error. Partially hepatectomized control animals demonstrated a peak of ODC activity at 4 hours, in keeping with other studies (186). Polyamine metabolism appeared disorganised therefore. Although it has been stated that the sequential appearance in the blood of ODC and thymidine kinase is typical of liver regeneration and is consistent for each species, the time course of ODC activity has been shown to be independent of

the onset of DNA replication in regenerating livers (186) and it seems that this component of the response is particularly sensitive to manipulations.

The attenuation of the proliferative response in this group may be due to the avoidance of a protracted period of portal stasis. Although endotoxin levels were not measured, it is apparent from previous studies that systemic and portal endotoxaemia follows a period of portal venous occlusion in rats (215). Furthermore endotoxin has been strongly implicated in the stimulation of hepatocyte proliferation (60,61,266). It is not known however, if the avoidance of portal stasis entirely prevents this endotoxaemia.

As previously discussed on page 101 it is unlikely that the observed effect can be attributed to ischaemic hepatocyte damage with greater loss of functional hepatocyte mass. Aspartate aminotransferase levels in this group followed the pattern of partially hepatectomized controls, with no significant differences at any of the time points studied.

In summary then, the possible reasons for the delay in the initiation of the regenerative response in this and previously discussed groups of animals are therefore likely to be:

1. Sympathetic denervation following dissection of the sympathetic plexus around the hepatic artery.

2. Diversion of hepatic synthetic function to the synthesis of acute phase proteins.

3. Consequent to the elevation of corticosteroid levels associated with the metabolic response to surgical trauma.

4. Diversion of hepatic synthetic function to the production of heat shock proteins.

5. Related to hyperglucagonaemia occurring after portocaval shunting.

The results from this and the previous group demonstrate that warm ischaemia significantly delays the first wave of DNA synthesis and mitosis in the partially hepatectomised rat liver. Furthermore, the magnitude of the regenerative response is attenuated by portal venous decompression, with respect to those animals in which portal stasis occurred. A possible reason for this would be the avoidance of portal and systemic endotoxaemia associated with portal stasis.

10. HEPATIC ARTERIAL LIGATION.

The purpose of this group was to establish whether the alterations in hepatocyte proliferation kinetics observed in animals exposed to liver dissection were attributable to hepatic denervation.

10.1 Methods.

Midline laparotomy and partial hepatectomy (with modification) were performed. A full liver dissection was then completed. Briefly, this was as follows: the falciform, left lateral and gastrohepatic ligaments were divided. The pyloric vein was ligated and divided, and the hepatic artery dissected free from the portal vein. Both left and right phrenic veins were divided and ligated. The oesophageal branches of the left gastric artery were diathermized and divided. The suprarenal inferior vena cava was dissected free from adjacent fatty connective tissue. The right adrenal vein was ligated and divided. Following the complete freeing of all remaining attachments to the liver, the portal vein and superior and inferior vena cavae were meticulously stripped of adventitial tissue using microsurgical technique. The hepatic artery was ligated and double-tied using 6/0 silk. All adventitial tissue around the hepatic artery was dissected free.

10.2 Results.

10.2.1 Survival.

There was 100% survival to sacrifice in this group (n=5).

10.2.2 AST.

A marked increase in plasma AST was noted, with levels reaching 691+-43 by 24 hours. This was not significantly different from that observed in partially hepatectomized controls, or for either of the ischaemic groups. It was however significantly less ($p<0.05$) than the AST elevation observed in stored allografts at this time (see page 137).

10.2.3 Thymidine kinase.

Thymidine kinase activity in this group was recorded as 36308+-6063. This was of similar magnitude to the peak observed in the ph control group (36021+-8060) (see page 83).

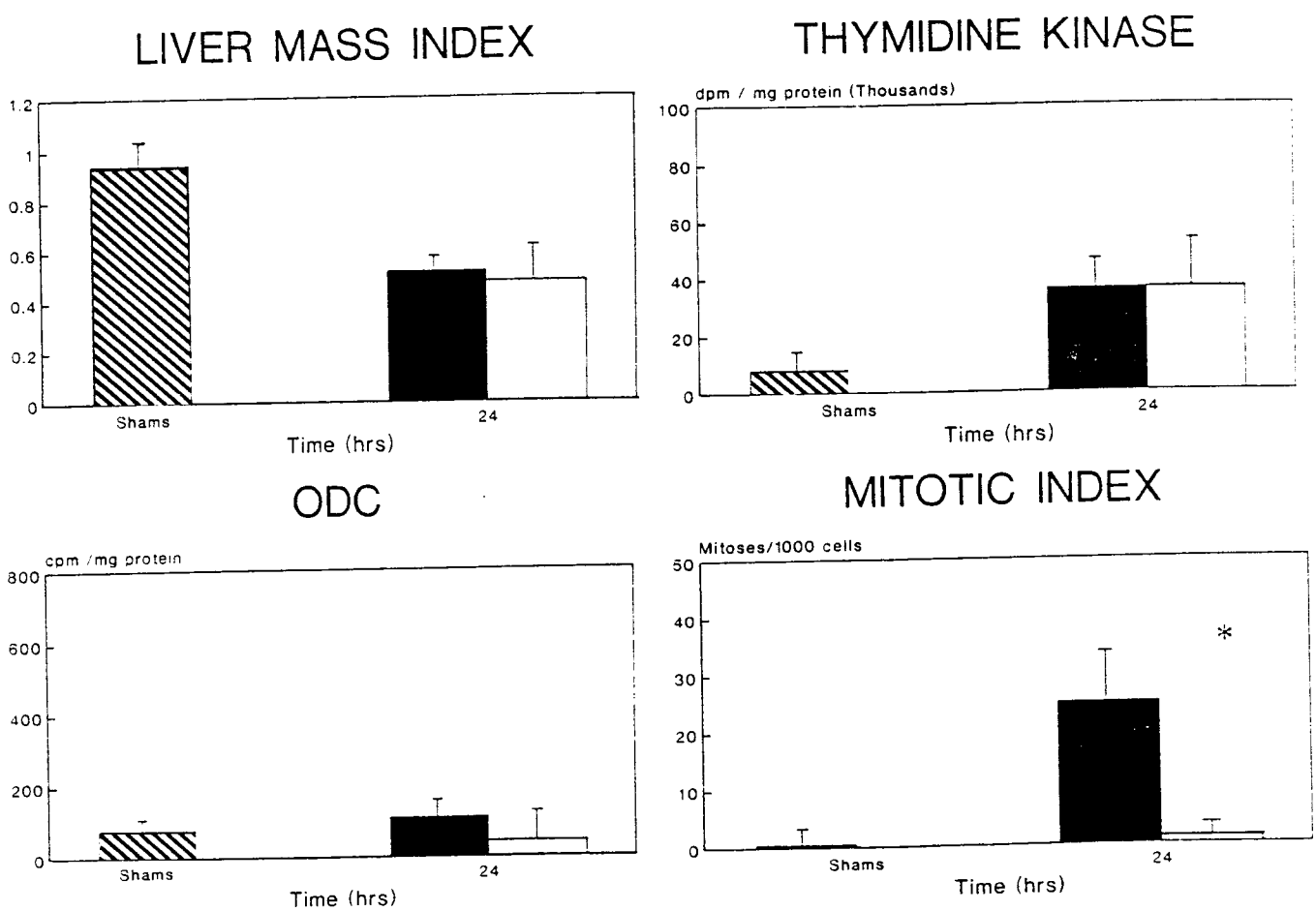
10.2.4 Mitotic index.

The mitotic index of 5+-3 at 24 hrs did not correlate accurately with thymidine kinase activity in this group, and was less than that of ph controls (25+-8) ($p<0.01$).

10.2.5 Liver mass index and tissue water.

The liver mass index recorded at 24 hours was 0.476+-0.33. This was not significantly different from partially hepatectomized controls. Tissue water at 24 hrs was 70%+-0.8%, and no different from controls (Table 7.2).

Figure 10.1 *Influence of hepatic dearterialization on hepatic regeneration in fasted rats. Partial hepatectomy was performed as a control (solid bars) and compared to partial hepatectomy plus hepatic arterial ligation (clear bars). Parameters of liver regeneration measured were the liver mass index (LMI), thymidine kinase (dpm per mg protein) and mitotic index (mitotic figures per 1000 cells). Aspartate aminotransferase (AST) was measured in plasma, and expressed in International Units (IU). Animals were sacrificed 24 hrs after partial hepatectomy. Results were expressed as means +- SE. * $p<0.05$ by ANOVA.*



10.3 Discussion.

Thymidine kinase activity at 24 hours in dearterialized, partially hepatectomized animals paralleled the peak activity in partially hepatectomized animals at this time. The liver mass index was similar to that of controls, suggesting a comparable rate of regenerative growth. However, the mitotic activity was significantly diminished with respect to ph controls. This represented a disparity between regenerative activity as indexed by thymidine kinase and that reflected by cellular division. Since both DNA synthesis and liver mass were comparable to controls, the low level of mitotic activity may not reflect a truly blunted response. The reasons for this are unclear however.

A previous study of liver mass gain after hepatic arterial ligation and hemihepatectomy in dogs demonstrated normal liver mass increase as compared to hemihepatectomized controls (301). This suggested that arterial inflow has little influence on the course of liver regeneration, as measured by increase in liver mass, and size on liver scan. It was also demonstrated that hepatic necrosis did not follow the permanent ligation of the hepatic artery (301). Our findings in rats are therefore confirmatory of these observations. Furthermore, in the latter study it was shown that hepatic arterial perineurectomy without hepatic arterial ligation had no effect on the course of liver regeneration, as measured by increase in liver mass and size (301). The results of other studies of hepatic arterial ligation have demonstrated considerable variation in the degree of hepatic necrosis and mortality, with large interspecies variations (231).

Recently, a role for the sympathetic nervous system, noradrenaline and the alpha-1 adrenergic receptor in the control of liver regeneration has been described (62).

Sympathetic denervation by microsurgical dissection of the hepatic artery, or administration of the alpha-1 blocker prazosin, results in abolition of the 24 hour DNA synthetic peak in rats *in vivo* (62). The alpha-1 adrenoceptor has been shown to mediate this catecholamine effect, by downregulation of the EGF receptor and enhanced EGF mitogenic effects in primary hepatocyte culture (64).

The results in our study are therefore contradictory to those of Cruise *et al* (62), in that no difference in the regenerative response at 24 hours in dearterialized animals could be demonstrated. Sympathetic denervation may diminish the release of catecholamines from sympathetic nerve terminals, but will not prevent exposure of adrenergic receptors to circulating catecholamines. The administration of an alpha-1 blocker would obliterate any effect due to circulating catecholamines, as observed by Cruise *et al* (62).

It is important to note that any differences observed in this group may have been due either to hepatic denervation or due to hepatic arterial ligation. It was felt necessary to completely ligate the hepatic artery in order to assure that no sympathetic innervation persisted. Since there was no demonstrable difference in the regenerative response between these animals and partially hepatectomized controls, the cause of the delay in initiation of hepatocyte proliferation in those animals subjected to full hepatic dissection in other groups is therefore unlikely to be due to hepatic denervation.

11. IN SITU LIVER FLUSHING.

The role of nonparenchymal cells in both regeneration (20) and transplantation (183,189) has been the focus of recent study. During the transplantation procedure, it is possible that the nonparenchymal cell population may incur an injury resulting from flushing of the liver prior to storage. This part of the study aimed to ascertain the effect of such flushing on the regenerative response under conditions likely to occur in clinical transplantation.

11.1 Methods.

Animals were divided into 5 groups:

Group I	ph (n=43);
Group II	ph + Ringer's lactate flush at 37°C (n=6);
Group III	ph + Ringer's lactate flush at 4°C (n=30);
Group IV	ph + University of Wisconsin flush at 4°C (n=32);
Group V	ph + Euro-Collins' solution flush at 4°C (n=31);

Midline laparotomy and partial hepatectomy were performed as described on page 81. Full hepatic dissection as described on page 90 was not performed. The liver was isolated by clamping the following structures in order: bile duct, hepatic artery, portal vein, infrahepatic inferior vena cava and suprahepatic vena cava. A primed

isolated by clamping the following structures in order: bile duct, hepatic artery, portal vein, infrahepatic inferior vena cava and suprahepatic vena cava. A primed cannula was passed into the portal vein distal to the clamp and secured. A venotomy was made in the infrahepatic suprarenal inferior vena cava distal to the clamp using a 23G needle. Prograde flushing of the liver was commenced. A total of 10 ml was used to flush the isolated liver, with the composition of the first 5 ml varying according to the experimental group. The subsequent 5 ml flush utilised Ringer's solution in all cases. In a pilot study, a volume of 10 ml was found to be sufficient to reduce the haematocrit of the vena caval effluent to less than 1% (n=6). The temperature of the flushing solution was as stated. The haematocrit of the vena caval effluent was recorded in a representative sample of animals (n=10), and never exceeded 2%. Perfusion pressure was standardized by manometry. This employed a column of water connected by a T-piece to the flushing catheter. Perfusion pressure was maintained at less than 30cm water in all cases. On completion of the liver flush, the portal vein and inferior vena caval venotomies were repaired using 9/0 nylon-140 m needle, and the clamps removed. The total ischaemic time to perform the liver flushing was recorded, and never exceeded 5 minutes.

11.2 Results.

11.2.1 Survival.

Of the 109 animals in groups II-IV, 99 survived until sacrifice (91%). The 10 animals that died were evenly distributed amongst the three groups, with no discernible cause evident at autopsy. The mortality of group I animals is presented on page 82.

11.2.2 AST.

There was no significant difference between the experimental groups, nor were the AST values significantly elevated over partially hepatectomized controls (Table 11.1). AST levels in group II animals (914+-133) were of similar magnitude to those in other groups at 24 hours.

11.2.3 Thymidine kinase.

Thymidine kinase levels in groups II, III, IV and V were diminished at 24 hrs with respect to controls ($p < 0.01$). The recorded peak occurred at 48 hrs in all groups, with the highest value occurring in the UW flush group (Figs. 11.1-11.3).

11.2.4 Mitotic index.

The mitotic index in groups II, III, IV and V was diminished at 24 hrs with respect to controls ($p < 0.05$). The recorded peaks occurred at 48 hrs in all groups in parallel with the elevation in thymidine kinase activity demonstrated at this time. In group IV (UW flush) this was significantly greater than the mitotic index in group I (ph) at 48 hrs ($p < 0.01$) (Figs. 11.1-11.3).

11.2.5 Liver mass index and tissue water.

The liver mass indices were diminished in groups II, III, IV and V at 48 hrs ($p < 0.01$). By 72 hrs there were no significant differences in the liver mass index between any of the groups and sham-operated animals (Figs. 11.1-11.3). No differences in tissue water could be demonstrated between the groups.

11.2.6 Ornithine decarboxylase.

ODC was depressed in group V at 4 hrs ($p < 0.05$), whilst in groups III and IV it tended to be less than that of group I (ph). There were no significant differences in ODC at the remaining sampling periods in any of the groups studied (Figs. 11.1-11.3).

11.2.7 Haematocrit.

The haematocrit of the vena caval effluent after 10 ml flush was less than 2% in a representative sample group ($n = 10$).

Table 11.1 Influence of in situ liver flushing on aspartate aminotransferase (AST) in fasted rats. Partial hepatectomy was performed as a control (PH) and compared to Ringers Lactate flush (RL flush), UW/RL flush (UW flush) and Euro-Collins/RL flush (EC flush).

TIME	PH	RL FLUSH	UW FLUSH	EC FLUSH
4 hrs	328+-40	545+-46	370+-71	508+-106
24 hrs	390+-31	377+-90	982+-388	553+-69
48 hrs	237+-16	370+-42	562+-169	487+-39
72 hrs	192+-32	163+-19	280+-52	159+-28
96 hrs	111+-9	188+-52	135+-25	102+-15

Figure 11.1 *Influence of liver flushing with Ringer's lactate solution on hepatic regeneration in fasted rats. Partial hepatectomy was performed as a control (first bar) and compared to partial hepatectomy plus Ringer's solution flush at 4° C (second bar) and Ringer's solution flush at 37° C (third bar). Parameters of liver regeneration measured were the liver mass index (LMI), thymidine kinase (dpm per mg protein), mitotic index (mitotic figures per 1000 cells) and ornithine decarboxylase (ODC) (cpm per mg protein). Animals were sacrificed 4, 24, 48, 72 and 96 hrs after partial hepatectomy. Results were expressed as means +- SE. * p<0.05 and ** p<0.01 by ANOVA.*

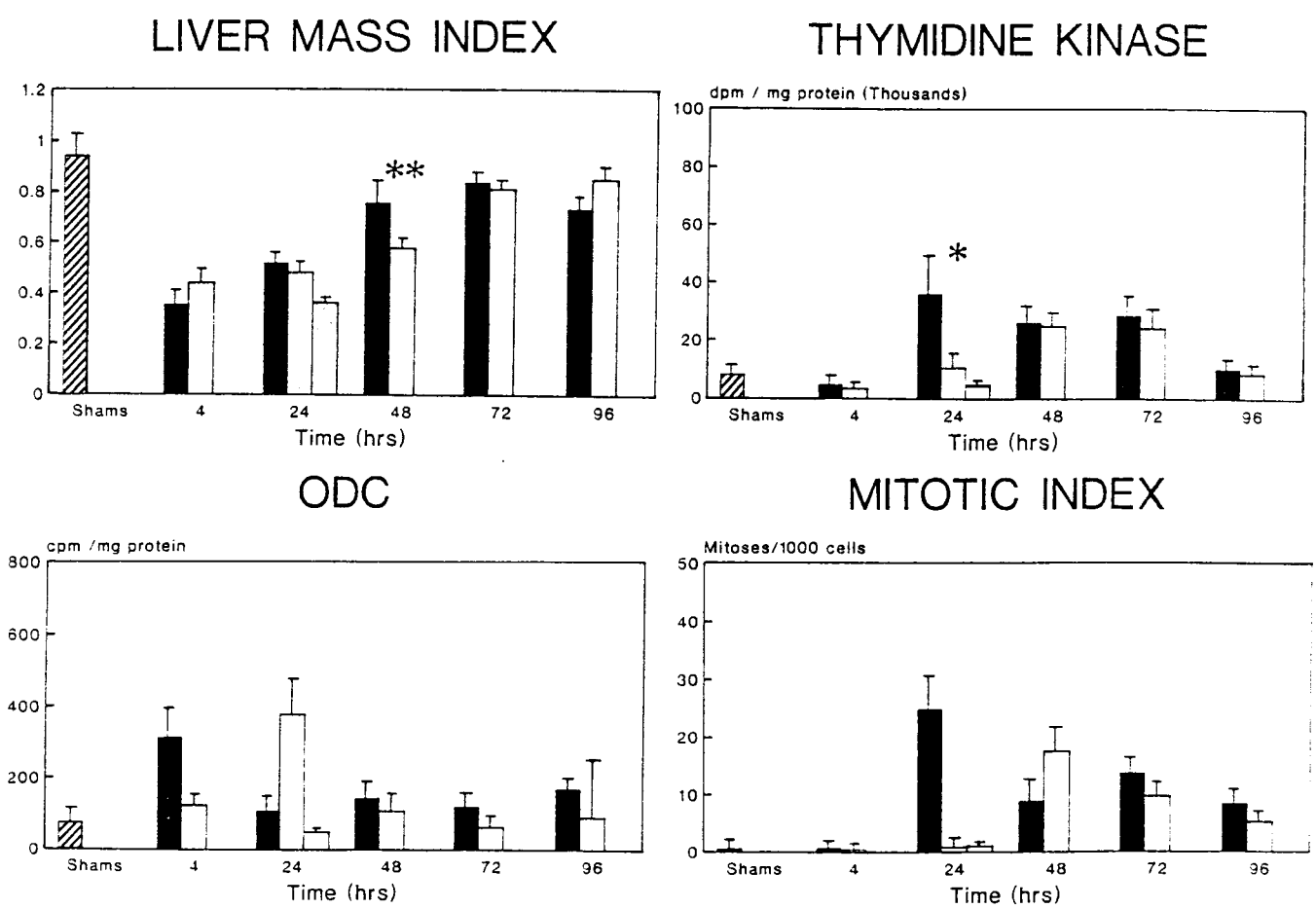


Figure 11.2 *Influence of liver flushing with University of Wisconsin solution on hepatic regeneration in fasted rats. Partial hepatectomy was performed as a control (solid bars) and compared to partial hepatectomy plus University of Wisconsin flush at 4°C (clear bars). Parameters of liver regeneration measured and times were as for Fig.11.1 Results were expressed as means +- SE. * p<0.05 and ** p<0.01 by ANOVA.*

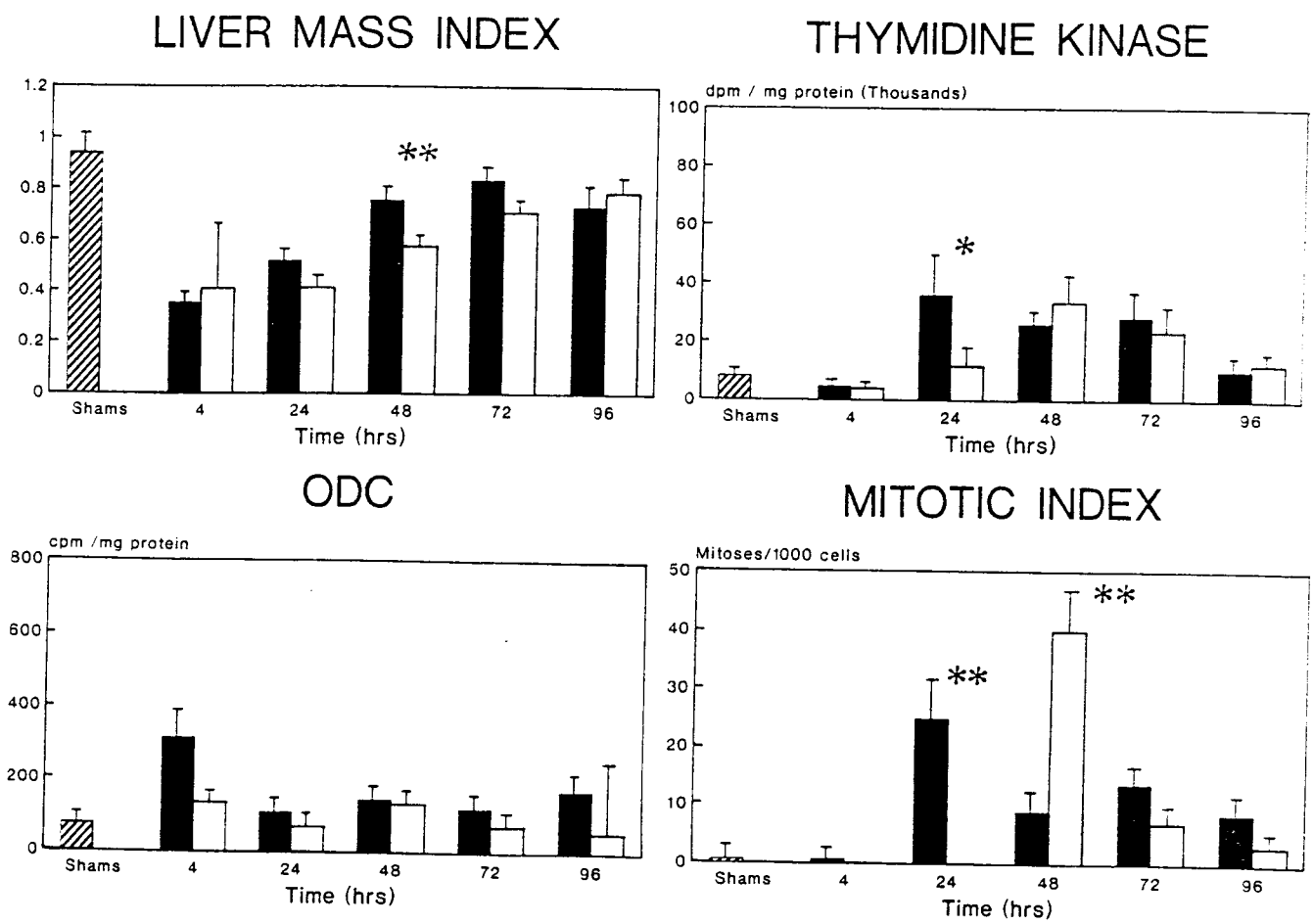
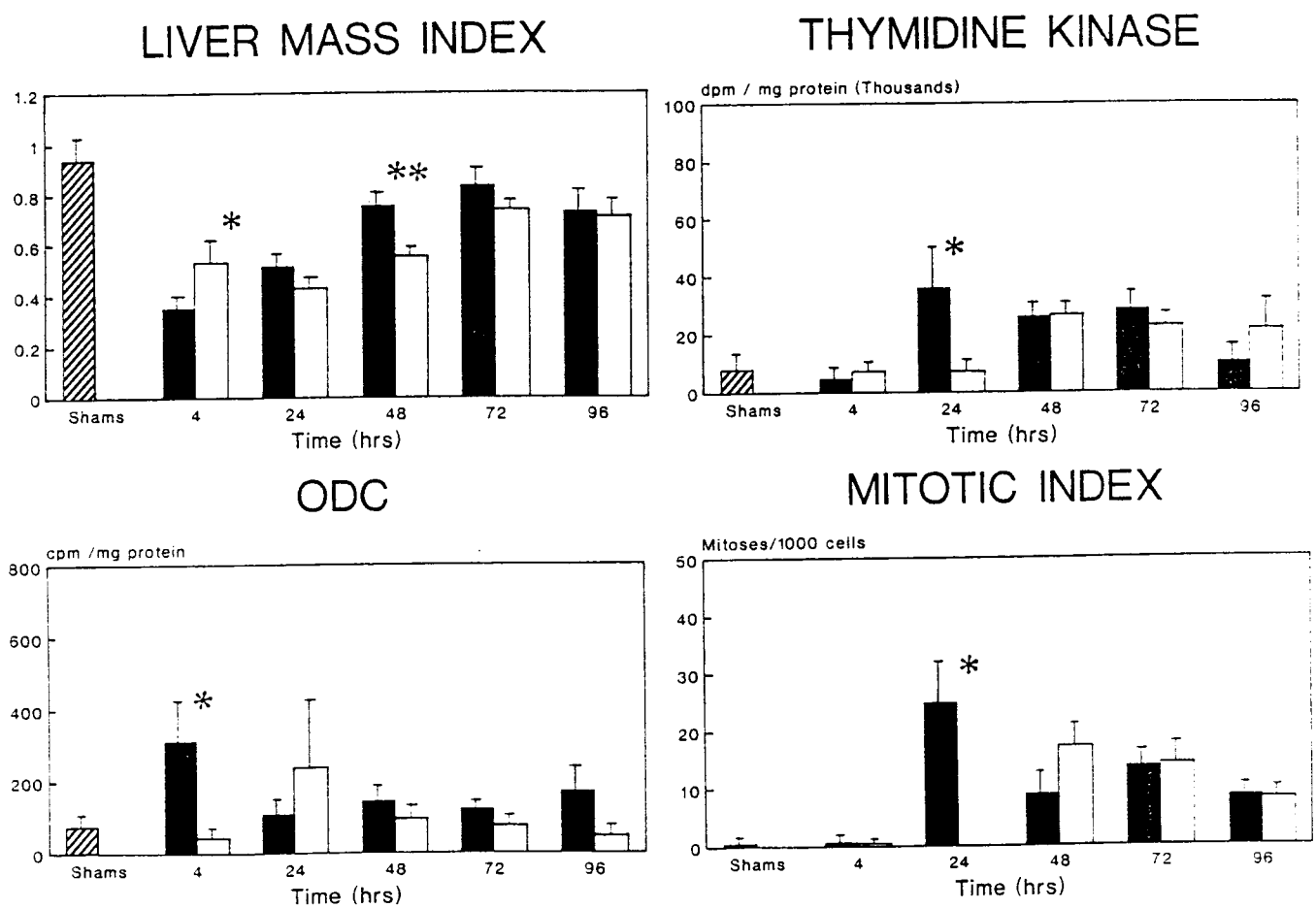


Figure 11.3 *Influence of liver flushing with Euro-Collins solution on hepatic regeneration in fasted rats. Partial hepatectomy was performed as a control (solid bars) and compared to partial hepatectomy plus Euro-Collins solution flush at 4°C (clear bars). Parameters of liver regeneration measured and times were as for Fig.11.1 Results were expressed as means +- SE. * p<0.05 and ** p<0.01 by ANOVA.*



11.3 Discussion.

The results of this component of the study indicate that the first wave of DNA synthesis and hepatocyte mitotic activity was inhibited by *in situ* flushing of the liver with either Ringer's Lactate, Euro-Collins or University of Wisconsin solutions. This was shown by the significantly diminished thymidine kinase activity and mitotic indices at 24 hours. Compared with the peak in ODC activity occurring at 4 hours in partially hepatectomized animals, ODC activity in each of the experimental groups was either significantly diminished (group V) or tended to be less (groups III and IV) at this time. Further evidence of the delay in initiation of the regenerative process in all experimental groups was a significantly diminished liver mass index at 48 hours. This finding was similar to other experimental groups (partial hepatectomy plus ischaemia or autograft) in which a delay in the initiation of the process was demonstrated. However, liver regeneration seemed unaffected in the longer term. By 72 hrs the liver mass had been restored to that of sham-operated animals in all groups.

Of note was the elevation in mitotic activity in group IV (UW flush) at 48 hours. This was not associated with a simultaneous increase in thymidine kinase activity at this time, nor was there a significant difference in ODC activity. Taken together, the elevated mitotic activity in this group seems unlikely to represent a truly enhanced regenerative response over the other groups in which the liver was flushed.

Aspartate aminotransferase has been established as a useful marker of liver graft viability (124). Significantly, AST levels in the animals in which liver flushing was performed were no greater than in partially hepatectomized animals. This would

suggest that any damage imposed during the flushing procedure was confined to the microvasculature, with no significant hepatocyte damage.

The role of the nonparenchymal and endothelial cells in maintenance of normal liver function following transplantation is being recognized increasingly (183). The control of liver regeneration may be governed by a paracrine mechanism involving the synthesis of TGF beta by nonparenchymal cells. Damage to these cells by preservation could influence the the regenerative response. Evidence for the role of TGF beta is provided by the finding that TGF beta, a Kupffer cell product, is able to inhibit the mitogenic effects of TGF alpha in primary hepatocyte culture (20). Sinusoidal lining cells are involved in the storage lesion during liver preservation (189). The observed delay in the initiation of the regenerative process may therefore reflect alteration in nonparenchymal cell function, following damage imposed during the flushing procedure. However, reperfusion injury appears to be confined to the endothelial cells with relative sparing of the Kupffer cells (41).

Increasead survival has been reported in liver grafts after rinsing with warm Ringer's Lactate, with associated lower AST values and improvement in microcirculation (289). The results of group II, in which *in situ* liver flushing was performed using Ringer's Lactate at 37°C did not demonstrate significantly different results to those in which cold flushing was utilized. In this group the thymidine kinase activity and mitotic index were diminished at 24 hrs, as in groups III, IV and V. This suggests that hypothermia *per se* is not responsible for the observed results.

The causes of a delay in the initiation of the regenerative process have been previously discussed on page 96, and will not be repeated here. Irrespective of the cause of the initial delay, the regenerative response was established by 72 hrs in all groups, as indicated by the restitution of liver mass.

In conclusion there appears to be no long-term effect of liver flushing on hepatocyte DNA synthesis or cytokinesis. In Chapter 12 we will extend our investigation beyond the effects of liver flushing, and examine the possible effects of liver preservation on hepatocyte proliferation.

12. PARTIAL ORTHOTOPIC LIVER AUTO- AND ALLOTRANSPLANTATION.

In the transplant procedure, several insults are imposed on the liver which may have a bearing on its normal physiologic processes. These have been identified as warm and cold hepatic ischaemia, reperfusion injury, flushing of the liver and its possible effects upon endothelial cells, the effects of portal venous stasis, liver preservation, denervation, and the ill-defined effects of the procedure itself. Where possible, it was endeavoured to isolate these factors in order that they might be examined individually. The liver transplant procedure combines several of these factors and it was the purpose of this component of the study therefore to examine the overall effect of the combination on the regenerative capacity of the partial liver transplant.

12.1 Methods.

12.1.1 Orthotopic reduced liver autograft.

Midline laparotomy was performed, followed immediately by partial hepatectomy (with modification). The bowel was retracted to the left and covered with a saline-soaked gauze swab. A similar swab was placed on the liver. Great care was exercised throughout the procedure to keep the liver and intestines moist, and to minimize their handling. Liver dissection was then completed. Briefly, this was as follows: the

falciform, left lateral and gastrohepatic ligaments were divided. The pyloric vein was ligated and divided, and the hepatic artery dissected free from the portal vein. The gastroduodenal branch of the hepatic artery was cauterized in order that the subsequent anastomosis could be performed proximal to its origin. Both left and right phrenic veins were divided and ligated. The oesophageal branches of the left gastric artery were cauterized and divided. The suprarenal inferior vena cava was dissected free from adjacent fatty connective tissue. The right adrenal vein was ligated and divided. Remaining posterior attachments were freed.

Heparin was administered in a dose of 12 IU sodium heparin/kg (made up to 1ml in 0.9% NaCl) by single injection into the dorsal vein of the penis immediately prior to application of the clamps. The bile duct was ligated and a polyethylene stent inserted and secured into the proximal portion. Vascular clamps were then applied to the hepatic artery, portal vein and infrahepatic vena cava and these were then transected in this order. Caution was observed in the placement of the clamps in order that sufficient length be preserved for the subsequent anastomosis. To obtain an adequate cuff of suprahepatic vena cava, a modified haemostat was applied across the vein including a portion of the diaphragm, and the vein divided at the liver margin. The liver was lifted out of the abdomen to ensure that no attachments remained, and replaced in the orthotopic position. Re-implantation was then begun with the vessels sutured in the order of suprahepatic IVC, portal vein, infrahepatic IVC and hepatic artery.

After completion of the suprahepatic vena caval and portal venous anastomoses, the clamps on these vessels were released and hepatic reperfusion was allowed to occur. Ischaemic time was recorded in all cases. The mean duration of portal vein occlusion was 22 mins +- 1 min. The infrahepatic vena cava was then sutured. The

portal vein, supra- and infrahepatic vena cavae were all sutured by direct end-to-end anastomosis. Microvascular technique was employed using 8/0 nylon-140 m needle for the suprahepatic IVC and 9/0 nylon-140 m needle for the infrahepatic IVC and portal vein. The hepatic artery anastomosis was performed using the sleeve technique (66) and 10/0 nylon-70 m needle. This technique is a form of end-in-end anastomosis which utilizes only 2 sutures. Long-term patency of the hepatic arterial anastomosis in rat liver allografts using this technique has been documented (113). Finally, bile duct continuity was re-established over the polyethylene stent and secured with a 7/0 silk tie. The stent was then left in situ. Delivery of anaesthetic gas (ether) was diminished during the anhepatic phase in accordance with the decreased anaesthetic requirements of the animal at this time.

12.1.2 Orthotopic reduced liver allograft.

a) Donor procedure.

Midline laparotomy was performed, followed immediately by partial hepatectomy (with modification). The liver dissection was performed as described for the orthotopic autograft. The bile duct was then transected and a polyethylene cannula inserted into the proximal portion and secured. The animal was fully heparinised using 12 IU sodium heparin/kg (made up to 1ml in 0.9% NaCl) by single injection into the dorsal vein of the penis immediately prior to application of the clamps. Vascular clamps were applied to the proximal portal vein, hepatic artery, suprahepatic IVC and infrahepatic IVC. A primed cannula was inserted into the portal vein via a venotomy and secured. Cold perfusion of the liver was then commenced using 5 ml of University of Wisconsin solution at 4 $^{\circ}$C. The effluent was allowed to drain through a venotomy in the inferior vena cava between the clamps. The vessels were then transected and the liver harvested for storage.

b) Storage.

The flushing cannula was secured in the portal vein and the liver placed in Ringer's lactate solution for storage at 4°C. A plastic container holding the liver in Ringer's lactate was placed in a second container of slushed ice, and this in turn placed in a refrigerator at 4°C. All livers used in this study were stored for 4 hrs. On completion of storage, and immediately prior to re-implantation, the liver was flushed with 5 ml of Ringer's lactate solution at 4°C.

c) Recipient procedure.

The mass of the recipient animal was chosen to closely approximate the mass of the donor. The preparation of the recipient was timed in order that re-implantation would take place 4 hrs after harvesting of the donor liver. Laparotomy, full heparinisation and dissection of the liver were performed as in the donor animal. Vascular clamps were applied to the portal vein, hepatic artery and infrahepatic vena cavae. The structures of the portal triad were transected at the hilum of the liver, in order to preserve length. To obtain an adequate cuff of suprahepatic vena cava, a modified haemostat was applied across the vein including a portion of the diaphragm, and the vein divided at the liver margin. The native liver was lifted from its position and discarded. The donor liver was placed in the orthotopic position and implantation commenced. The suprahepatic vena caval anastomosis was sutured first by direct end-to-end anastomosis using 8/0 nylon-140 m needle. The portal vein was completed next using the same technique and 9/0 nylon-140 m needle. The mean duration of portal vein occlusion was 21 min +- 1 min. On completion of the suprahepatic vena caval and portal venous anastomoses, the clamp on the portal vein was released. The liver gradually resumed its normal colour as it was perfused with portal blood. The first 1 ml of hepatic venous effluent was allowed to drain

from the infrahepatic vena cava, following the temporary removal of the clamp. The infrahepatic IVC clamp was then immediately re-applied and the suprahepatic IVC clamp released. This manouevre ensured that no residual UW solution, stagnant hepatic venous blood or air reached the systemic circulation. The infrahepatic IVC anastomosis was then completed using 9/0 nylon-140 m needle. These clamps were then released and inferior vena caval flow re-established. The portal vein, supra- and infrahepatic vena cavae were all sutured by direct end-to-end anastomosis. Microvascular technique was employed using 8/0 nylon-140 m needle for the suprahepatic IVC and 9/0 nylon-140 m needle for the infrahepatic IVC and portal vein. The hepatic artery anastomosis was performed using the sleeve technique (66) and 10/0 nylon-70 m needle. Bile duct continuity was re-established over the polyethylene stent and secured with a 7/0 silk tie. In all cases the liver was observed closely for the return of its normal colour and for evidence of bile production. The gut was observed for evidence of the return of normal portal venous circulation.

12.2 Results.

12.2.1 Survival.

In the autograft group, 32 out of 80 animals survived to sacrifice (40%). In the allografts, 30 out of 68 animals survived to sacrifice (44%). At autopsy, haemoperitoneum was noted in approximately 40% of animals, suggesting technical failure (anastomotic bleeding) as the probable cause of death. No obvious cause for mortality was noted at autopsy in the remaining animals. These animals were usually those in which ischaemic times were delayed beyond 25 mins for technical reasons.

12.2.2 Aspartate aminotransferase (AST).

In autografts an increase in AST over control values was noted at 4 and 24 hours ($p<0.05$), with a tendency to be greater at other time intervals. In the allografts, significant increases in AST over partially hepatectomized controls occurred at 4 hrs, 24 hrs and 48 hrs ($p<0.01$) (Table 12.1). There was a significant increase in AST in allografts as compared with levels in autografts at 24 hrs ($p<0.05$) and at 48 hrs ($p<0.01$).

*Table 12.1 Aspartate aminotransferase (AST) after liver autograft and allograft in fasted rats. Results are expressed as means +- SE. * $p<0.05$ and ** $p<0.01$ by ANOVA, with respect to partially hepatectomized controls (ph).*

TIME	PH	AUTOGRAFT	ALLOGRAFT
4 hrs	328+-40	998+-228*	1300+-339**
24 hrs	390+-31	910+-164*	1617+-470**
48 hrs	237+-16	496+-102	985+-259**
72 hrs	192+-32	328+-121	256+-30
96 hrs	111+-9	106+-34	173+-20

12.2.3 Thymidine kinase (TK).

In the autografts and allografts, activity was depressed up to 3.5 fold at 24 hours with respect to partially hepatectomized controls ($p<0.01$). Peak thymidine kinase activity was observed at 48 hrs in both auto- and allografts (Figure 12.1-2).

12.3.4 Mitotic index.

In both autografts and allografts, mitotic activity was significantly diminished at 24 hrs (1+-0) ($p<0.01$). Maximal mitotic activity was demonstrated at 48 hours in both groups, (autografts 13+-7) and (allografts 20+-6) corresponding with the elevation in thymidine kinase at this time. No significant differences in mitotic index occurred at 72 hrs. The mitotic index for allografts was significantly less than controls at 96 hrs ($p<0.05$) (Figure 12.1-2).

12.3.5 Liver mass index (LMI).

There were no differences between groups at 4 hours, reflecting the uniformity of partial hepatectomy. The LMI for both autografts and allografts was reduced at 24 hrs ($p<0.05$) and 48 hrs ($p<0.01$) with respect to controls. The greatest increment in LMI was shown in the second 48 hour period in both groups. Restitution of liver mass to that of sham-operated animals occurred in both autografts and allografts by 96 hours (Figure 12.1-2).

12.3.6 Tissue water.

The mean liver tissue water was greater in the autografts (73.3%) than in controls (70%) at 24 hours ($p<0.01$). In the allografts, tissue water was significantly greater than ph controls at 24 and 48 hrs ($p<0.01$) (Table 12.2).

*Table 12.2 Liver tissue water after liver autograft and allograft in fasted rats. Results are expressed as means +- SE. * $p<0.05$ and ** $p<0.01$ by ANOVA, with respect to partially hepatectomized controls.*

TIME	AUTOGRAFT	ALLOGRAFT
4 hrs	71.6%+-0.5%	75.0%+-1.2%
24 hrs	73.3%+-0.9%*	78.8%+-2.2%**
48 hrs	74.9%+-0.9%**	75%+-0.6%**
72 hrs	73.1%+-0.4%	73.3%+-6.9%
96 hrs	71.7%+-1.0%	73.4%+-5.5%

12.2.7 Ornithine decarboxyase (ODC).

In both the transplant groups, the pattern of ODC activity was no different to that of controls. The mean values in all groups demonstrated a wide standard error (Figure 12.1-2).

Figure 12.1 *Influence of reduced orthotopic liver autotransplantation on hepatic regeneration in fasted rats. Partial hepatectomy was performed as a control (clear bars) and compared to the reduced autograft (solid bars). Parameters of liver regeneration and sampling times were as previously indicated. Results were expressed as means +- SE. * p<0.05 and ** p<0.01 by ANOVA.*

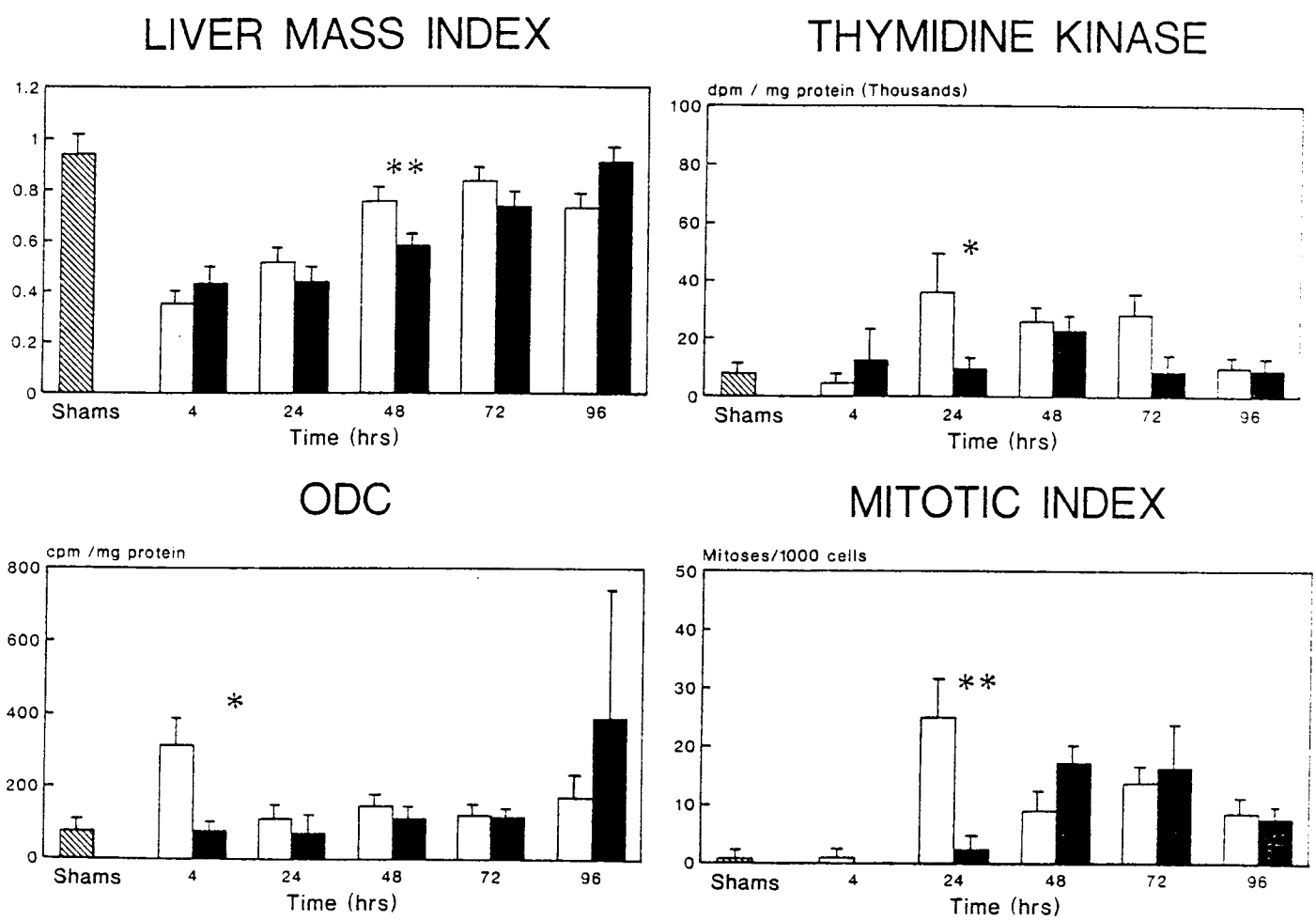
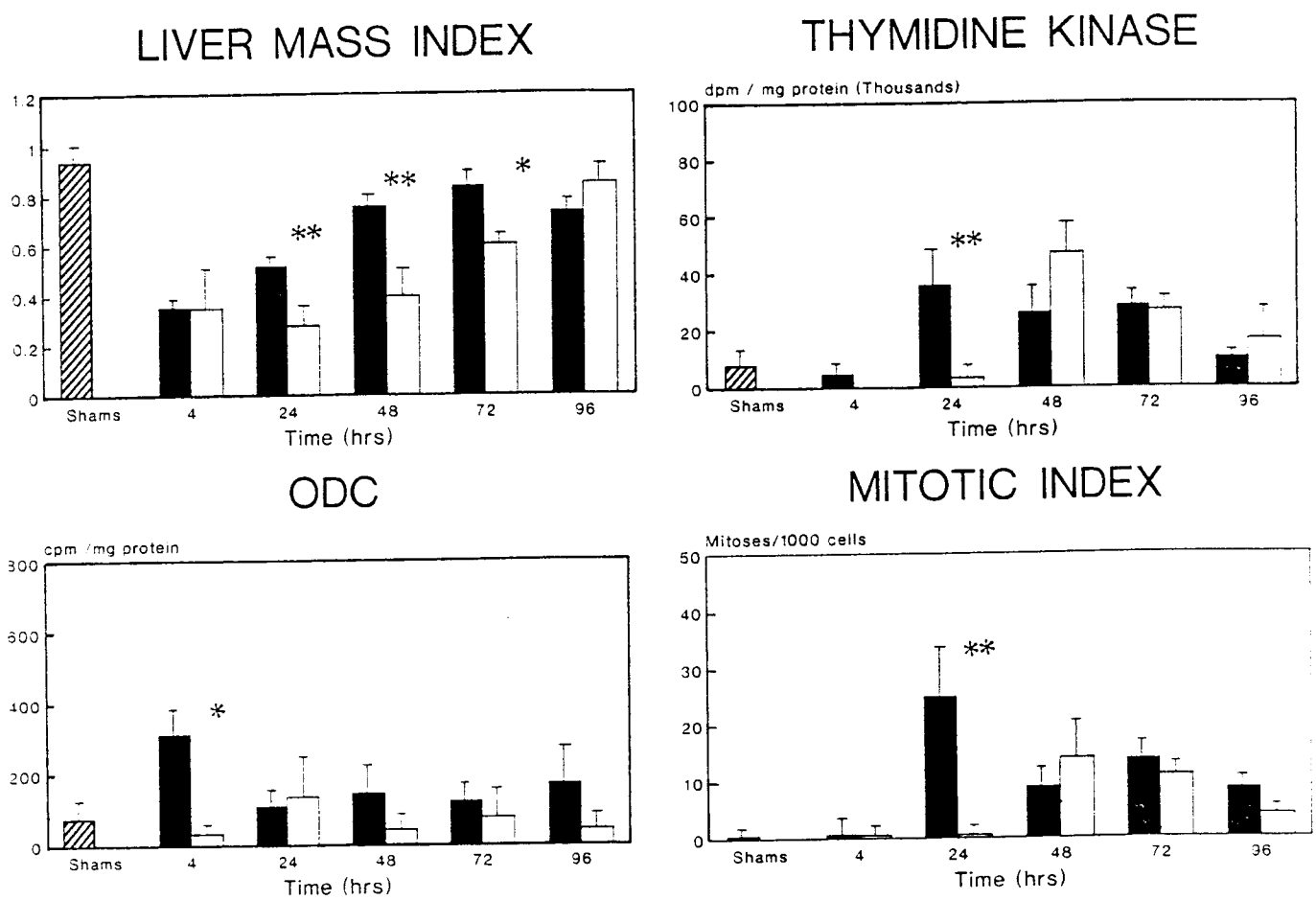


Figure 12.2 *Influence of reduced orthotopic liver allotransplantation on hepatic regeneration in fasted rats. Partial hepatectomy was performed as a control (solid bars) and compared to the reduced allograft (clear bars). Parameters of liver regeneration and sampling times were as previously indicated. Results were expressed as means +- SE. * p<0.05 and ** p<0.01 by ANOVA.*



12.4 Discussion.

The model for partial orthotopic liver transplantation.

Orthotopic rat liver transplantation was first carried out by Lee *et al* (161,162) and later modified by Zimmerman (308). This model represented a significant advance in liver transplantation research, since it was a reliable model of solid organ transplantation that utilized inexpensive laboratory animals. In the first report, a portojugular extracorporeal shunt was utilized (161). However, this proved cumbersome and was the source of a number of technical difficulties and was therefore later abandoned (162). The first report of the technique utilized hepatic arterial anastomosis (161). However, it was subsequently reported by Lee that "the rat liver deprived of hepatic arterial flow not only tolerates transplantation well, but also functions normally in the host for an indefinite period" (162). Anastomosis of the hepatic artery was therefore abandoned in subsequent modifications of the technique (162,308).

Difficulty in completing the suture anastomoses of the suprahepatic vena cava and portal vein within the limited safe anhepatic period, and problems in the control of the shunts created the necessity for a more expedient technique of rat liver transplantation. In response to this need, Kamada and Calne developed cuff techniques to facilitate the procedure (143). This enabled anhepatic times to be reduced from an average of 25 mins for suture anastomosis (161), to approximately 14 mins (143). This shortened anhepatic time resulted in improved results and the redundancy of extracorporeal shunting (143). In the opinion of Kamada and Calne, such shunts are unnecessary if the anhepatic phase does not exceed 26 mins (143). This technique was however criticized since it did not provide for reanastomosis of

the hepatic artery, at a time when the importance of hepatic arterialization was being increasingly recognized (50,97,120,277,286). It should however be noted that the rat liver has a remarkable ability to survive dearterialized liver transplantation (143). Increased isograft survival has since been demonstrated in arterialized orthotopic rat liver allografts (74). Furthermore, improvement in graft morphology, elimination of unspecific cell-mediated *in vitro* reactivity and specific transplantation tolerance in fully allogeneic combinations has been demonstrated (74). The hepatic artery has been shown to be important in the prevention of biliary ischaemia and the subsequent development of biliary obstruction (97,120). Induction of MHC class II antigens does not occur in arterialized liver isografts, unlike nonarterialized isografts (97). In the light of these recent observations, there can be little dispute over the advantage of the arterialized rat liver transplantation model.

Rat liver transplantation, as modified by Kamada *et al* has become an accepted model for the study of transplantation immunology and liver preservation (143,288). However, the use of the cuff technique precludes the autograft procedure, and the numerous advantages associated with it.

This laboratory has previously reported on an alternate technique for intact arterialized liver transplantation in the rat (72,113). In this model direct suture anastomosis is employed for the venae cavae and the portal vein. The artery was anastomosed by using the sleeve technique (66). An intraluminal polyethylene stent for the bile duct is utilized as described by Kamada (143). In response to the need for a model of reduced-size liver transplantation in small, inexpensive laboratory animals, we have developed a model of reduced-size liver transplantation in the rat. This was tested in both the autograft and allograft applications, and found to be a reliable model of arterialized liver transplantation. Anhepatic times averaged 22

mins (+-1 min) in the autograft group and 21 mins (+-1 min) in the allografts. A recognized limitation in the technique is the need for a skilled microsurgeon capable of performing the superior vena caval and portal venous anastomoses in less than 25 mins. We have encountered significantly increased mortality when ischaemic times are extended beyond this. The advantages of the cuff techniques are recognized in this regard. However, the necessity for an autograft model precluded their use in this experiment. Furthermore, it is felt that rearterialization is vital in any study of liver regeneration. We believe that the sleeve technique has significant advantages in the hepatic arterial anastomosis (113). Unlike the performance of rapid portal venous anastomoses, it is an easily aquired and reproducible skill.

Discussion of results.

The results of this part of the study indicate a similar pattern of hepatocyte proliferation in both autograft and allograft groups to that observed in animals subjected to hepatic ischaemia only. This suggests that the single most important factor governing alterations in hepatocyte proliferative kinetics is warm hepatic ischaemia. The autografts differed little in the pattern of their response to the allografts. This would indicate that cold ischaemia, such as incurred during storage of the liver, has no additional effect on the subsequent regenerative response.

Hepatic reperfusion injury has been observed in rat liver allotransplantation following 60 mins of cold preservation, manifesting primarily as an injury to the microvasculature (293). The reintroduction of blood-borne oxygen with generation of free oxygen radicals and resultant endothelial damage has been proposed as a possible mechanism of this injury (40,293). Significantly, no hepatocellular injury could be demonstrated following 30 mins warm ischaemia (293), suggesting that absorbed toxins from the gut are unlikely to be responsible. The results of our study

confirm those of Thurman *et al* (293), with significantly greater AST levels in allografts (warm ischaemic time 21 mins, cold ischaemic time 4 hrs) than in autografts (warm ischaemic time 22 mins). Damage after cold storage in this study may be due primarily to the effect of cold hypoxia on the endothelium. This has been proposed as a possible mechanism of injury in liver preservation (40). The possibility exists that damage to the microvasculature caused by reperfusion injury may contribute to the subsequent immunologic and biliary complications seen in clinical transplantation.

The loss of 17% of viable hepatocytes has been demonstrated following 60 mins of cold ischaemia (293). This deficit, added to the 68% partial hepatectomy performed in our study would result in an effective 75% hepatectomy, and a 25% partial graft. Interpretation of AST values should be seen in this context. In this study, AST values were greater in those animals in which storage of the liver had occurred, yet the proliferative response did not differ from those which were not stored (autografts). This would counter the contention that storage results in additional hepatocyte necrosis, greater loss of functional hepatocyte mass and a greater regenerative stimulus. At no time did recipients of stored livers demonstrate greater DNA synthesis or mitotic activity than either autografts or partially hepatectomised controls.

In the experiments on hepatic ischaemia and *in situ* flushing, it was demonstrated that the initiation of the regenerative response was retarded. In allografts, where ischaemia, flushing and storage all occurred, the peak of DNA synthesis and mitotic activity was recorded at 48 hrs. Therefore, whilst ischaemia and flushing both result in a delay in the initiation of regeneration, their effect is not additive in combination.

Of note is the increased tissue water observed in the allografts, as compared to either the autografts or partially hepatectomized controls. This would be consistent with the swelling of hepatocytes known to occur with preservation (270). Cytokine-mediated activation of the sinusoidal endothelium may account for the accumulation of intracellular and interstitial fluid (227).

Increased plasma catecholamine concentrations have been demonstrated after intact rat (50) and pig (114) liver allografts. The role for the sympathetic nervous system, noradrenaline and the alpha-1 adrenergic receptor in the control of liver regeneration (62) has been previously described on page 119. Increased levels of circulating catecholamines following liver transplantation may therefore have a positive effect on the proliferative response following partial liver transplantation.

A previous study of auxiliary and nonauxiliary liver allotransplantation performed in semiallogeneic rat combinations (109) has relevance in the discussion of the current study. Firstly, evidence of hypertrophy in the heterotopic 30% liver transplant was demonstrated in animals where the native liver was totally hepatectomized (109), although definitive markers of hepatocyte proliferation were not measured. Our findings, of 30% partial allografts in the orthotopic position confirm these findings. Secondly, compared to partially hepatectomized recipients, a state of donor-specific transplantation tolerance was observed in partial heterotopic allografts where total hepatectomy of the native liver was performed (109). This suggests that total recipient hepatectomy results in a state of immunologic privilege in the recipient, possibly due to the removal of the entire population of native Kupffer cells. This would tend to diminish the rate of rejection in this study, in which a fully allogeneic combination was used (outbred LE X LE).

In summary, the results of this group suggest that although a delay in the initiation of the regenerative process was present, the hepatocyte proliferative response in autografts and allogeneic allografts appears intact, with restoration of normal liver mass by 96 hrs in both transplanted groups. This is further discussed in the following chapter.

13. CONCLUSIONS.

The conclusions of this study, in brief, are as follows:

1. Hepatic ischaemia of 40 minutes duration delays the onset of liver regeneration following partial hepatectomy.

2. Portal venous stasis appears to enhance the proliferative response.

3. Ischaemic hepatocyte damage does not result in a greater regenerative stimulus by amplifying the loss of functional hepatocyte mass.

4. Portal venous occlusion results in significant mortality. This is alleviated by portal decompression by temporary portocaval shunt.

5. Hepatic denervation by surgical means has no apparent effect on the early regenerative response.

6. *In situ* liver flushing has a similar effect on the initiation of DNA synthesis to that observed in ischaemia. That is, the process is delayed by 24 hrs but is nevertheless complete by 96 hrs. There is no advantage of any one flushing solution over another in this regard.

7. The regenerative process is complete by 96 hours in male Long-Evans rats, as measured by the restitution of the liver-body mass ratio.

8. Acceptable models of liver autograft and allograft have been demonstrated; these are successful provided that the necessary microsurgical skill is available.

9. The technical procedure of liver transplantation has no direct implications in its own right on liver regeneration.

10. The effects of ischaemia and liver flushing on liver regeneration are not additive when in combination, that is in the stored liver allograft.

11. Despite an early delay in the initiation of the process, the regenerative response of the partial liver graft is largely unaffected by the multiple insults to which it is exposed during experimental transplantation.

We have thus examined the effect of some of the diverse insults to which the liver is exposed at the time of transplantation, on the livers' regenerative capacity.

Additional factors that may impact on the liver graft are not all potentially unfavourable. The immunosuppressive drugs cyclosporin (150) and FK 506 (89) have been shown to enhance DNA synthesis following partial hepatectomy, provided they are given prior to the operation. Allograft rejection has been shown to similarly enhance regeneration of intact liver grafts in rats (290).

We believe then that the major factors that may alter the hepatocyte proliferation kinetics of the partial liver graft have now been examined in the rat. From this and other studies (9,248) it would appear that the ability of the partial graft to restore the normal liver to body ratio by regenerative growth is unimpeded in the long term. A concern however, is the delay in the initiation of the process. Liver regeneration in rats is normally complete by 7 days (32), whilst in man this process may take 3

months, increasing at a rate of approximately 70 ml per day (295). A delay of 24 hours in the rat may therefore translate into a 2 week delay in man. This would imply that regenerative activity in the nascent partial liver graft may only commence on postoperative day 14. This might have significant implications if a rejection episode were also to occur. The immunosuppressive agents cyclosporin (150) and FK 506 (89) are reported to enhance regeneration if given prior to partial hepatectomy. Cyclosporin and FK 506 is reported to ameliorate the injury caused by ischaemia and reperfusion (147,149,151,254,255). A study is presently under way to determine the effect of the administration of these substances to rats following partially hepatectomy and hepatic ischaemia.

The implications of this are that sufficient liver parenchyma will need to be transplanted to sustain bodily requirements during this period. Rejection and graft infection are compounding threats at this time.

Hepatic regeneration is controlled by an equilibrium between humoral and hepatic growth factors and their specific hepatocellular receptors (166). Metabolic signals resulting from a smaller liver mass may result in the G_0 to G_1 transition in transplanted liver graft. Subsequently autocrine effectors such as TGF alpha would induce transition to the S phase in the stimulated cell, whilst co-mitogenic factors act on cells in the G_0 phase to result in either positive or negative effects on the cell. These factors include noradrenalin, vasopressin and angiotensin (194). It is likely that ischaemia acts to modify the effect of these so-called co-mitogenic factors. At a cellular level, the mechanisms whereby ischaemia may do this may include obliteration of the TGF alpha/EGF or alpha-1 adrenergic receptor by cellular damage at the time of reperfusion, or by impairing the cellular production of mitogens such as TGF alpha by a global depression of cellular metabolism. The

time course of the witnessed depression of parameters of cellular proliferation would suggest that permanent destruction of the receptor would be unlikely however, as the response has normalized by 48 hours. Other possibilities include the nonspecific depression of co-mitogenic substance release.

Unanswered questions and further studies.

In view of the apparent enhancement of the proliferative response following portal venous occlusion, and in the knowledge that endotoxaemia is associated with portal stasis (215), an important question is the effect of portal venous stasis on the expression of mitogenic cytokines. Portal and systemic endotoxaemia may form part of the physiologic process of liver regeneration.

Computerized axial tomography (295) and magnetic resonance imaging may provide accurate assessments of the volume increase of liver transplants, but they provide no direct information about the proliferative status of the transplanted liver. The development of a noninvasive parameter of liver regeneration is therefore important. Alfa foetoprotein and epidermal growth factor have been suggested as such noninvasive parameters by Koch and Leffert (154). Further possibilties may relate to oncogene or heat shock protein expression in the transplantation context. Progress in the elucidation of the control mechanism of liver regeneration at a cellular level may however be necessary prior to the use of such a parameter in the clinical context. The ever present trap is the measurement of serum parameters of which the true significance is controversial or worse, unknown.

Cyclosporin (150) and FK 506 (89) are known to enhance the proliferative response following partial hepatectomy. Obliteration of the observed delay in initiation of liver regeneration following transplantation is therefore a possible effect of these

drugs. This in turn would beg the question of whether the immunosuppression provided by these drugs alters the regenerative response of reduced liver transplantation in different allogeneic combinations. A enhanced proliferative response has previously been observed in intact liver grafts in fully allogeneic combinations (290).

The measurement of hepatocyte-specific synthetic function following transplantation is important, as is the question of how this is temporally related to liver regeneration. A delay in regenerative activity following transplantation may occur at a time when the liver is concerned with the synthesis of essential proteins.

Given the near normal ability of the liver to regenerate following transplantation, what is the minimum hepatocyte mass that can be safely transplanted into a recipient? It would appear that the limitation in this regard may be the maintenance of normal liver function in the period between transplantation and restitution of liver size to normal, rather than concern as to the liver segment's regenerative capacity. Further studies of this nature are therefore needed, since the proliferative status and kinetics of cellular proliferation of human transplanted liver are not known.

The answers to these questions may yet provide insight into the outcome of reduced liver transplantation surgery and the nature of the ideal liver mass.

REFERENCES.

1. Adkison D, Hollwarth ME, Benoit JN, Parks DA, McCord JM, Granger DN. Role of free radicals in ischaemia-reperfusion injury to the liver. Acta Physiol Scand 1986; 548: 101-107.

2. Andus T, Bauer J, Gerok W. Effects of cytokines on the liver. Hepatology 1991: 13; 364-375.

3. Atalla S, Toledo-Pereyra LH, MacKenzie GH, Cederna JP. Influence of oxygen-derived free radical scavengers on ischaemic livers. Transplantation 1985; 40: 584-590.

4. Backlund WM, Stevens J, Hamit HF, Jordan GL. Hepatic ischaemia in dogs. JAMA 1965; 194: 1116-1121.

5. Baker H de C. Ischaemic necrosis in the rat liver. J Pathol Bacteriol 1956; 71: 135-143.

6. Bardella L, Cairo G, Schiaffonati L. Post-ischaemic recovery and acute phase reaction in the liver cells. In: Gentilini P, Dianzani MU, eds. Frontiers in gastrointestinal research. Basel: Karger, 1984: 63-74.

7. Battersby C, Balderson G, Winch J, Burnett W. Acute occlusion of the portal vein in the calf. J Surg Res 1971; 11: 95-100.

8. Battersby C, Hickman R, Saunders SJ, Terblanche J. Liver function in the pig: 1. The effects of 30 minutes' normothermic ischaemia. Br J Surg 1974; 61: 27-32.

9. Bax NMA, Vermiere BMJ, Dubois N, Madern G, Meradji M, Molenaar JC. Orthotopic nonauxiliary homotransplantation of part of the liver in dogs. J Paed Surg 1982; 17: 906-912.

10. Bernard C. Lecons sur les proprietes physiologique et les alterations pathologique des liquides de Porganisme (Quoted by Johnstone). In: Bernard C, eds. Paris: JB Balliere et Fils, 316-320.

11. Bernuau D, Rogier E, Moreau A, Bernuau J, Feldmann G. Inhibitory effect of the acute inflammatory reaction on liver regeneration after partial hepatectomy in the rat. Gastroenterology 1986; 90: 268-273.

12. Bianchi PA, Crathorn AR, Shooter KV. Thymidine kinases and deoxyribonucleic acid synthesis in normal and regenerating rat liver. Biochim et Biophys Acta 1962; 61: 728-735.

13. Bismuth H. Surgical anatomy and anatomical surgery of the liver. World J Surg 1982; 6: 3-9.

14. Bismuth H, Houssin D. Reduced-size orthotopic liver graft in hepatic transplantation in children. Surgery 1984; 95: 367-372.

15. Bismuth H, Morino M, Castaing D, Gillon MC, Descorps Dec A, Saliba F, Samuel D. Emergency orthotopic liver transplantation in two patients using one donor liver. Br J Surg 1989; 76: 722-724.

16. Blankensteijn JD, Terpstra OT. Liver preservation: the past and the future. Hepatology 1991; 13: 1235-1250.

17. Bollum FJ, Potter VR. Nucleic acid metabolism in regenerating rat liver. VI. Soluble enzymes which convert thymidine to thymidine phosphates and DNA. Cancer Res 1959; 19: 561-565.

18. Bollum FJ, Anderegg JW, McElya AB, Potter VR. Nucleic acid metabolism in regenerating rat liver. VII. Effect of X-radiation on enzymes of DNA synthesis. Cancer Res 1960; 20: 138-143.

19. Bowers BB, Branum GD, Rotolo FS, Watters CR, Meyers WC. Bile flow - an index of ischaemic injury. J Surg Res 1987; 42: 565-569.

20. Braun L, Mead JE, Panzica M, Mikumo R, Bell GI, Fausto N. Transforming growth factor beta mRNA increases during liver regeneration: a possible paracrine mechanism of growth regulation. Proc Natl Acad Sci USA 1988; 85: 1539-1543.

21. Broelsch CE, Emond JC, Thistlethwaite JR, Whitington PF, Zucker AR, Baker AL, Aran PF, Rouch DA. Liver transplantation, including the concept of reduced-size liver transplants in children. Ann Surg 1988; 208: 410-420.

22. Broelsch CE, Emond JC, Thistlethwaite JR, Rouch DA, Whitington PF, Lichtor JL. Liver transplantation with reduced-size donor organs. Transplantation 1988; 45: 519-523.

23. Broelsch CE, Emond JC, Whitington PF, Thistlethwaite JR, Baker AL, Lichtor JL. Application of reduced-size liver transplants as split grafts, auxiliary orthotopic grafts, and living related segmental transplants. Ann Surg 1990; 212: 368-375.

24. Broelsch CE, Whitington PF, Emond JC. Evolution and future perspectives for reduced-size hepatic transplantation. Surg Gynecol Obstet 1990; 171: 353-360.

25. Brues AM, Drury DR, Brues MC. Quantitative study of cell growth in regenerating liver. Arch Pathol 1936; 22: 658-673.

26. Bucher NLR, McGowan JA. Regulatory mechanisms in hepatic regeneration. In: Wright R, Millward-Sadler GH, Albert KGMM, Karran S, eds. Liver and biliary disease 2nd edition. London: WB Saunders, 1985: 251-263.

27. Bucher NLR, Scott JF, Aub JC. Regeneration of the liver in parabiotic rats. Cancer Res 1951; 11: 457-465.

28. Bucher NLR. Regeneration of mammalian liver. Internat Rev Cytol 1963; 15: 245-300.

29. Bucher NLR, Swaffield MN, DiTroia JF. The influence of age upon the incorporation of thymidine-2-C14 into the DNA of regenerating rat liver. Cancer Res 1964; 24: 509-512.

30. Bucher NLR, Swaffield MN. The rate of incorporation of labeled thymidine into the deoxyribonucleic acid of regenerating rat liver in relation to the amount excised. Cancer Res 1964; 24: 1611-1625.

31. Bucher NLR. Experimental aspects of liver regeneration. NEJM 1967; 277: 686-696.

32. Bucher NLR, Malt RA. Regeneration of the liver and kidney. Boston: Little,Brown, 1971.

33. Bucher NLR, Swaffield MN. Regeneration of liver in rats in the absence of portal splanchnic organs and a portal blood supply. Cancer Res 1973; 33: 3189-3194.

34. Bucher NLR, Swaffield MN. Regulation of hepatic regeneration in rats by synergistic action of insulin and glucagon. Proc Natl Acad Sci USA 1975; 72: 1157-1160.

35. Bucher NLR, Patel U, Cohen S. Hormonal factors concerned with liver regeneration. Hepatotrophic factors. Ciba Foundation Symposium, 1978: 95-110.

36. Bucher NLR. Thirty years of liver regeneration: a distillate. Cold Spring Harbour Conferences on Cell Proliferation 1982; 9.

37. Buckberg GD, Ono H, Joseph WL, Tocornal JA, Fonkalsrud EW, Longmire WP. Hypotension following revascularization of the ischaemic liver: factors influencing its occurrence and prevention. Surgery 1968; 63: 446-458.

38. Cairo G, Bardella L, Schiaffonati L, Bernelli-Zazzera A. Synthesis of heat shock proteins in rat liver after ischaemia and hyperthermia. Hepatology 1985; 5: 357-361.

39. Cajone F, Ragnotti G, Bernelli-Zazzera A. State and function of liver polysomes during recovery from ischaemia. Exp Molec Pathol 1971; 14: 392-403.

40. Caldwell-Kenkel JC, Thurman RG, Lemasters JJ. Selective loss of nonparenchymal cell viability after cold ischaemic storage of rat livers. Transplantation 1987; 45: 834-837.

41. Caldwell-Kenkel JC, Thurman RG, Lemasters JJ. Reperfusion injury to endothelial cells following cold ischaemic storage of rat liver in Euro-Collins solution. Hepatology 1987; 7: 1048-1048.

42. Caldwell-Kenkel JC. Kupffer cell activation and endothelial damage after storage of rat livers : effects of reperfusion. Hepatology 1991; 13: 83-95.

43. Callery MP, Mangino MJ, Flye MW. Kupffer cell prostaglandin-E2 production is amplified during hepatic regeneration. Hepatology 1991; 14: 368-372.

44. Callery MP, Kamei T, Flye MW. Kupffer cell tumor necrosis factor-alpha production is suppressed during liver regeneration. J Surg Res 1991; 50: 515-519.

45. Camargo ACM, Cornicelli J, Cardoso SS. Alteration in lipid content of liver in rat after partial hepatectomy. Proc Soc Exp Biol Med 1966; 122: 1151-1154.

46. Carr B, Huang TH, Buzin CH, Itakura K. Induction of heat-shock gene expression without heat shock by hepatocarcinogens and during hepatic regeneration in rat liver. Cancer Res 1986; 46: 5106-5111.

47. Carr BI, Laishes BA. Carcinogen-induced drug resistance in rat hepatocytes. Cancer Res 1981; 41: 1715-1719.

48. Carr BI, Hayashi I, Branum EL, Moses HL. Inhibition of DNA synthesis in rat hepatocytes by platelet-derived type beta transforming growth factor. Cancer Res 1986; 46: 2330-2334.

49. Carr BI, Huang TH, Itakura K, Noel M, Marceau N. TGF beta gene transcription in normal and neoplastic liver growth. J Cell Biochem 1989; 39: 477-487.

50. Chaland P, Braillon A, Gaudin C, Sekiyama T, Bernuau D, Adam R, Bismuth H, Benhamou J. Orthotopic liver transplantation with hepatic artery anastomoses. Haemodynamics and response to haemorrhage in conscious rats. Transplantation 1990; 49: 675-678.

51. Chan K, Kost DP, Michalopoulos G. Multiple sequential periods of DNA synthesis and quiescence in primary hepatocyte cultures maintained on the DMSO-EGF on/off protocol. J Cell Physiol 1989; 141: 584-590.

52. Chau AYS, Goldbloom VC, Gurd FN. Clostridial infection as a cause of death after ligation of the hepatic artery. Arch Surg 1951; 63: 390-402.

53. Cherqui D, Emond JC, Pietrabissa A, Michel M, Roncella M, Brown SB, Whitington PF, Broelsch CE. Segmental liver transplantation from living donors. Report of the technique and preliminary results in dogs. HPB Surgery 1990; 2: 189-204.

54. Child CG, Barr D, Holswade GR, Harrison CS. Liver regeneration following portocaval transposition in dogs. Ann Surg 1953; 138: 600-608.

55. Child CG. Eck's fistula. Surg Gynecol Obstet 1953; 96: 375-376.

56. Chopra J, Joist JH, Webster RO. Loss of 51Chromium, lactate dehydrogenase and 111Indium as indicators of endothelial cell injury. Lab Invest 1987; 57: 578-584.

57. Christophi C, Morgan B, Wale R, McInnes I. The effect of venovenous bypass on portal vein bacteraemia in orthotopic liver transplantation. Transplant Proc 1989; 21: 3859-3860.

58. Cornell RP. Endotoxin-induced hyperinsulinaemia and hyperglucagonaemia after experimental liver injury. Am J Physiol 1981; 241: E428-E435.

59. Cornell RP. Role of the liver in endotoxin-induced hyperinsulinaemia and hyperglucagonaemia in rats. Hepatology 1983; 3: 188-192.

60. Cornell RP. Restriction of gut-derived endotoxin impairs DNA synthesis for liver regeneration. Am J Physiol 1985; 249: R563-R569.

61. Cornell RP. Gut-derived endotoxin elicits hepatotrophic factor secretion for liver regeneration. Am J Physiol (Regulatory Integrative Comp. Physiol.18) 1985; 249: R551-R562.

62. Cruise JL, Knechtle SJ, Bollinger RR, Kuhn C, Michalopoulos G. Alpha-1 adrenergic effects and liver regeneration. Hepatology 1987; 7: 1189-1194.

63. Cruise JL, Muga SJ, Lee Y, Michalopoulos GK. Regulation of hepatocyte growth: Alpha-1 adrenergic receptor and ras p21 changes in liver regeneration. J Cell Physiol 1989; 140: 195-201.

64. Cruise JL. Alpha-1 adrenergic receptors in liver regeneration. Dig Dis Sci 1991; 36: 485-488.

65. Drapanas T, Becker DR, Alfano GS, Potter WH, Stewart JD. Some effects of interrupting hepatic blood flow. Ann Surg 1955; 142: 831-835.

66. Duminy FJ. A new microvascular "sleeve" anastomosis. Journal of Surgical Research 1989; 46: 189-194.

67. Edwards JL, Koch A. Parenchymal and littoral cell proliferation during liver regeneration. Lab Invest 1964; 13: 32-43.

68. Eiseman B, Karran S. Measuring liver growth. World Journal of Surgery 1979; 3: 781-782.

69. Emond JC, Whitington PF, Thistlethwaite JR, Alonso EM, Broelsch CE. Reduced-size orthotopic liver transplantation: use in the management of children with chronic liver disease. Hepatology 1989; 10: 867-872.

70. Emond JC, Whitington PF, Thistlethwaite JR, Cherqui D, Alonso EA, Woodle IS, Vogelbach P. Transplantation of two patients with one liver. Analysis of a preliminary experience with 'split liver grafting'. Ann Surg 1990; 212: 14-22.

71. Emond JC, Whitington PF, Broelsch CE. Overview of reduced-size liver transplantation. Clin Transplantation 1991; 5: 168-173.

72. Engelbrecht GHC, Duminy F, Hickman R, Terblanche J. A technique for liver autograft in the rat, including a novel method for bile duct anastomosis. SA J Sci 1989; 85: 391-394.

73. Engelbrecht GHC, Hickman R. Does arterialization alter the regenerative response after rat liver allograft? [Abstract]. S Afr J Surg 1990; 28: 115-115.

74. Engemann R, Ulrichs K, Thiede A, Muller-Ruccholz W, Hamelmann H. Value of a physiological liver transplant model in rats. Induction of specific graft tolerance in a fully allogeneic strain combination. Transplantation 1982; 33: 566-568.

75. Engerson TD, McKelvey TG, Rhyne DB. Conversion of xanthine dehydrogenase to oxidase in ischaemic rat tissues. J Clin Invest 1987; 79: 1564-1570.

76. Esquivel CO, Koneru B, Karrer F, Todo S, Iwatsuki S, Gordon RD, Makowka L, Marsh WJ. Liver transplantation before 1 year of age. J Pediatr 1987; 110: 545-548.

77. Exton JH. Role of phosphoinositides in the regulation of liver function. Hepatology 1988; 8: 152-166.

78. Fausto N. Hepatic regeneration. In: Zakim D, Boyer TD, eds. Hepatology. A textbook of liver disease, Volume 1, 2nd edition. Philadelphia: WB Saunders, 1990: 49-65.

79. Fausto N, Van Lancker JL. Molecular mechanisms of liver regeneration. IV. Thymidylic kinase and deoxyribonucleic acid polymerase activities in normal and regenerating liver. J Biol Chem 1965; 240: 1247-1255.

80. Fausto N. Studies on ornithine decarboxylase activity in normal and regenerating livers. Biochim Biophys Acta 1969; 190: 193-201.

81. Feindel CM, Harper R, Wallace AC, Wall WJ. Tolerance of the liver to ischaemia: an experimental study. Can J Surg 1981; 24: 147-150.

82. Feingold KR. Localization of tumor necrosis factor-stimulated DNA synthesis in the liver. Hepatology 1991; 13: 773-779.

83. Fleig WE, Hoss G. Partial purification of rat Hepatic Stimulator Substance and characterization of its action on hepatoma cells and normal hepatocytes. Hepatology 1989; 9: 240-248.

84. Fortner JG, Kinne DW, Shiu MH, Howland WS, Kim DK, Castro EB, Yeh SDJ, Benua RS. Clinical liver heterotopic (auxiliary) transplantation. Surgery 1973; 74: 739-751.

85. Francavilla A, Eagon P, DiLeo A, Polimeno L, Panella C, Aquilino AM, Ingrosso M, Van Thiel DH. Sex hormone-related functions in regenerating male rat liver. Gastroenterology 1986; 91: 1263-1270.

86. Francavilla A, Ove P, Polimeno L, Sciascia C, Coetzee ML, Starzl TE. Epidermal growth factor and proliferation in rat hepatocytes in primary culture isolated at different times after partial hepatectomy. Cancer Res 1986; 46: 1318-1323.

87. Francavilla A, Ove P, Polimeno L, Coetzee M, Makowka L, Rose J, Van Thiel DH, Starzl TE. Extraction and partial purification of Hepatic Stimulator Substance in rats, mice and dogs. Cancer Res 1987; 47: 5600-5605.

88. Francavilla A, Ove P, Polimeno L, Coetzee M, Makowka L, Barone M, Van Thiel DH, Starzl TE. Regulation of liver size and regeneration: importance in liver transplantation. Transplant Proc 1988; 20: 494-497.

89. Francavilla A, Barone M, Todo S, Zeng Q, Porter KA,Sakr Starzl TE. Augmentation of rat liver regeneration by FK 506 compared with cyclosporin. Lancet 1989: 25; 1248-1249.

90. Francavilla A, Gavaler JS, Makowka L, Barone M, Mazzaferro V, Ambrosino G, Iwatsuki S, Guglielmi FW. Estradiol and testosterone levels in patients undergoing partial hepatectomy. Dig Dis Sci 1989; 34: 818-822.

91. Francavilla A, Panella C, Polimeno L, Giangaspero A, Mazzaferro V, Pan C, Van Thiel DH, Starzl TE. Hormonal and enzymatic parameters of hepatic regeneration in patients undergoing major liver resections. Hepatology 1990; 12: 1134-1138.

92. Fredericks W. Plasma ODC as an indicator of ischaemic injury in rat liver. Cell Biochem Function 1987; 2: 217-221.

93. Frederiks WM, Myagkaya GL, Bosch KS, Fronik GM, H van Veen, IMC Vogels, J James. The value of enzyme leakage for the prediction of necrosis in liver ischaemia. Histochemistry 1983; 78: 459-472.

94. Frederiks WM, Vogels IMC, Fronik GM. Plasma ornithine carbamyl transferase as an indicator of ischaemic injury of rat liver. Cell Biochem Funct 1984; 2: 217-220.

95. Freeman BA, Crapo JD. Biology of disease: free radicals and tissue injury. Lab Invest 1982; 47: 412-426.

96. Garcia-Alonzo I, Portugal V, Iturburu I, de Tejada IL, Mendez J. Modifications induced by cyclosporin A on ischaemic liver regeneration. Surg Res Comm 1990; 9: 227-233.

97. Gassel HJ, Engemann R. Preservation of rat liver allografts. Transplantation 1987; 44: 726-726.

98. Gaub J, Iversen J. Rat liver regeneration after 90% partial hepatectomy. Hepatology 1984; 4: 902-904.

99. German J. The heat-shock response is for hepatologists, too. Proc Natl Acad Sci USA 1985; 5: 516-520.

100. Glinos AD, Gey GO. Humoral factors involved in the induction of liver regeneration in the rat. Proc Soc Exp Biol Med 1952; 80: 421-425.

101. Gohda E, Tsubuochi H, Nakayama H, Hirono S, Sakiyama O, Takahashi K, Miyazaki H, Hashimoto S. Purification and partial characterization of hepatocyte growth factor from plasma of a patient with fulminant hepatic failure. J Clin Invest 1988; 81: 414-419.

102. Gores GJ, Herman B, Lemasters JJ. Plasma membrane bleb formation and rupture: a common feature of hepatocellular injury. Hepatology 1990; 11: 690-697.

103. Gottlieb LI, Fausto N, Van Lancker JL. Molecular mechanism of liver regeneration. The effect of puromycin on deoxyribonucleic acid synthesis. J Biol Chem 1964; 239: 555-559.

104. Granger DN, Rutili G, McCord JM. Role of superoxide radicals in feline intestinal ischaemia. Gastroenterology 1981; 81: 22-29.

105. Grisham JW. A morphologic study of deoxyribonucleic acid synthesis and cell proliferation in regenerating rat liver; autoradiography with thymidine-H3. Cancer Res 1962; 22: 842-849.

106. Grisham JW, Porta E. Origin and fate of proliferated hepatic ductal cells in the rat: electron microscopic and autoradiographic studies. Exper Molec Path 1964; 3: 242-261.

107. Grisham MB, Hernandez LA, Granger DN. Xanthine oxidase and neutrophil infiltration in intestinal ischaemia. Am J Physiol 1986; 251: G567-G574.

108. Grun M, Liehr H, Heine WD, Rasenack U. Fulminant hepatic failure after galactosamine injection as result of KC-failure in germ-free rats (Abstract). J Reticuloendothel Soc 1977; 22: 19.

109. Gugenheim J, Houssin D, Tamisier D, Franco D, Martin E, Lang P, Bismuth H. Spontaneous long-term survival of liver allografts in inbred rats. Influence of the hepatectomy of the recipients' own liver. Transplantation 1981; 32: 445-450.

110. Hall RR. Hyperkalaemia following temporary occlusion of the portal vein and hepatic artery. Br J Surg 1972; 59: 125-128.

111. Hanley MR. Mitogenic neurotransmitters. Nature 1989; 340: 97.

112. Harris KA, Wallace AC, Wall WJ. Tolerance of the liver to ischaemia in the pig. J Surg Res 1982; 33: 524-530.

113. Hickman R, Engelbrecht GHC, Duminy F. A technique for liver transplantation in the rat. Transplantation 1989; 48: 1080-1080.

114. Hickman R, James MFM, Janicki P. Plasma catecholamines in the anhepatic pig. S Afr J Surg 1991; 29: 168.

115. Hickman R, Tyler M, Rose Innes C, Lotz Z, Fourie J. How rapidly do hyperinsulinaemia and hyperglucagonaemia develop after portacaval shunting? J Surg Res 1992 (In Press).

116. Higgins GM, Anderson RM. Experimental pathology of the liver 1. Restoration of the liver in the white rat following partial surgical removal. Arch Pathol 1931; 12: 186-202.

117. Holecek M, Simek J, Palicka V, Zadak Z. Effect of glucose and branched chain amino acid (BCAA) infusion on onset of liver regeneration and plasma amino acid pattern in partially hepatectomized rats. J Hepatol 1991; 13: 14-20.

118. Holper K, Olcay I, Kitihama A, Miller RH, Brettschneider L, Drapanas T, Trejo RA, Di Luzio NR. Effect of ischaemia on hepatic parenchymal and reticuloendothelial function in the baboon. Surgery 1974; 76: 423-432.

119. Houck KA, Michalopoulos GK. Altered responses of regenerating hepatocytes to norepinephrine and transforming growth factor type beta. J Cell Physiol 1989; 141: 503-509.

120. Howden B, Jablonski P, Grossman H, Marshall VC. The importance of the hepatic artery in rat liver transplantation. Transplantation 1989; 47: 428-431.

121. Huggett AC, Krutzsch HC, Thorgeirson SS. Characterization of a hepatic proliferation inhibitor (HPI): effect of HPI on the growth of normal liver

cells-comparison with transforming growth factor beta. J Cell Biochem 1987; 35: 305-314.

122. Huguet C, Nordlinger B, Bloch P, Conard J. Tolerance of the human liver to prolonged normothermic ischaemia. Arch Surg 1978; 113: 1448-1451.

123. Ichihara A. Mechanisms controlling growth of hepatocytes in primary culture. Dig Dis Sci 1991; 36: 489-493.

124. Iu S, Harvey PRC, Makowka L, Petrunka CN, Ilson RG, Strasberg SM. Markers of allograft viability in the rat. Relationship between transplantation viability and liver function in the isolated perfused liver. Transplantation 1987; 44: 562-569.

125. Ives DH, Morse PA, Potter VR. Feedback inhibition of thymidine kinase by thymidine triphosphate. J Biol Chem 1963; 238: 1467-1474.

126. Jacob AI, Goldberg PK, Bloom N, Degenshein A, Kozinn PJ. Endotoxin and bacteria in portal blood. Gastroenterology 1977; 72: 1268-1270.

127. Janne J, Poso H, Raina A. Polyamines in rapid growth and cancer. Biochim Biophys Acta 1978; 473: 241-293.

128. Jirtle RL, Michalopoulos G. Effects of partial hepatectomy on transplanted hepatocytes. Cancer Res 1982; 42: 3000-3004.

129. Johansson S, Andersson N, Andersson G. Pretranslational and posttranslational regulation of the EGF receptor during the prereplicative phase of liver regeneration. Hepatology 1990; 12: 533-541.

130. Johnstone FRC. Acute ligation of the portal vein. Surgery 1957; 41: 958-971.

131. Jolly PC, Foster JH. Hepatic inflow stasis. Surgery 1963; 54: 45-55.

132. Kaczmarek L. Protooncogene expression during the cell cycle. Lab Invest 1986; 54: 365-376.

133. Kahn D, Stadler J, Terblanche J, Hickman R. Thymidine kinase: an inexpensive index of liver regeneration in a large animal model. Gastroenterology 1980; 79: 907-911.

134. Kahn D, Hickman R, McLeod H, Terblanche J. The stimulatory effect of a partially hepatectomized auxiliary graft upon the host liver. Observations on the regenerative response in orthotopic and heterotopic grafts. S Afr Med J 1982; 61: 362-365.

135. Kahn D, Hickman R, Dent DM, Terblanche J. For how long can the liver tolerate ischaemia? Eur Surg Res 1986; 18: 277-282.

136. Kahn D, Hickman R, Terblanche J, Von Sommoggy ST. Partial hepatectomy and liver regeneration in pigs - the response to different resection sizes. J Surg Res 1988; 45: 176-180.

137. Kahn D, Hickman R, Terblanche J, Kirsch RE. Hepatic stimulator substance in extracts from regenerating porcine liver. Eur Surg Res 1988; 20: 168-174.

138. Kahn D, Eagon PK, Porter LE, Elm MS, Makowka L, Podesta L, Starzl TE, Van Thiel DH. Effect of tamoxifen on hepatic regeneration in male rats. Dig Dis Sci 1989; 34: 27-32.

139. Kalayoglu M, Sollinger HW, Stratta RJ, D'Allesandro AM, Hoffmann RM. Extended preservation of the liver for clinical transplantation. Lancet 1988; 1: 617-619.

140. Kallenbach M, Roome NO, Schulte-Hermann R. Kinetics of DNA synthesis in feeding-dependant and independant hepatocyte populations of rats after partial hepatectomy. Cell Tissue Kinet 1983; 16: 321-322.

141. Kallio A, Poso H, Jänne J. Inhibition of prereplicative polyamine accumulation in regenerating rat liver. Biochim Biophys Acta 1977; 479: 345-353.

142. Kam I, Lynch S, Svanas G, Todo S, Polimeno L, Francavilla A, Penkrot R, Takaya S, Ericzon BG, Starzl TE, Van Thiel DH. Evidence that host size determines liver size: studies in dogs receiving orthotopic liver transplants. Hepatology 1987; 7: 362-366.

143. Kamada N, Calne RY. Orthotopic liver transplantation in the rat. Technique using cuff for portal vein anastomosis and biliary drainage. Transplantation 1979; 28: 47-50.

144. Kamada N, Calne RY. A surgical experience with five hundred thirty liver transplants in the rat. Surgery 1983; 93: 64-69.

145. Kan M, Huang J, Mansson P, Yasamitsu H, Carr B, McKeehan WL. Heparin-binding growth factor type 1 (acid fibroblast growth factor): a potential biphasic autocrine and paracrine regulator of hepatocyte regeneration. Proc Natl Acad Sci USA 1989; 86: 7432-7436.

146. Katsumoto F, Miyazaki K, Nakayama F. Stimulation of DNA synthesis in hepatocytes by Kupffer cells after partial hepatectomy. Hepatology 1989; 9: 405-410.

147. Kawano K, Kim YI, Kaketani K, Kobayashi K. The beneficial effect of cyclosporin on liver ischaemia in rats. Transplantation 1989; 48: 759-764.

148. Kam I, Lynch S, Svanas G, Todo S, Polimeno L, Francavilla A, Penkrot R, Takaya S, Ericzon BG, Starzl TE, Van Thiel DH. Evidence that host size determines liver size: studies in dogs receiving orthotopic liver transplants. Hepatology 1987; 7: 362-366.

149. Kawano K, Kim YI, Goto S, Nagai T, Egashira T, Yamanaka Y, Kobayashi M. Evidence that azathioprine, as well as cyclosporin, ameliorates warm ischaemia in the rat liver. Transplantation 1990; 49: 1002-1003.

150. Kim YI, Calne RY, Nagasue N. Cyclosporin A stimulates proliferation of the liver cells after partial hepatectomy in rats. Surg Gynecol Obstet 1988; 166: 317-322.

151. Kim YI. Alleviation of 3.5 hr warm ischaemic injury of the liver in pigs by cyclosporin pretherapy. Transplantation 1991; 51: 731-733.

152. Kirsch RE, Saunders SJ, Frith O'C, Rawlings E, Woodburn V. The effect of intragastric feeding with amino acids on liver regeneration after partial hepatectomy in the rat. Am J Clin Nutr 1979; 32: 738-740.

153. Kirsch RE, Frith O'C, Vinik A, Terblanche J. Insulin, glucagon and liver regeneration. S Afr Med J 1980; 58: 854-856.

154. Koch KS, Leffert HL. Comments on Van Thiel DH *et al* "Rapid growth of an intact human liver transplanted into a recipient larger than the donor". Gastroenterology 1987;93:1414-1419. Hepatology 1989; 9: 789-790.

155. Koff RS. Commentary. Gastroenterology 1991: 101; 1445-1446.

156. Koj A. Acute-phase reactants. Their synthesis, turnover and biologic significance. In: Allison AC, eds. Structure and function of plasma proteins. London: Plenum, 1974: 73-125.

157. La Breque DR, Steele G, Fogerty S, Wilson M, Barton J. Purification and physical-chemical characterization of Hepatic Stimulator Substance. Hepatology 1987; 7: 100-106.

158. Lambotte L, de Hemptinne B, Alvarez-Lopez A, Besse T. Effects of calcium blocking agents and prostaglandins I2 or E2 on the tolerance of the rat liver to ischaemia. Transplant Proc 1988; 20: 986-986.

159. Lee PC. ApppA, heat shock stress and cell division. Hepatology 1985; 5: 516-520.

160. Lee S, Keiter JE, Rosen H, Williams R, Chandler JG, Orloff MJ. Influence of blood supply on regeneration of liver transplants. Surg Forum 1969; 20: 369-371.

161. Lee S, Charters AC, Chandler JG, Orloff MJ. A technique for orthotopic liver transplantation in the rat. Transplantation 1973; 16: 664-669.

162. Lee S, Charter AC, Orloff MJ. Simplified technique for orthotopic liver transplantation in the rat. Am J Surg 1975; 130: 38-40.

163. Leffert H, Alexander NM, Faloona G, Rubalcava B, Unger R. Specific endocrine and hormonal receptor changes associated with liver regeneration in adult rats. Proc Natl Acad Sci USA 1975; 72: 4033-4036.

164. Leffert H, Koch KS. Proliferation of hepatocytes. Ciba Foundation Symposium 1978; 55: 61-82.

165. Leffert HL, Koch KS. Two ionic signals as prominent regulators of liver regeneration. In: Berk PD, Chalmers TC, eds. Frontiers in liver disease. New York: Thieme-Stratton, 1981: 54-59.

166. Leffert HL, Koch KS, Lad PJ, Shapiro IP, Skelly H, de Hemptinne B. Hepatocyte regeneration, replication, and differentiation. In: Arias IM, Jakoby WB, Popper H, Schacter D, Shafritz DA, eds. The Liver: Biology and Pathobiology. New York: Raven Press. Ltd., 1988: 833-850.

167. Leong GF, Grisham JW, Hole BV, Albright ML. Effect of partial hepatectomy on DNA synthesis an mitosis in heterotopic partial autografts of rat liver. Cancer Res 1964; 24: 1496-1501.

168. Levi JU, Zeppa R. Source of the humoral factor that initiates hepatic regeneration. Ann Surg 1971; 174: 364-370.

169. Lilly JR, Hall RJ. Liver transplantation and Kasai operation in the first year of life: therapeutic dilemma in biliary atresia. J Paediatr 1987; 110: 561-562.

170. Lindroos PM, Michaelopoulos GK. HGF (Hepatopoeitin A) rapidly increases in plasma before DNA synthesis and liver regeneration stimulated by PH and CCl_4 adminstration. Hepatology 1991; 13: 743-749.

171. Lombard MN, Nadal C, Fiszer-Szafa B, Le Rumeur E, Zajdela F. Interference of sex-related factors in the response of liver cells to experimental mitotic stimuli. Cell Tissue Kinet 1979; 12: 379-391.

172. Luk GD. Essential role of polyamine metabolism in hepatic regeneration. Inhibition of deoxyribonucleic acid and protein synthesis and tissue regeneration by difluoromethylornithine in the rat. Gastroenterology 1986; 90: 1261-1267.

173. MacKenzie RJ, Furnival CM, Wood CB, O'Keane MA, Blumgart LH. The effects of prolonged hepatic ischaemia before 70% partial hepatectomy in the dog. Br J Surg 1977; 64: 66-69.

174. Maddrey W, Van Thiel DH. Liver transplantation: an overview. Hepatology 1988; 8: 948-959.

175. Makino R, Hayashi K, Sugimura K. *C-myc* transcript is induced in rat liver at a very early stage of regeneration or by cycloheximide treatment. Nature 1984; 310: 697-698.

176. Malatack JJ, Schaid DJ, Urbach AH, Gartner JC, Zitelli BJ, Rockette H, Fischer J, Starzl TE. Choosing a pediatric recipient for orthotopic liver transplantation. J Paediatr 1987; 111: 479-489.

177. Mann FC. Restoration and pathologic reactions of the liver. J Mt Sinai Hosp 1944; 11: 65-74.

178. Marotto ME, Thurman RG, Lemasters JJ. Early midzonal cell death during low-flow hypoxia in the isolated, perfused rat liver: protection by allopurinol. Hepatology 1988; 8: 585-590.

179. Marubayashi S, Takenaka M, Dohi K, Ezaki H, Kawasaki T. Adenine nucleotide metabolism during hepatic ischaemia and subsequent blood reflow periods and its relation to organ viability. Transplantation 1980; 30: 294-296.

180. Marubayashi S, Dohi K, Yamada K, Kawasaki T. Changes in the levels of endogenous coenzyme Q homologs, alpha-tocopherol, and glutathione in rat liver after hepatic ischaemia and reperfusion, and the effect of pretreatment with coenzyme Q_{10}. Biochim Biophys Acta 1984; 797: 1-9.

181. Marubayashi S, Dohi K, Kawasaki T. Role of free radicals in ischaemic rat liver cell injury. Prevention of damages by vitamin E, coenzyme Q_{10}, or reduced glutathione administration. Surg Forum 1985; 36: 136-138.

182. Marubayashi S, Dohi K, Ochi K, Kawasaki T. Role of free radicals in ischaemic rat liver cell injury: prevention of damage by alpha-tocopherol administration. Surgery 1986; 99: 184-191.

183. Marzi I, Zhong Z, Lemasters JJ, Thurman RG. Evidence that graft survival is not related to parenchymal cell viability in rat liver transplantation. Transplantation 1989; 48: 463-468.

184. Marzi I, Knee J, Menger MD, Harbauer G, Buhm V. Hepatic microcirculatory disturbances due to portal vein clamping in the orthotopic rat liver transplantation model. Transplantation 1991; 52: 432-436.

185. McCord JM. Oxygen-derived free radicals in postischaemic tissue injury. NEJM 1985; 312: 159-163.

186. McGowan JA, Fausto N. Ornithine decarboxylase activity and the onset of deoxyribonucleic acid synthesis in regenerating liver. Biochem J 1978; 170: 123-127.

187. McGowan JA, Strain AJ, Bucher NLR. DNA synthesis in primary cultures of adult rat hepatocytes in a defined medium: effects of epidermal growth factor, insulin, glucagon, and cyclic-AMP. J Cell Physiol 1981; 180: 353-363.

188. McJunkin FA, Breuhaus HC. Homologous liver as a stimulus to hepatic regeneration. Arch Pathol 1931; 12: 900-908.

189. McKeown CMB, Edwards V, Phillips MJ, Harvey PRC, Petrunka CN, Strasberg SM. Sinusoidal lining cell damage: the critical injury in cold preservation of liver allografts in the rat. Transplantation 1988; 46: 178-191.

190. Mead JE, Fausto N. Transforming growth factor alpha may be a physiological regulator of liver regeneration by means of an autocrine mechanism. Proc Natl Acad Sci USA 1989; 86: 1558-1562.

191. Metzger J, Dore SP, Lauterburg BH. Oxidant stress during reperfusion of ischaemic liver: no evidence for a role of xanthine oxidase. Hepatology 1988; 8: 580-584.

192. Michalopoulos G, Houck K, Dolan M, Novicki DL. Control of proliferation of hepatocytes by two serum hepatopoeitins. Fed Proc 1983; 42: 1023-1023.

193. Michalopoulos G, Houck KA, Dolan ML, Luetteke NC. Control of hepatocyte replication replication by two serum factors. Cancer Res 1984; 44: 4414-4419.

194. Michalopoulos GK. Liver regeneration: molecular mechanisms of growth control. FASEB J 1990; 4: 176-187.

195. Minuk GY, Gauthier T, Benarroch A. Changes in serum and hepatic polyamine concentrations after 30%, 70% and 90% partial hepatectomy in rats. Hepatology 1990; 12: 542-546.

196. Minuk G Y, Gauthier T, Gaharie A, Murphy L J. The effect of GABA on serum and hepatic polyamine concentrations after partial hepatectomy in rats. Hepatology 1991: 14; 685-689.

197. Mittnacht S. Ischaemic mitochondrial dysfunction and reversal with chlorpromazine. J Biol Chem 1979; 254: 9871-9878.

198. Miyata T, Todo S, Imventarza Y, Ueda H, Furukawa H, Starzl TE. Endogenous endotoxaemia during orthotopic liver transplantation in dogs. Transplant Proc 1989; 21: 3861-3862.

199. Miyazawa K, Tsubouchi H, Naka D, Takahashi K, Okigaki M, Arakaki N, Nakayama H, Hirono S. Molecular cloning and sequence analysis of cDNA for human Hepatocyte Growth Factor. Biochem Biophys Res Comm 1989; 163: 967-973.

200. Montecuccoli G, Novello F, Stirpe F. Effect of protein deprivation and of starvation on protein synthesis in resting and regenerating rat liver. J Nutr 1972; 102: 507-514.

201. Moolten FL, Oakman NJ, Bucher NLR. Accelerated response of hepatic DNA synthesis to partial hepatectomy in rats pretreated with growth hormone or surgical stress. Cancer Res 1970; 30: 2353-2357.

202. Moreno AH. Studies on the outflow tracts of the liver. II. On the outflow tracts of the canine liver with particular reference to its regulation by the hepatic vein sphincter mechanisms. Ann Surg 1962; 155: 427-432.

203. Morgan GR. Comparison of UW vs. RL as flushing solutions in the isolated perfused rat liver. Transplantation 1990; 50: 350-351.

204. Morimoto T, Kusumoto K, Isselhard W. Impairment of grafts by short-term warm ischaemia in rat liver transplantation. Transplantation 1991; 52: 424-431.

205. Morley CGD, Kuku S, Rubenstein AH, Boyer JL. Serum hormone levels following partial hepatectomy in the rat. Biochem Biophys Res Comm 1975; 67: 653-661.

206. Nakamura T, Nawa K, Ichihara A. Partial purification and characterization of hepatocyte growth factor from serum of hepatectomized rats. Biochem Biophys Res Comm 1984; 122: 1450-1459.

207. Nakamura T, Nawa K, Ichihara H, Kaise N, Nishino T. Purification and subunit structure of hepatocyte growth factor from rat platelets. FEBS Lett 1987; 224: 311-316.

208. Nakamura T, Arakaki R, Ichihara A. Interleukin-1 is a potent growth inhibitor of adult rat hepatocytes in primary culture. Exp Cell Res 1988; 179: 488-497.

209. Nakamura T, Nishizawa T, Hagiya M, Seki T, Shimonishi M, Sugimura A, Tashiro K, Shimizu S. Molecular cloning and expression of human hepatocyte growth factor. Nature 1989; 342: 440-443.

210. Nishimura T, Yoshida Y, Watanabe F, Koseki M, Nishida T, Tagawa K, Kawashima Y. Blood level of mitochondrial aspartate aminotransferase as an indicator of the extent of ischaemic necrosis of the rat liver. Hepatology 1986; 6: 701-707.

211. Nitta N, Yamamoto S, Ozaki N, Morimoto T, Mori K, Yamaoka Y, Ozawa K. Is the deterioration of liver viability due to hepatic warm ischaemia or reinflow of pooled-portal blood in intermittent portal triad cross-clamping? Res Exp Med 1988; 188: 341-350.

212. Nolan JP. Endotoxin, reticuloendothelial function, and liver injury. Hepatology 1981; 1: 458-465.

213. Nolan JP. Intestinal endotoxins as mediators of hepatic injury - an idea whose time has come again. Hepatology 1989; 10: 887-891.

214. Nordlinger B, Douvin D, Javaudin L, Bloch P, Aranda A, Boschat M, Huguet C. An experimental study of survival after two hours of normothermic hepatic ischaemia. Surg Gynecol Obstet 1980; 150: 859-864.

215. Olcay I, Kitihama A, Miller RH, Drapans T, Trejo RA, Di Luzio NR. Reticuloendothelial dysfunction and endotoxaemia following portal vein occlusion. Surgery 1974; 75: 64-70.

216. Oloffson P, Nylander G, Olsson P. Endotoxin - transport routes and kinetics in intestinal ischaemia. Acta Chir Scand 1985; 151: 635-639.

217. Otte JB, Yandza T, De Ville D, Goyet J. Paediatric liver transplantation: Report on 52 patients with a two year survival of 86%. J Ped Surg 1988; 23: 250-253.

218. Otte JB, De Ville De J, Alberti D, Palladur D, De Hemptinne B. The concept and technique of the split liver in clinical transplantation. Surgery 1990; 107: 605-611.

219. Otto G, Wolff H, David H. Preservation damage in liver transplantation: electron-microscopic findings. Transplant Proc 1984; 17: 1247-1247.

220. Pachter HL. Inflow occlusion in hepatic trauma. Ann Surgery 1979; 190: 423-428.

221. Parks DA, Granger DN. Ischaemia-reperfusion injury: a radical view. Hepatology 1988; 8: 680-682.

222. Peignoux M, Bernuau J, Benhamou JP. Total hepatectomy and hepatic vascular exclusion in the rat: a comparison, with special reference to the influence of body temperature. Clin Sci 1982; 62: 273-277.

223. Peterson DA, Kelly B, Gerrard JM. Allopurinol can act as an electron transfer agent. Is this relevant during reperfusion injury? Biochem Biophys Res Comm 1986; 137: 76-79.

224. Pichlmayr R, Bretschneider HJ, Kirchner E, Ringe B, Lamesch P, Gubernatis G, Hauss J, Niehaus KJ. *Ex situ* operation an der leber. Eine neue moglickeit in der leberchirurgie. Langenbecks Arch Chir 1988; 373: 122-126.

225. Pichlmayr R, Ringe B, Gubernatis G, Hauss J, Bunzendahl H. Transplantation einer Spenderleber auf zwei Empfanger (Splitting-Transplantation): Eine neue Methode in der Weiterentwicklung der Lebersegmenttransplantation. Langenbecks Arch Chir 1988; 373: 127-130.

226. Pichlmayr R, Grosse H, Hauss J, Gubernatis G, Lamesch P, Bretschneide HJ. Technique and preliminary results of extracorporeal liver surgery (bench procedure) and of surgery on the in situ perfused liver. Br J Surg 1990; 77: 21-26.

227. Pober JS. Cytokine-mediated activation of vascular endothelium. Am J Pathol 1988; 133: 426-433.

228. Pober JS, Cotran RS. The role of endothelial cells in inflammation. Transplantation 1990; 50: 537-544.

229. Polimeno L, Azzarone A, Dell'Aquilla P, Amoruso C, Barone M, Angelini A, van Thiel D H, Francavilla A. Relationship between plasma and hepatic cytosolic levels of ornithine decarboxylase (ODC) and thymidine kinase (TK) in 70% hepatectomised rats. Dig Dis Sci 1991: 36; 289-292.

230. Poso H, Pegg AE. Effect of alpha-diflouromethylornithine on polyamine and DNA synthesis in regenerating rat liver. Reversal of inhibition of DNA synthesis by putrescine. Biochim Biophys Acta 1982; 696: 179-186.

231. Prakash A, Tanga MC. Hepatic artery ligation. An experimental study. Int Surg 1968; 50: 167-174.

232. Price JB, Voorhees AB, Britton RC. Partial hepatic autotransplantation with complete revascularization in the dog. Arch Surg 1967; 95: 59-64.

233. Pringle JH. Notes on the arrest of hepatic haemorrhage due to trauma. Ann Surg 1908; 48: 541-549.

234. Raab KH, Webb TH. Inhibition of DNA synthesis in regenerating rat liver by hydrocortisone. Experientia 1969; 25: 1240-1247.

235. Rabes HM. Kinetics of hepatocellular proliferation as a function of the microvascular structure and functional state of the liver. In: Hepatotrophic Factors; Ciba Foundation Symposium 55, Holland: Elsevier 1978: 31-59.

236. Rabes HM, Brandle H. Synthesis of RNA, protein and DNA in the liver of normal and hypophysectomised rats after partial hepatectomy. Cancer Res 1969: 29; 817-822.

237. Rabes HM, Iseler G, Czichos S, Tuczek HV. Synchronization of hepatocellular DNA synthesis in regenerating rat liver by continuous infusion of hydroxyurea. Cancer Res 1977: 37; 1105-1111.

238. Rabes HM, Tuczek HV. Zellproliferation in der partiell resezierten Rattenleber nach akuter intoxikation durch allylformat. Beitr Path Anat 1971: 143; 14-19.

239. Rabes HM, Wirsching R, Tuczec H, Iseler G. Analysis of cell cycle compartments of hepatocytes after partial hepatectomy. Cell Tissue Kinet 1976; 9: 517-532.

240. Raffucci FL, Wangensteen OH. Tolerance of dogs to occlusion of entire afferent vascular inflow to the liver. Surg Forum 1951; 1: 191-194.

241. Raia S, Nery JS, Mies S. Liver transplantation from live donors. Lancet 1989; 2: 497-497.

242. Raper SE, Burwen SJ, Barker ME, Jones AL. Translocation of epidermal growth factor to the hepatocyte nucleus during rat liver regeneration. Gastroenterology 1987; 92: 1243-1250.

243. Rappaport A M. The microcirculatory acinar concept of normal and pathological hepatic structure. Beitr Pathol 1976: 157; 215-243.

244. Rasmussen TN, Jorgenson PE, Almdal T, Poulsen SS, Olsen PS. Effect of gastrin on liver regeneration after partial hepatectomy in rats. Gut 1990; 31: 92-95.

245. Richman RA, Claus TH, Pilkis SJ, Friedman DL. Hormonal stimulation of DNA synthesis in primary cultures of adult rat hepatocytes. Proc Natl Acad Sci USA 1976; 73: 3589-3593.

246. Ritossa F. A new puffing induced by temperature shock and DNP in Drosophila. Experientia 1962; 18: 571-573.

247. Roncone A, Pienaar H, Mahlati G, Rose-Innes C, McLeod H, Kahn D, Hickman R. Ex vivo versus in situ resection of segmental liver grafts in pigs - a comparison in immediate or 4 hour stored grafts. Transplantation (in press).

248. Rossi G, De Carlis L, Doglia M, Fassati LR, Tarenzi L, Galmarini D. Orthotopic transplantation of partially hepatectomized liver in the pig. Transplantation 1987; 43: 362-365.

249. Rubin RA, O'Keefe EJ, Earp HS. Alteration of epidermal growth factor-dependant phosphorylation during rat liver regeneration. Proc Natl Acad Sci USA 1982; 79: 776-780.

250. Russell D, Snyder SH. Amine synthesis in rapidly growing tissues: ornithine decarboxylase activity in regenerating rat liver, chick embryo, and various tumors. Proc Natl Acad Sci 1968; 60: 1420-1427.

251. Russell WE, Bucher NLR. Vasopressin modulates liver regeneration in the Brattleboro rat. Am J Physiol 1983; 245: G321-G324.

252. Russell WE. Transforming growth factor beta (TGF-beta) inhibits hepatocyte DNA synthesis independantly of EGF binding and EGF receptor autophosphorylation. J Cell Physiol 1988; 135: 253-261.

253. Russell WE, Coffey RJ, Ouellette AJ, Moses HL. Type beta transforming growth factor reversibly inhibits the early proliferative response to partial hepatectomy in the rat. Proc Natl Acad Sci USA 1988; 85: 5126-5130.

253. Rymsa B, Wang J, De Groot H. O2- release by activated Kupffer cells upon hypoxia-reoxygenation. Am J Physiol 1991; 261: G602-G607.

254. Sakr MF, Zetti GM, Farghali H, Hassanein TH, Gavaler JS, Starzl TE, Van Thiel DH. Protective effect of FK 506 against hepatic ischaemia in rats. Transplant Proc 1991; 23: 340-341.

255. Sakr MF, Zetti GH, Hassanein TI, Garghali H, Nalesnik MA, Gavaler JS, Starzl TE, van Thiel DH. FK 506 ameliorates the hepatic injury associated with ischemia and reperfusion in rats. Hepatology 1991: 13; 947-951.

256. Sand TE, Thoresen GH, Refnes M, Christoffersen T. Growth regulatory effects of glucagon, insulin and epidermal growth factor in cultured hepatocytes. Dig Dis Sci 1992: 37; 84-92.

257. Schiff M. Sur une nouvelle fonction du foie et des effets de la ligature de la veine porte (quoted by Johnstone). Arch Sc Physiques et Naturelles de Geneve 1877; 58: 293.

258. Seglen PO. Preparation of isolated rat liver cells. Methods Cell Biol 1976; 13: 29-83.

259. Selden AC. Growth factors and the liver. Gut 1991; 32: 601-603.

260. Serafin D, Stone HH, Kolb LD, Martin JD Jr. Alterations in reticuloendothelial functions as produced by the administration of bacterial toxins. Am Surg 1968; 34: 714-716.

261. Shaw BW. Venous bypass in clinical liver transplantation. Ann Surg 1984; 200: 524-534.

262. Shi YE, Yager JD. Effects of the liver tumor promoter ethinyl estradiol on epidermal growth factor-induced DNA synthesis and epidermal growth factor receptor levels in cultured rat hepatocytes. Cancer Res 1989; 49: 3574-3580.

263. Sigel B, Baldia LB, Brightman SA , Dunn MR, Proce RIM. Effect of blood flow reversal in liver autotransplantation upon the site of hepatocyte regeneration. J Clin Invest 1968: 47; 1231-1237.

264. Sigel B, Baldia LB, Dunn MB, Menduke H. Humoral control of liver regeneration. Surg Gynecol Obstet 1967; 124: 1023-1031.

265. Siimes MA, Dallman PR. Nucleic acid and polyamine synthesis in the rat during short-term protein deficiency: responsiveness of the liver to partial hepatectomy. J Nutrition 1974; 104: 47-58.

266. Simek J, Sobotka L, Cervinkova Z, Smejkalova J. The stimulatory effect of E coli endotoxin on DNA synthesis in regenerating rat liver. Sb Ved Pr Lek Fak Univ Karlovy Hradci Kralove 1978; 21: 589-593.

267. Singer PA, Lantos JD, Whitington PF, Broelsch CE, Siegler M. Equipoise and the ethics of segmental liver transplantation. Clin Res 1988; 36: 539-545.

268. Singer PA, Siegler M, Whitington PF, Lantos JD, Emond JC, Thistlethwaite JR, Broelsch CE. Ethics of liver transplantation with living donors. NEJM 1989; 321: 620-622.

269. Smith B. Segmental liver transplantation from a living donor. J Ped Surg 1969; 4: 126-132.

270. Southard JH. Advances in organ preservation. Transplant Proc 1989; 21: 1195-1196.

271. Sporn MB, Roberts AB. Transforming growth factor-beta: new chemical forms and new biological roles. Biofactors 1988; 1: 89-93.

272. Starzl TE, Francavilla A, Halgrimson CG, Francavilla FR, Porter KA, Brown TH, Putnam, CW. The origin, hormonal nature, and action of hepatotrophic substances in portal venous blood. Surg Gynecol Obstet 1973; 137: 179-199.

273. Starzl TE, Porter KA, Kashiwagi N, Putnam CW. Portal hepatotrophic factors, diabetes mellitus and acute liver atrophy, hypertrophy and regeneration. Surg Gynecol Obstet 1975; 141: 843-858.

274. Starzl TE, Porter KA, Kashiwagi N, Lee IY, Russell WJI, Putnam CW. The effect of diabetes mellitus on portal blood hepatotrophic factors in dogs. Surg Gynecol Obstet 1975; 140: 549-562.

275. Starzl TE, Terblanche J. Hepatotrophic factors. In: Popper H, Schaffner F, eds. Progress in liver diseases. New York: Grune and Stratton, 1979: 135-151.

276. Starzl TE, Iwatsuki S, Van Thiel DH. Evolution of liver transplantation. Hepatology 1982; 2: 614-636.

277. Steffen R, Ferguson DM, Krom RAF. A new method for orthotopic liver transplantation with arterial cuff anastomosis to the recipient common hepatic artery. Transplantation 1989; 48: 166-168.

278. Stenger RJ, Confer DB. Hepatocellular ultrastructure during liver regeneration after subtotal hepatectomy. Exp Molec Path 1966; 5: 455-474.

279. Stewart JD, Potter WH, Hubbard RS, Andersen MN. Potassium movement in acute liver damage. Ann Surg 1953; 138: 593-599.

280. Stirling GA, Laughlin J, Washington SLA. The effects of starvation on the proliferative response after partial hepatectomy. Exp Molec Pathol 1973; 19: 44-52.

281. Stowell RE. Nucleic acids and cytologic changes in regenerating rat liver. Arch Pathol 1948; 46: 164-178.

282. Strong RW, Lynch SV, Ong TH, Matsunami H, Koido Y, Balderson GA. Successful liver transplantation from a living donor to her son. NEJM 1990; 322: 1505-1507.

283. Stryer L. Part IV. Information: storage, transmission, and expression of genetic information. In: Stryer L, ed. Biochemistry. San Francisco: WH Freeman and Co., 1975: 555-692.

284. St. Hilaire RJ, Jones AL. Epidermal growth factor: its biologic and metabolic effects with emphasis on the hepatocyte. Hepatology 1982; 2: 601-613.

285. Sugino K. The role of lipid peroxidation in endotoxin-induced hepatic damage and the protective effect of antioxidants. Surgery 1987; 101: 746-752.

286. Sumimoto R, Shinomiya T, Yamaguchi A. Influence of hepatic arterial blood flow in rats with liver transplants. Examination of donor liver-derived serum class I MHC antigen in rats with liver transplants with or without hepatic arterial reconstruction. Transplantation 1990; 51: 1138-1139.

287. Sumimoto R, Yamaguchi A, Teramoto K, Matsuda M, Yamadera H, Ishii E, Kamada N. Lack of effect of portal diversion on the outcome of liver allograft in rats. Transplantation 1990; 50: 893-895.

288. Sumimoto R, Goto S, Kamada N. A rat liver preservation experiment. Transplantation 1990; 50: 178-179.

289. Takei Y, Gao W, Hijioka T, Savier E, Lindert KA, Lemasters JJ, Thurman RG. Increase in survival of liver grafts after rinsing with warm Ringer's solution due to improvement of hepatic microcirculation. Transplantation 1991; 52: 225-230.

290. Teramoto K, Shimizu K, Tsukada K, Kamada N. DNA synthesis in hepatocytes during liver allograft rejection in rats. Transplantation 1990; 50: 199-201.

291. Terblanche J, Porter KA, Starzl TE, Moore J, Patzelt L, Hayashida N. Stimulation of hepatic regeneration after partial hepatectomy by infusion of a cytosol extract from regenerating dog liver. Surg Gynecol Obstet 1980; 151: 538-544.

292. Terblanche J, Krige JEJ, Bornman PC. Simplified hepatic resection with the use of prolonged vascular inflow occlusion. Arch Surg 1991; 126: 298-301.

293. Thurman RG, Marzi I, Seitz G, Thies J, Lemasters JJ, Zimmerman F. Hepatic reperfusion injury following orthotopic liver transplantation in the rat. Transplantation 1988; 46: 502-506.

294. Tissieres A. Summary. In: Heat shock: from bacteria to man. Cold Spring Harbor: Cold Spring Harbor Laboratory Press. 1982; 419-431.

295. Van Thiel DH, Gavaler JS, Kam I, Francavilla A, Polimeno L, Schade RR, Smith J, Diven W. Rapid growth of an intact human liver transplanted into a recipient larger than the donor. Gastroenterology 1987; 93: 1414-1419.

296. Van-Hoorn Hickman R, Kahn D, Green J, MacLeod H, Terblanche J. Is there a regeneration stimulator substance in the effluent from perfused partially hepatectomized livers? Hepatology 1981; 1: 287-293.

297. Vintermeyr OK, Doskeland SO. Cell cycle parameters of adult rat hepatocytes in a defined medium. A note on the timing of nucleolar DNA replication. J Cell Physiol 1987; 132: 12-21.

298. Weinbren K. The portal blood supply and regeneration of the rat liver. Br J Exp Pathol 1955; 36: 583-591.

299. Weinbren K, Stirling GA, Washington SLA. Development of a proliferative response in liver parenchyma deprived of portal blood flow. Br J Exp Pathol 1972; 53: 54-58.

300. Weinbren K, Woodward E. Delayed incorporation of 32P from orthophosphate into deoxyribonucleic acid of rat liver after subtotal hepatectomy. Brit J Exp Pathol 1964: 5; 442-449.

301. Wexler M, Slapak M, Mizumoto R, Latzina A, Giles G, Soto E, McDermott WV. Regeneration and maintenance of integrity of canine liver. Arch Surg 1970; 101: 267-276.

302. Winner BJ. Analysis of variance. Statistical principles in experimental design. Kogahusha: McGraw-Hill, 1971: 149-257.

303. Wollenberg GK, Harris L, Farber E, Hayes MA. Inverse relationship between epidermal growth factor induced proliferation and expression of high affinity surface epidermal growth factor receptors in rat hepatocytes. Lab Invest 1989; 60: 254-259.

304. Yokoyama I, Todo S, Miyata T, Selby R, Tzakis AG, Starzl TE. Endotoxaemia and human liver transplantation. Transplant Proc 1989; 21: 3833-3841.

305. Younger LR, King J, Steiner DF. Hepatic proliferative response to insulin in severe alloxan diabetes. Cancer Res 1966; 26: 1408-1414.

306. Zarnegar R, Muga S, Enghild J, Michalopoulo G. NH2-terminal amino acid sequence of rabbit Hepatopoeitin A, a heparin-binding polypeptide growth factor for hepatocytes. Biochem Biophys Res Comm 1989; 163: 1370-1376.

307. Zarnegar R, Michalopoulo G. Purification and biological characterization of human Hepatopoeitin A, a polypeptide growth factor for hepatocytes. Cancer Res 1989; 49: 3314-3320.

308. Zimmerman FA, Butcher GW, Davies HS, Brons G, Kamada N, Turel O. Techniques for orthotopic liver transplantation in the rat and some studies of the immunologic responses to fully allogeneic liver grafts. Transplant Proc 1979; 11: 571-577.

309. Barbason H, Van Cantfort J. Nyctohemeral rhythms in the liver. In: Popper H, Schaffner F, eds. Progress in liver disease. New York: Grune and Stratton, 1976: 5; 136-148.

310. Van Cantfort J, Barbason H: Relation between circadian rhythms of mitotic rate and cholesterol-7 alpha-hydroxylase activity in the regenerating liver. Cell Tissue Kinet 1972: 5; 525-530.

311. Bengmark S, Hafström L. Immediate effects of short-term hepatic inflow occlusion in pigs. Acta Chir Scand 1972: 138; 597-603.