"A REVIEW OF LIVING DONOR LIVER TRANSPLANTATION – WHY IS REGENERATION MORE RAPID IN THE RECIPIENT COMPARED TO THE DONOR?"

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DISSERTATION PRESENTED FOR THE MMED (SURGERY) DEGREE

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To my wife, children and parents for their support
in all my endeavours in life

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PART 1

REVIEW OF THE LITERATURE

CHAPTER 1

LIVER TRANSPLANTATION

A) INTRODUCTION

Liver transplantation has been established as the treatment of choice for most patients with end-stage irreversible, acute and chronic liver disease and is performed on a routine basis in most major centres throughout the world, [1] with patient survival rates of approximately 85% at one year and 75% at five years after transplantation.[2]

B) HISTORY

The first attempt at liver transplantation was performed in dogs in 1955 where the auxiliary liver was engrafted heterotopically in either the pelvis or right paravertebral gutter. The portal vein was anastomosed to the inferior vena cava and the hepatic artery to the aorta or iliac artery.[3, 4] There was no immunosuppression.

Human liver transplantation

Thomas Starzl in 1963 first attempted human liver transplantation in a 3-year old boy with biliary atresia. The patient died of severe blood loss.[5] Initial results with human liver transplantation remained poor with survival rates between 0-23 days.

The management of the haemodynamic and metabolic problems by anaesthesiologists who specialize in liver transplantation, the use of modern blood component and coagulation factor replacement therapy, and improved surgical methods to control operative bleeding have improved the outcome of liver transplantation.[6, 7]

The massive blood loss that routinely characterized earlier operations to a large extent has been reversed, and liver transplantation today is associated with minimal blood loss. The introduction of the venovenous bypass has led to the development of new transplant programs since the haemodynamic crisis during the anhepatic phase of liver transplantation was addressed.[8]

Other factors which led to the improvement in the results included the improvement in the knowledge of liver anatomy, improved surgical techniques, use of more potent immunosuppressant drugs, and the management of the haemodynamic problems. There has also been improved histological monitoring for early signs of graft damage, better post operative monitoring and the introduction of a close working transplant team.

At present the reported 1 and 3 years survival rates in USA are 77% and 68% respectively and 73% and 65% in Europe.[9]

Deaths within the first 6 months are caused by primary non-function of the allograft, hepatic artery thrombosis, infection, multi-organ failure or allograft rejection. Late death is commonly caused by atherosclerotic disease or malignancy.

C) INDICATIONS FOR LIVER TRANSPLANTATION

The indications for liver transplantation are listed in Table1.1

The list of indications continues to expand, so that liver transplantation should now be considered in nearly all patients with advanced chronic liver disease and fulminant hepatic failure.

Liver diseases for which liver transplantation has been performed can be divided generally into 4 groups.

- Advanced irreversible chronic liver failure
- Fulminant hepatic failure
- Hepatic malignancies
- Inherited metabolic disorders

Some metabolic liver disease eg antitrypsin deficiency result in liver damage and the patients are transplanted for liver failure.

Other inherited disorders, such as type II hypercholesterolinaemia, the enzyme defect resides in the hepatocytes, but other end organs are damaged. In primary hyperoxaluria the patient has end stage renal failure and renal transplantation

results in damage of the renal allograft; the enzyme defect has to be reversed by a liver transplant.

The chronic liver diseases consist of the hepatocellular diseases such as auto-immune diseases, cryptogenic cirrhosis, and the cholestatic liver diseases, such as primary sclerosing cholangitis.

Liver transplantation for hepatic malignancies has been performed with better prognosis in patients with small tumours (<5cm) without vascular invasion.

One of the major problems in the management of a patient with liver disease is to determine the optimal timing of the liver transplant procedures.

TABLE 1.1: INDICATIONS FOR LIVER TRANSPLANTATION

1	**Chronic liver disease**	
	a)	Primary biliary cirrhosis
	b)	Primary sclerosing cholangitis
	c)	Chronic drug induced cholestasis and biliary cirrhosis
	d)	Chronic virus induced liver disease
	e)	Chronic drug induced liver disease
	f)	Alcoholic liver disease
	g)	Idiopathic autoimmune chronic active hepatitis and cirrhosis
	h)	Wilson disease
	i)	Congenital hepatic fibrosis
	j)	Budd chiari syndrome
	k)	Venous occlusive disease
2	**Fulminant hepatic failure**	
	a)	Viral hepatitis
	b)	Drug induced liver disease
	c)	Quinidine
	d)	Wilson disease
3	**Hepatic malignancies**	
	a)	Hepatocellular carcinoma
4	**Metabolic liver disease**	
	a)	Hemophilia A
	b)	Type ii hypercholesterolaemia
	c)	Primary hyperoxaluria type1
	d)	Antitrypsin deficiency

D) TIMING OF LIVER TRANSPLANTATION

The following parameters are used to decide when the transplant should be done in a patient with chronic liver disease.

The most common clinical indications are:

- Intractable ascites
- Severe encephalopathy
- Variceal haemorrhage
- Diminishing quality of life

Complications of severe hepatic dysfunctions, like severe ascites, variceal haemorrhage, hepatorenal syndrome, recurrent spontaneous bacterial peritonitis, severe encephalopathy and sustained severe jaundice are late manifestations of liver disease and represent an urgent consideration for liver transplantation

Recently, the model for end-stage liver disease (MELD) score was introduced for recipient selection in the United States of America with the focus on maximizing the utility of organ allocation and transplant benefit.

The adoption of this scoring system for liver allocation has been successful in implementing a system based on medical urgency rather than waiting time. It has reduced the mortality of patients on the waiting list significantly. However, despite this shift to sicker patients, there has been no difference in one year patient and graft survival after the implementation of MELD.[10, 11] It is a good predictor of pre-transplant survival but a weak predictor of post-transplant survival.[11]

Clinical and biochemical indications for liver transplantation are listed in Table 1.2.

TABLE 1.2: CLINICAL AND BIOCHEMICAL INDICATIONS FOR LIVER TRANSPLANTATION

1. **Acute liver failure**
    a. Serum bilirubin>10-20mg/dl and increasing
    b. Prothrombin time(PT) >10seconds above normal and increasing
    c. Progressive hepatic encephalopathy(coma)
2. **Chronic liver disease**.
    a. Cholestatic liver disease
        i. Bilirubin>10-15mg/dl
        ii. Intractable pruritus
        iii. Malnutrtion
        iv. Recurrent cholangitis
    b. Hepatocellular liver disease
        i. Serum albumin <2.5gm/dl
        ii. Hepatic encephalopathy
        iii. Prothrombin time >5seconds above control values and increasing
    c. Factors common to both types of liver disease
        i. Portal hypertension with bleeding from oesophageal varices
        ii. Intractable ascites
        iii. Recurrent spontaneous bacterial peritonitis
        iv. Hepatorenal syndrome
        v. Recurrent episodes of biliary sepsis
        vi. Development of hepatocellular carcinoma

E) CONTRAINDICATIONS TO LIVER TRANSPLANTATION

The contraindications to liver transplantation are listed in Table 1.3

At present, the absolute contraindications to liver transplantation are extrahepatic malignancy, septicaemia and severe underlying cardiopulmonary or systemic disease.[12,13] Interestingly, human immuno deficiency virus (HIV) infection is no longer an absolute contraindication to liver transplantation provided strict criteria for disease stage are fulfilled. Early results of liver transplantation in HIV-positive patients are encouraging.[14, 15]

Relative contraindications include; prior extensive abdominal surgery, portal vein thrombosis, HBsAg and HBeAg positivity, and hepatoma greater than 5cm.[12]

TABLE 1.3: CONTRAINDICATIONS TO LIVER TRANSPLANTATION

1. **Absolute contraindications**
    a. Active sepsis outside the hepatobiliary tree
    b. Malignancy outside the liver
    c. Advanced cardiopulmonary disease
    d. Acquired Immune Deficiency Syndrome (AIDS)
2. **Relative contraindications**
    a. Portal vein thrombosis
    b. Cholangiocarcinoma
    c. Age greater than 60 years
    d. HBsAg and HBeAg positivity
    e. Prior Porto systemic shunt surgery.

F) SURGICAL TECHNIQUE

Technical considerations for living donor liver transplantation (LDLT) are total hepatectomy with caval preservation, and to achieve as much length on the donor vessels as safely as possible. The recipient explant can be performed without venovenous bypass or portocaval decompression.[16]

The complete transplant procedure is composed of 4 main stages:

- Donor surgery
- Recipient surgery
- Implantation of the graft
- Haemostasis and bile duct reconstruction

i) Donor surgery

This could either be (1) Right lobectomy (2) Left lobectomy or (3) Left lateral segmentectomy. For the right lobectomy, the right lobe is mobilised with transection of short hepatic veins. Those along the left margin of the hepatic inferior vena cava are left intact.

The vessels are isolated by dissection of the hepatic hilum. The right portal branch and right hepatic artery are clamped for a short time to produce the discoloration needed for parenchymal division. Transection lines are then made by electrocautery on the surface of the liver just to the right of the discoloration line.

Parenchymal transection of the liver is performed by ultrasonic dissection without interruption of the inflow to the liver. The right hepatic duct is transected at its bifurcation prior to parenchymal transection. Perfusion of the graft is performed through the portal vein and hepatic artery using University of Winsconsin solution.

ii) Recipient surgery

Adequate exposure is usually achieved by a bilateral subcostal incision, with an upper T extension and excision of the xiphoid process. The

recipient hepatectomy usually represents the most difficult stage of the entire liver transplant procedure.

It may be difficult to dissect the structures of the hepatic hilum individually because of prior surgery or because of the massive formation of varices. Total hepatectomy is undertaken with preservation of the inferior vena cava, with or without veno-venous bypass. The hepatic artery, portal vein, suprahepatic and infrahepatic vena cava are clamped. The hepatic artery and portal vein are divided and the liver excised with the vena cava left intact. The stump of the middle and left hepatic veins are closed with a running suture.

iii) Veno-venous bypass

The most critical period of the recipient operation is the anhepatic phase, when the native liver has been removed and the inferior vena cava and portal veins have been occluded. This obligatory clamping of the splanchnic and infrahepatic venous system results in a massive sequestration of blood volume in the mesenteric venous circulation and the circulation of the lower body. Sequelae include severe oedema of the whole gastrointestinal tract, renal venous hypertension and an increase in bleeding from the thin walled venous collaterals. Other complications include volume overload, haemodynamic instability, cardiac arrhythmias and arrest on reperfusion, due to the accumulation of potassium and other toxic substances in the stagnant venous blood.

These problems were overcome by the introduction of the pump-driven veno-venous bypass system (between the left femoral/ external iliac and left axillary vein), without systemic heparinization, during the anhepatic phase of the adult recipient operation. This resulted in a significant reduction in morbidity and mortality.[17] It also made liver transplantation an option in patients, previously assessed as too unstable to qualify for the procedure. Early placement of the veno-venous bypass greatly facilitates the mobilization of the liver from the hepatic fossa and the dissection of the vena cava. Appropriate vena caval cuffs and sufficient portal vein length must be developed before the graft is brought into the field for anastomosis.

Veno-venous bypass is used selectively rather than routinely nowadays.

iv) Graft implantation

After adequate haemostasis of the liver bed has been assured, the vena cava is prepared. It is much easier to deal with small leaks at this stage, rather than at the moment of revascularization.

After the donor liver is positioned in the recipient hepatic bed, the vena caval anastomosis is performed with polypropylene sutures. Prior to completion of the anterior side of the suture line, the liver is flushed with a minimum of 250ml of a "flush out" solution to wash out the high-potassium containing preservation solution and the air entrapped inside the liver.

The portal veno-venous bypass cannula is then removed and the portal vein is trimmed to the appropriate length. The portal venous anastomosis is then performed, and the portal, infrahepatic and suprahepatic vascular clamps removed, and the liver revascularised. After controlling major leaks with interrupted sutures, the veno-venous bypass is interrupted and the remaining cannulae removed. An "expansion" or "growth factor" is used to prevent suture line stenosis, especially for the portal vein and hepatic artery.[18] The donor hepatic artery is anastomosed to the recipient hepatic artery. Bile duct anastomosis is principally duct-to-duct choledocho-choledochostomy unless there is a specific indication for a biloenteric approach. e.g. Primary sclerosing cholangitis (PSC).

G) PRESERVATION SOLUTION

Improvements that ultimately provided better survival rates following liver transplantation included the use of better preservation solutions from the original saline to Collins solutions and later of Wisconsin (gold standard) solutions, which extended the preservation time from 6 to 24 hours. The newer preservation solutions, including HTK and Celsior, have been shown to be equivalent to Wisconsin solution.

- Eurocollins Solution
- University of Wisconsin Solution

- HTK Solutions
- Celsior Solution

i) <u>Eurocollins Solution</u>

Originally Eurocollins was used as the preservation solution fluid in liver transplantation.[19, 20] With this solution, the liver could be stored for up to six hours. This covered major logistical problems in that the donor and recipient operations had to be carefully co-ordinated.

ii) <u>The University of Wisconsin solution</u>

Folkert Belzer developed the University of Wisconsin (UW) solution. This solution was first used to preserve the canine pancreas for 72 hours.[21] subsequently; successful storage of the canine liver for 24 and 48 hours in experimental liver transplantation was reported.[22] This was followed by the report in 1988 of the use of UW solution in human liver transplantation. Tolerance of cold ischaemia was significantly improved from 8 hours or less to over 20 hours with Collins solution.[23] The 12 constituents in UW solution were all added to counterbalance specific metabolic processes thought to be responsible for preservation injury. The compositions of University of Wisconsin solution are listed in table 1.4

TABLE 1.4: COMPOSITION OF UNIVERSITY OF WISCONSIN SOLUTION

Component	Amount
K-lactobionate	100mM
KH2PO4	25Mm
MgSO4	5Mm
Raffinose	30Mm
Adenosine	5Mm
Gluthathione	3Mm
Allopurinol	1Mm
Dexamethasone	16mg
Regular insulin	40u/l
PenicillinG	200,000u/l
Hydroxyl ethyl starch	50g/l
Osmolarity	320mOsm/l

Solution is brought to ph 7.4 at room temperature with NaoH.

The most important constituents of UW solution are lactobionate and raffinose as impermeants to suppress hypothermia induced tissue swelling.[23] Lactobionate is a large sized organic anion, which by nature of its size and negative charge is impermeable to most tissue membranes.

Raffinose, a trisaccharide, and hydroxyl ethyl starch, were both added for additional osmotic support and to prevent expansion of extracellular space.

The phosphate buffer was added to the solution to prevent tissue acidosis from anaerobic metabolism by the ischaemic cells.

Adenosine and phosphate were added as precursors for ATP-production as well as to support energy utilizing reactions.[24] Allopurinol and gluthathione were added to prevent toxic oxygen radical related damage.

Recently, some modifications have been made. The potassium salts have been replaced successfully by sodium salts. Hydroxyl ethyl starch has been omitted without negatively influencing the preservation injury.[25, 26]

Despite numerous possible modifications, UW solution remains currently the “gold standard” cold preservation solution, with preservation time from 18-24 hours.[27, 28, 29]

The newer preservation solutions, including HTK and Celsior, have also been shown to be equivalent to Wisconsin solution.

iii) HTK (Bretschneider) solution

Bretschneider developed this solution with a goal of protecting the myocardium during cardioplegic period.[30] The composition of Bretschneider solution are listed in table 1.5

TABLE 1.5: COMPOSITION OF BRETSCHNEIDER HTK4 SOLUTION

Component	Amount
Histidine	180mM
Histidine-Hcl	18mM
K-ketoglutarate	1mM
Tryptophane	2mM
Potassium	9Mm
Sodium	15Mm
Magnesium	4mM
Mannitol	30mM
Osmolarity	300mOsm/l
Ph	7.1

Histidine was used as a buffering agent. Ketoglutarate and tryptophane were added to deliver energy. These substances are able to produce ATP, while inhibiting glycolysis and reducing lactate production and tissue acidosis.

Potassium was added to achieve cardiac arrest, while magnesium was added to reduce calcium efflux and stabilize ionic membrane status.

Mannitol was chosen as an agent to increase the osmotic gradient and to prevent tissue oedema.

Since this solution is based on its substantial buffering capacity, high volume flush-outs are used allowing equilibration across the cell membrane.[31]

This solution was successfully used in kidney transplantation and it has also been used in liver transplantation. Several European centres have introduced HTK solution in their liver transplantation program.

iv) Celsior solution

Celsior was introduced as a new cardioplegic solution, with the aim of preventing free radical injury. This remains an important cause of early graft dysfunction. The composition of Celsior solution are listed in table 1.6

The major feature of Celsior solution is the presence of three anti-oxidants, reduced glutathione, mannitol and histidine.[32, 33, 34] Anti-oxidant effects are provided by mannitol and histidine because of their capacity to scavenge hydroxyl radicals.[33]

Celsior solution has mainly been used in cardiac surgery and cardiac transplantation.

TABLE 1.6: COMPOSITION OF CELSIOR SOLUTION

Component	Amount
Mannitol	60mM
Lactobionate	80mM
Glutamic acid	20mM
Histidine	30Mm
Reduced glutathione	3mM
Potassium	15mM
Sodium	100mM
Magnesium	13mM
Calcium	0.25mM
Chloride	41.5mM
pH (at 20C)	7.3
Osmolarity	360mOsm/l

v) Flush out solutions

Ringers lactate is suitable because of its similarity to the extracellular fluid. Several studies have shown that the use of warm ringers lactate flush has an advantage over a cold rinse.[35, 36] The use of 4% serum albumin as a rinse solution has been shown to compare favourably with the use of ringers lactate solution.[37]

vi) Carolina rinse solution

This solution was developed with the aim of minimizing reperfusion injury and improving graft survival.[38] It consists of 10 empirical components, including adenosine, nisodipine, desferrioxamine and fructose. The most essential component is adenosine, [37] which inhibits platelets aggregation, [39] improving microcirculation and conversion to ATP. This improves survival.

Clinical evidence has demonstrated that Carolina rinse solution is more effective in reducing cholestatic injury than in decreasing hepatocellular damage.[40] There are indications that this solution improves graft function and microcirculation, but its exact role in human liver transplantation is not yet known.[41]

H) IMMUNOSUPPRESSION

The development of the field of immunosuppression was critically important in liver transplantation. In 1944, Medawar showed that graft rejection is an immunological event that has both specificity and memory.[42, 43]

Immunosuppressive regimens are required to control the allogenic response in clinical liver transplantation; however they may lead to severe complications such as cardiovascular disease and infectious diseases.

In the precyclosporin era, where corticosteroids and azathioprine were used as immunosuppressants, the result was suboptimal. One year survival was 30%.

Agents used as immunossuppressants can be divided into:

Corticosteroids

Antimetabolites

Calcineurin inhibitors

Antibody inductors

Antibiotics

i) Corticosteroids

In the early days of liver transplantation, corticosteroids were the main immunosuppressive agent. Steroids remain the most frequently used immunosuppressive drug in conjuction with calcineurin inhibitors.

The mode of action of corticosteroid is non-specific and includes blocking T-cell derived and antigen presenting cell derived cytokine expression e.g. Interleukins 1-3 and 6.[44]

Steroids are used both as treatment of acute rejection, and in maintenance therapy.

Side effects include hypertension, osteoporosis and the risk of fungal and bacterial infections.

ii) Antimetabolites

The first antimetabolite used in liver transplant was Azathioprine which is an imidazolyl derivative of mercaptopurine. Its mode of action is by inhibiting the synthesis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and proteins, and inhibits the proliferation of lymphocytes.

The most recent additions to the antimetabolite arena are Mycophenolate mofetil (MMF) and Mycophenolic acid (MPA) .[45]

They both inhibit the de-novo purine nucleotide synthesis. Side effects include neutropenia and diarrhoea.[46]

iii) Calcineurin Inhibitors

The calcineurin inhibitors remain the major component of most immunossuppression protocols and include:

Cyclsporin A (CyA)

Tacrolimus (TAC)

Calne and colleagues in 1978 and 1979 first described cyclosporine A as an immunosuppressant with increased survival rate of liver transplant recipient from 45% to 70%.[47-51]

Cyclosporine A causes selective suppression of cell mediated immunity via inhibition of T-cell activation. It is metabolised in the liver via cytochrome P450-3A pathway.

Side effects include nephrotoxicity, hypertension, and gingival hyperplasia.[52]

In 1984 Tacrolimus was discovered in Japan. It is 100 times more potent than CyA. It exerts its action by binding to FK binding protein which inhibits calcineurin. Side effects are similar to CyA. Including, nephrotoxicity, neurotoxicity and hyperkalaemia.[53]

Moreover, a multicenter trial has shown that liver transplant patients receiving TAC based immunosuppressant compared to CyA have a lower rate of rejection within the first year.[54]

iv) <u>Antibodies</u>

Anti-lymphocyte antibodies has been used both as part of induction therapy, as well as in the treatment of steroid resistant acute rejection.

The therapy can be seen as depleting or receptor modulating or both.

The antibodies used in liver transplantation may be monoclonal or polyclonal.

Polyclonal antibodies

These are gamma globulin fractions from animals inoculated with human lymphocytes, thymocytes or cultured lymphoblast. They causes depletion of T-cells by apoptosis, antibody mediated cytolysis and internalization of the cell surface receptors. Side effects include tachycardia, chills, gastro-intestinal distubances, bronchospasm and fluctuation of blood pressure.[46]

Monoclonal antibodies

Monoclonal anti T-cell antibodies e.g. Muromonab-CD3 (OKT3) are murine derived antibody directed to a specific portion of T-cells.

The mode of action is by binding to the CD3 antigen on the surface of T-lymphocytes resulting in rapid fall in number of mature lymphocytes.OKT3 was first used in liver transplantation in 1987 for prophylaxis against acute cellular rejection and later to reduce CNI exposure and treatment of steroid resistant rejection.[46, 55] Side effect includes; pyrexia, chills, dyspnoea, chest pain to severe life threatening pulmonary oedema.

IL-2 receptor antibodies e.g. Basiliximab, Daclizumab. They bind to the IL-2R chain. Immunosuppression is achieved by competitive antagonism of IL-2 induced T-cell proliferation. Side effects are mild gastro-intestinal disturbances.[46]

v) <u>Antibiotic</u>

Rapamycin is a macrolide antibiotic with immunosuppressive, anti-tumour and anti-fungal properties. It blocks the response of T and B cell activation by cytokines which prevents cell-cycle progression and proliferation by binding to the immunophillin FKB12. Side effects include: Leukopenia and oral ulcerations.

Most liver transplant centres use triple-drug regimen (prednisone, either cyclosporine or tacrolimus, and azathioprine or MMF). Some centres use induction immunosuppression with antilymphocyte globulin such as OKT3 (muromonab-CD3, orthodone, orthopharmaceuticals Ravitan, NJ) or Atgam (antilymphocyte globulin UpJohn Co.Kalamazoo, Michigan). Antilymphocyte induction is used to avoid the potential nephrotoxicity of either cyclosporine or tacrolimus.

The history of immunosuppressive drug regimens are shown in table 1.7

TABLE 1.7: HISTORY OF IMMUNOSUPPRESSIVE DRUG REGIMENS

Year	Agent
1962	Azathioprine.
1963	Azathioprine plus corticosteroid
1966	Polyclonal antibodies; antilymphocyte globulin
1970	Cyclophosphamide substituted for azathioprine
1978	Cyclosporine use in humans
1980	Cyclosporine plus corticosteroids
1981	Development of monoclonal antibodies
1989	Tacrolimus plus corticosteroid
1990s	Development of newer agents (e.g., rapamycin etc.)

RESULTS OF IMMUNOSUPPRESSION

The introduction of immunosuppression in liver transplantation has led to improvement in patient and graft survival. One year and 5-year survival ranges between 85% and 75% respectively.[2]

CHAPTER 2

STRATEGIES TO IMPROVE DONOR SUPPLY

A) CRITICAL SHORTAGE OF DONOR ORGANS

Unfortunately, liver transplantation has become a victim of its own success in that the supply of donor organs has not been able to keep up with the demand. The majority of donors for liver transplantation are brain-dead cadaver donors following either a severe head injury, or a massive intracranial haemorrhage. The numbers of organ donors have remained relatively stable with only minor increases in retrieval rates in recent years, in spite of many efforts to increase organ donation. This has led to an increase in the number of patients on the liver transplantation waiting list, and an increase in the number of deaths on the waiting list.

B) STRATEGIES TO IMPROVE DONOR SUPPLY

1. Reduced sized adult liver transplantation
2. Split liver transplants
3. Living donor liver transplant

The critical shortage of donor livers for transplantation has been addressed in several ways, including the use of reduced size adult livers for paediatric recipients, the use of split liver transplants, and more recently, the use of living donor liver transplants.[56] The latter strategies have been the result of a better understanding of the anatomy of the liver and knowledge of the latent capacity of the liver to undergo regeneration after partial hepatectomy.

i) Reduced-sized liver transplantation

The critical shortage of donor livers was a particular problem in paediatric population. This problem has to a large extent been overcome by the use of reduced size adult livers.

The left lateral segments, the left lobe or the right lobe of the liver are transplanted. Patient and graft survival are similar to intact liver transplantation.[57]

Success with reduced size liver transplantation led to the development of split liver technique, in which one liver is split into two functioning units and used for two recipients.[58] This technique is far more challenging since extensive reconstruction of the segmental vessels and biliary system are required. The initial poor results were due primarily to the selection of poor-risk recipients.[59] It is now performed with good result in selected experienced centres.[60, 61]

ii) Auxiliary liver transplantation

Heterotopic (auxiliary) liver transplantation has been proposed for fulminant hepatic failure with the ultimate goal to bridge the patient to allow recovery of the native liver.[62] It has also been performed for end stage chronic liver disease.[63]

A recent modification involves partial resection of the native recipient liver and implantation of part of the donor liver in the orthotopic position i.e. auxilary partial orthotopic liver transplantation (APOLT).

The main advantage of this technique is that recovery of the native liver function eliminates the need for chronic immunosuppression. The disadvantage includes a higher number of vascular complications.

iii) Living donor liver transplantation

Living donor liver transplantation (LDLT) was first performed in children with acute liver failure and is now routinely performed in most major centres with a decrease in the waiting list for children, the group in which the gap between demand and supply of livers for transplantation was most acute.[64]

The experience gained in paediatric living related liver transplantation and the encouraging results, led to the development of adult to adult LDLT which provides several advantages to the recipient when compared to the cadaver donor. Advantages of LDLT over cadaver donor include:

1) The transplant procedure can be scheduled electively.

2) Cadaveric donor factors, such as hypotensive episodes and use of pressor support are avoided with LDLT because the donor is always healthy and haemodynamically stable with good liver functions.

3) Total ischaemic time is minimized, resulting in excellent initial function of the graft and more rapid recovery of the recipient.

In some centres like Japan, where brain death laws were not established, most orthotopic liver transplants were from living related donors. Unfortunately, one third of potential living related donors are unsuitable, either because of liver problems or due to underlying medical conditions.

The risk to the donor is low. The one year survival rates for the recipients are excellent and approach 100% in elective cases, while results from procedures performed under emergency circumstances remain poor.

Donor selection

Donor safety is of prime importance in LDLT because the procedure subjects a healthy person to a major surgery and potential morbidity and mortality.[65]

1) Donors must accept the risk of surgery and should agree to donation voluntarily and without coercion.

2) Donors need to be evaluated psychologically.

3) Blood groups need to be determined. The majority of donor/recipient combinations have been A.B.O compatible, but not exclusively.

4) Meeting with the potential donor, recipient and their immediate family members is extremely important. In this meeting importance must be given about living donor liver

transplantation, including operative and post-operative complications of both the donor and recipient procedures.

5) The need for long-term follow-up of donors and recipients especially for donors of right (R) lobe grafts.

6) Associated co-morbidity of donors needs to be discussed e.g., obesity is a contra-indication along with assisted risk of hypertension, diabetes and ischaemic heart disease.

7) Hepatitis B (Hep B) surface antigen positive donors are excluded from donation.

Imaging/Radiology

Pre-operative imaging is important for donor hepatectomy in order to ensure that remnant segments are left with their inflow (arterial and portal) and drainage systems intact (veins and bile ducts). This is important when the remnant liver is small.

Moreover, anomalous hepatic anatomy, particularly arterial, is common and can be a contra-indication to donation.

The investigation of choice to assess the donor liver is a Doppler/ultrasound to evaluate any gross pathology or portal vein thrombosis.

Newer generation multi-sliced CT and three-dimensional reconstruction are used for volumetric analysis and to identify important anatomic variants.[66, 67]

Magnetic resonant imaging (MRI) also provides acceptable high resolution imaging without the added risk of radiation and contrast exposure as compared to CT.[68-70]

Both modalities can detail portal, arterial and biliary roadmaps as one-off procedures.

Angiography is another modality which is not routinely performed, but some centres still prefer conventional angiography for vascular anatomy, [71] especially if there are anatomic anomalies.

Graft Selection

There is consensus on minimum graft size requirements in living donor liver transplantation. A graft to recipient weight ration (GRWR) of more than 0.8 is suggested to provide enough liver volume for the recipient.

The term “small for size syndrome” is used to refer to the clinical consequences of using a graft that is too small. In order not to have small for size syndrome, besides calculating the graft to recipient weight ratio GRWR, the recipient’s medical condition (Child’s classification.), the recipients portal flow dynamics, and segmental outflow problems must be taken into account.

Determining the correct size of the graft is vital. Adult donors/recipients have to be well matched in terms of body mass index (BMI). In most cases when the body weight of the recipient is 30% more than the donor, it is unlikely that the donor operation will yield a graft of sufficient size and leave an adequate remnant.[72]

With respect to living donor liver transplantation (LDLT) different formulae have been devised for guidance. To predict a safe remnant volume the graft is expressed as a percentage of the standard liver volume (SLV).[73] It is generally agreed upon that between 30%-40% of the SLV is sufficiently safe.

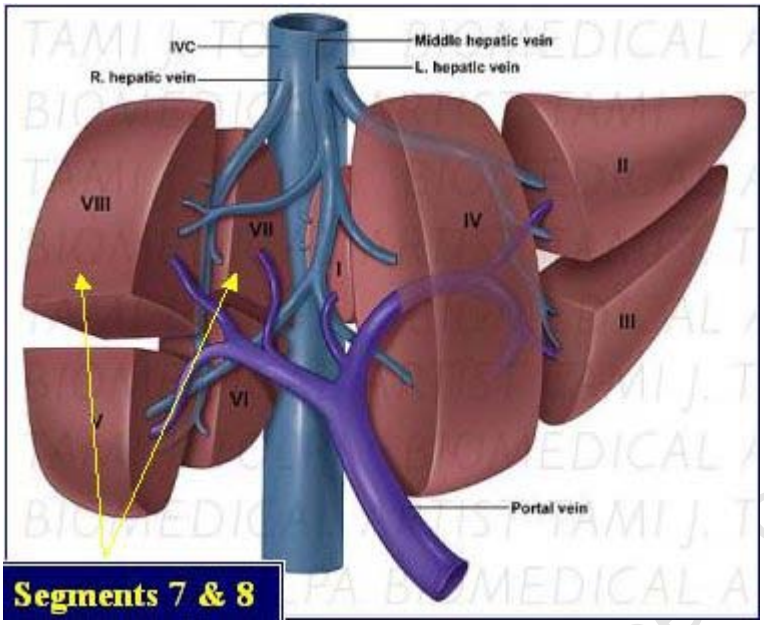
Grafts can be classified according to whether they are segmental (eg segment III) or contain multiple segments. The most common types of grafts are left lateral segmental, left lobe or right lobe, with or without the middle hepatic vein.[74] In general, left lateral grafts are for paediatric recipients, right lobe grafts for 30-60 kg recipients, and right extended lobe grafts for recipients >60kg.

Techniques to reduce small for size syndrome include portal flow reduction by shunting. Graft/recipient body weight ratio (GRWR) of >0,8-1% is acceptable. If recipients are graded Child B or C,

then a GRWR of >0.85% will be required in order to avoid small for size syndrome.[75]

$$\text{GRWR} = \frac{\text{Graft volume}}{\text{Recipient weight x 100}}$$

<u>FIGURE 2.:</u> THE LIVER EIGHT SEGMENT DIVISIONS



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Left lateral segment includes segment 2 and 3.

Left lobe includes segment 2, 3 and 4.

Right lobe includes segment 5, 6, 7 and 8.

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CHAPTER 3

LIVER REGENERATION

A) INTRODUCTION

Liver regeneration is a fascinating process that makes living related liver transplantation feasible. It is an important determinant of the success rate, especially for recipients of partial liver transplants.

B) HISTORY

The ancient Greeks were the first to recognise liver regeneration in the myth of Prometheus who was condemned to having a portion of his liver eaten by the eagles for stealing the secrets of fire from the Gods of Olympus - only to find out that the liver regenerates overnight, thus providing the eagle with eternal food and Prometheus with eternal torture.

Liver regeneration has been studied in both animals and humans. In 1931 Higgins and Anderson defined the characteristics of hepatic regeneration after 2/3rd partial hepatectomy in rats.[76] The qualitative account of the changes in rat liver weight following this procedure was described. The morphological, chemical and histochemical features following standard 2/3rd partial hepatectomy in the rat are well established. It was observed that within 18-24 hours post partial 2/3rd hepatectomy, there is restorative cell growth within the liver which is characterised by an increase in DNA synthesis and liver weight. The residual lobes almost double in size within 48 hours, and approach the original liver weight by seven days. Macroscopically, the regenerating liver appears pale and swollen during the first day due to the extensive temporary infiltrations of neutral fat. Microscopically, there are changes in the parenchymal cells at the periphery of the hepatic lobule which progresses towards the centre. Mitotic activity of hepatic cells is conspicuous during the subsequent 24-48 hours.

C) CONTROL OF LIVER REGENERATION

The liver has a latent capacity to undergo regeneration after injury or partial hepatectomy.[77] A number of mechanisms participate in the hepatic injury that occurs during and following liver transplantation. A variety of genes, cytokines, growth factors and cells are involved in liver regeneration.[78] The exact mechanism of regeneration and the interaction between cells and cytokines are not fully understood.

There seems to exist a sequence of stages that result in liver regeneration, while at the same time inhibitors control the size of the regenerated liver. Precise factors which initiate and terminate the regenerative response remain unresolved.[79, 80]

Previous studies have suggested that the regenerating liver itself may be a source of hepatotrophic factors.[81, 82] A liver cytosol prepared from regenerating liver after partial hepatectomy was able to potentiate the regenerative response, whereas cytosol from normal liver did not.[83]

Several of the immunosuppressive drugs used after liver transplantation has been shown to potentiate the regenerative response after partial hepatectomy. Both cyclosporine and tacrolimus are thought to be hepatotrophic.[84-87]

D) LIVER REGENERATION IN THE LIVING DONOR AND RECIPIENT

Liver regeneration in the recipient and in the donor after living donor liver transplantation has been studied to a limited extent. Various techniques which have been used to measure organ volume includes ultrasonography, magnetic resonance imaging, nuclear scintigraphy, conventional computed tomography CT, cine CT, and helical CT.[80, 88]

Computer tomography (CT) has been the standard method of determining liver mass. MRI has technical advantages over CT, particularly in liver donors and transplant recipients. This technique avoids potential complication associated with the use of traditional intravenous contrast agents and exposure to ionizing radiation, and can therefore be performed repeatedly with minimal risk to the patient.

Several studies have shown that the donor liver takes longer to restore liver mass than the transplanted liver in the recipient.[89, 90] In fact; liver mass in the donor has still not been restored to pre-operative size by one year after surgery.

Several factors may be responsible for this discrepancy in the regenerative response between the recipient and the donor. Firstly, the recipient has high levels of circulating hepatotrophic factors because of the liver disease. Secondly, the recipient also receives cyclosporine, which is known to be hepatotrophic in the post transplant period.[84-87] In contrast; the donor because of the normal liver preoperatively does not have any circulating hepatotrophic factors, and secondly does not receive cyclosporine post operatively.

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PART 2

THE STUDY

CHAPTER 4

AIM OF THE STUDY

A) THE PROBLEM

Living donor liver transplantation (LDLT) is now well established and performed on a routine basis in many major centres around the world. LDLT is feasible because of the capacity of both the remnant donor liver and the transplanted partial liver to undergo liver regeneration. However it has been demonstrated that liver regeneration in the recipient is rapid, whereas restoration of liver mass in the donor is delayed.[89, 90]

This discrepancy in the rate of regeneration could be due to the presence of hepatotrophic factors and the use of immunosuppression in the recipient.

B) AIM OF STUDY

The aims of the studies were to determine if hepatotrophic factors and immunosuppression (Cyclosporine) could modify the restoration of the liver mass after partial hepatectomy in rats.

CHAPTER 5

MATERIALS & METHODS

A) ANIMALS

This study was approved by the Faculty Animal Ethics Committee of the University of Cape Town. Adult male Long Evans rats weighing 200-250 grams were maintained under standard laboratory conditions and allowed *ad libitum* access to a standard rat pellet diet and water. Following an equilibration period, the rats were subjected to either standard 2/3rd partial hepatectomy (PH) or sham operation (SH). All surgical procedures were performed under light ether anaesthesia between 08h00 and 11h00. The animals were randomly allocated to the following treatment groups:

- Group 1 partial hepatectomy (PH) (n=30)
- Group 2 partial hepatectomy + cyclosporine + liver cytosol (PH + Cy + C) (n=30)
- Group 3 sham operation (SH) (n=30)
- Group 4 sham operation + cyclosporine + liver cytosol (SH + Cy + C) (n=30)

B) SURGICAL PROCEDURES:

A 2/3rd standard partial hepatectomy was performed via a midline laparotomy extending from the xiphisternum posteriorly for approximately 2 centimetres. The liver was extruded through the incision, and involved removal of the left lateral and middle lobes of the liver. The right lateral and caudate lobes were left intact. The abdominal wall and integuments were closed separately by continuous sutures. Sham operation consisted of a midline laparotomy and gentle manipulation of the liver.

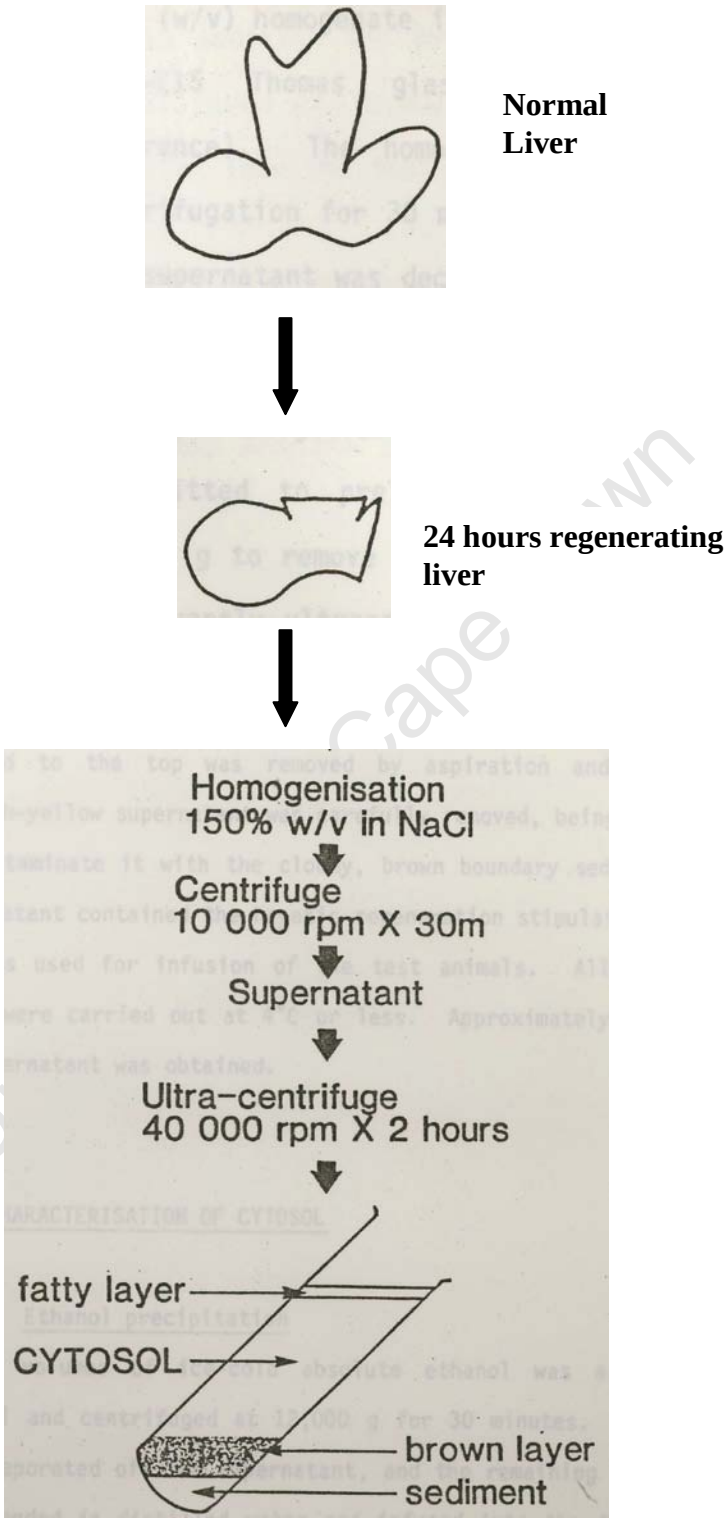
C) PREPARATION OF LIVER CYTOSOL

A separate group of 5 animals were subjected to 2/3rd partial hepatectomy and sacrificed 24 hours post-operatively. The remnant livers were removed, homogenized in 0,9 % normal saline solution with a motor driven 3431-E15 Thomas glass/Teflon tissue homogeniser. The homogenate was submitted to preliminary cold centrifugation for 30 minutes at 10,000 rpm to remove gross tissue. The supernatant was decanted and subsequently subjected to ultra centrifugation at 105,000 g for 2 hours in a Beckman model L3-65 Ultracentrifuge equipped with a type 40 rotor. This produced a multi-layered sediment and a clear reddish-yellow supernatant separated by an indistinct, cloudy boundary. The white lipid layer which floated to the top was removed and discarded, and the clear, reddish-yellow supernatant was carefully removed. The supernatant served as the liver cytosol, which contained the hepatic regeneration stimulator substance and was used for infusion of the test animals.

The steps in the preparation of liver cytosol are shown in figure 5.1.

All preparatory steps were carried out at 4^0C or less. Approximately 140-160 millilitres of supernatant was obtained.

FIGURE 5.1: PREPARATION OF LIVER CYTOSOL



D) INJECTION OF CYTOSOL

The animals in Groups 2 and 4 received a daily intraperitoneal injection of liver cytosol at a dose of 0.685 mg protein in 5 micro litres.

E) ORAL CYCLOSPORINE

The animals in Group 2 and 4 also received oral cyclosporine 4mg/kg twice daily.

F) SACRIFICE

Five animals from each of the above groups were sacrificed at 24, 48, 72 and 96 hours and at 1 and 2 weeks post-operatively. The animals were exsanguinated under ether anaesthesia via a midline laparotomy and the liver remnant removed.

G) INVESTIGATIONS

The blood specimens were used to measure the liver function tests (Biochemical).

The liver remnants were used to measure the liver weight/body weight ratio and for histological examination to determine the mitotic indices.

H) METHODS

Blood was taken from the rats at the specified times of the experiment into heparin coated tubes. The blood was spun at 3000 rpm for 30 minutes to separate the plasma from the cells. The plasma was stored at -20C until the AST and ALT assays were done using the following methods as shown in Tables 5.1 and 5.2.

TABLE 5.1: ALANINE TRANSAMINASE

Reagents:

1) Sodium dehydrogenase NADH

Mw 709.4

2) Buffer

13.6gms of Potassium phosphate KH_2PO_4
3.3gms of Sodium hydroxide N_AOH
Dissolve in distilled water and adjust the Ph to 7.4
Make up to one litre and store at 4^OC

3) L-Alanine

Dissolve 7.6gms L-Alanine in 50ml Buffer. Dissolves while adjusting pH to 7.4 with Potassium hydroxide (KOH). Make up to 100ml with Buffer. Store at -20^{o}C in 4.5ml aliquots.

6) Lactate Dehydrogenase (LDH)

25mg/2.5ml

7) Oxaloacetic Acid:

Dissolve 0.735gms Oxaloacetic Acid in 30ml Buffer.
Adjust pH to 7.4 and make up to 50ml
Store at -20^{o}C in 5ml aliquots.

Make up cocktail as follows:

36mgs Sodium dehydrogenase NADH
19ml Buffer
4.5ml L-Alanine
50ul LDH

Method:

50ul Sample
650ul Cocktail
Stand at room temperature for 15minutes to equilibrate
50ul Oxaloacetic acid
Read absorbance at 340nm on spectrophotometer every 30 seconds

Calculation:

$$\frac{\text{Total volume} \times \text{delta absorbance}}{6.22 \times 1 \times \text{sample volume}} \times \text{dilution} \quad \text{I.U./litre}$$

TABLE 5.2: ASPARTIC TRANSAMINASE

Reagents:

1) Sodium dehydrogenase NADH

   Mw 709.4

2) Buffer

   13.6gms of Potassium phosphate KH_2PO_4
   3.3gms of Sodium hydroxide N_AOH
   Dissolve in distilled water and adjust the Ph to 7.4
   Make up to one litre and store at 4^OC

3) Aspartic Acid:

   Dissolve 2.66gms Aspartic Acid in 50ml Buffer. Dissolves while adjusting pH to 7.4 with Potassium hydroxide (KOH). Make up to 100ml with Buffer. Store at -20^{o}C in 4.5ml aliquots.

4) Malate Dehydrogenase (MDA)

   25mg/2.5ml

5) Oxaloacetic Acid:

   Dissolve 0.735gms Oxaloacetic Acid in 30ml Buffer.
   Adjust pH to 7.4 and make up to 50ml
   Store at -20^{o}C in 5ml aliquots.

Make up cocktail as follows:

36mgs Sodium dehydrogenase NADH
19ml Buffer
4.5ml Aspartic Acid
50ul MDA

Method:

50ul Sample
650ul Cocktail
Stand at room temperature for 15minutes to equilibrate
50ul Oxaloacetic acid
Read absorbance at 340nm on spectrophotometer every 30 seconds

Calculation:

$$\frac{\text{Total volume} \times \text{delta absorbance}}{6.22 \times 1 \times \text{sample volume}} \times \text{dilution} \quad \text{I.U./litre}$$

LIVER WEIGHT/BODY WEIGHT RATIO:

The liver weight / body weight ratio was calculated by dividing the liver weight by the body weight at death.

MITOTIC INDEX:

The liver remnant was fixed in 10% formalin, dehydrated through 70%, 96% and 100% alcohols. Then cleared in Xylol and impregnated with wax. This was done using a Leica TP 1020 Tissue processor. These tissue samples were then embedded in wax blocks and then cut into 1-2 micron sections and fixed onto microscopes slides at 56^{o}C.

These sections were then dewaxed in xylol and rehydrated through alcohols (100% - 70%) and stained with Haematoxylin and Eosin

The dividing cells were counted in 10 fields using a microscope and the results were given as cells per 10 fields.

STATISTICAL ANALYSIS:

The statistical analysis of results was done using the Shapiro-Wilk, the T-test, Oneway analysis and Bonferroni test.

CHAPTER 6

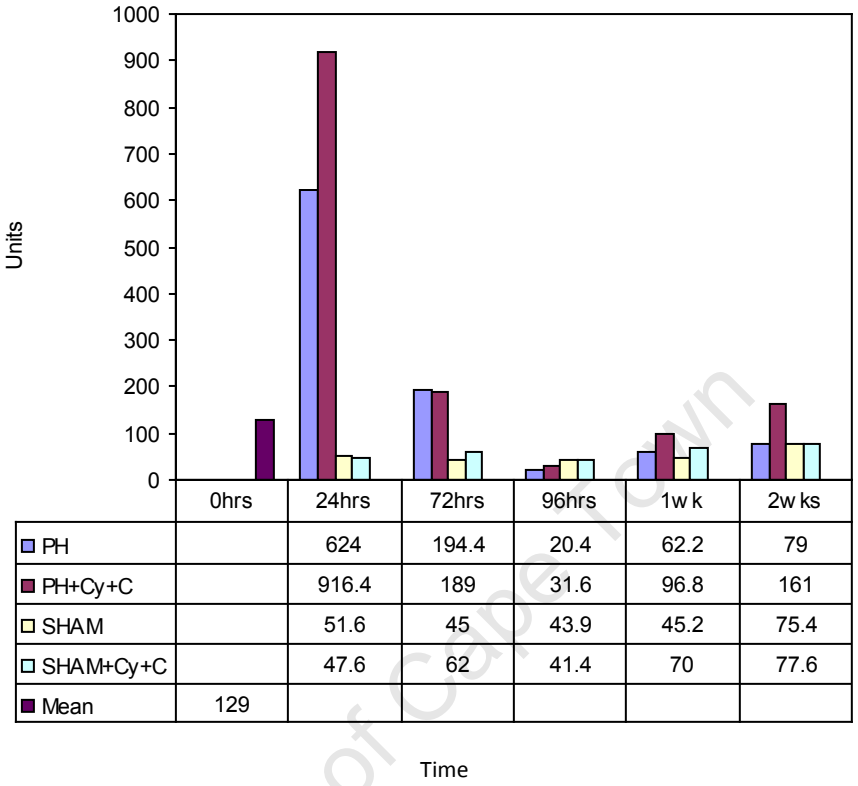
RESULTS

A) LIVER FUNCTION TEST

i) AST (IU/L)

The changes in serum aspartate transferase (AST) levels are shown in Figure 6.1. The serum AST levels remained unchanged after sham operation (Group 3). There was a significant increase in serum AST at 24 hours after partial hepatectomy (P=0.019). Thereafter, there was a gradual decrease in AST and a return to normal by 96 hours post-operatively. Serum AST levels were significantly higher at 24 hours in the animals subjected to partial hepatectomy and cytosol infusion (P=<0.001). The serum AST levels in the animals subjected to sham operation with an injection of cyclosporine and cytosol (P=0.018) were also slightly higher than after sham operation only (Group 3). AST increases after PH because of injury. AST is higher in the groups receiving cytosol because cytosol contains AST.

FIGURE 6.1: CHANGES IN SERUM ASPARTATE TRANSFERASE (AST) AFTER STANDARD 2/3 PARTIAL HEPATECTOMY AND ADMINISTRATION OF CYCLOSPORIN AND LIVER CYTOSOL

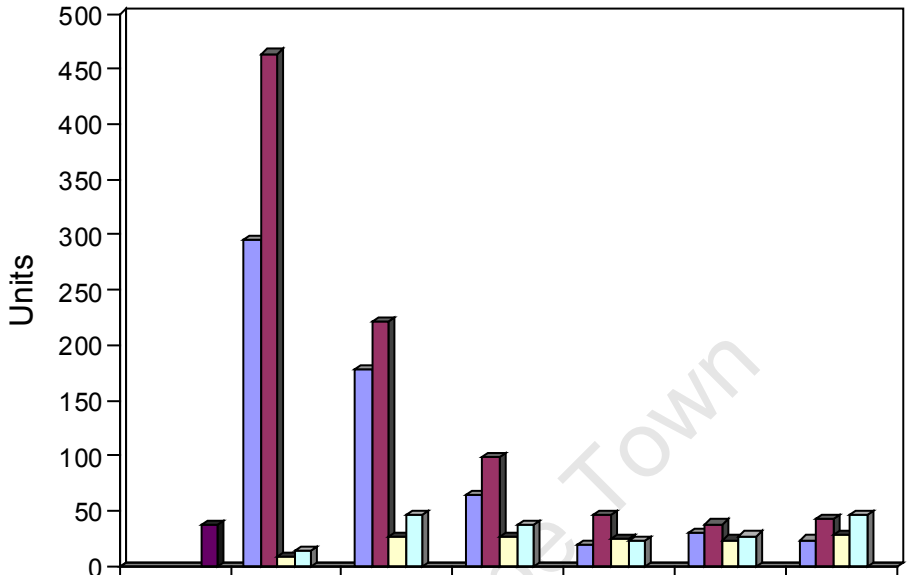


	0hrs	24hrs	72hrs	96hrs	1w k	2w ks
PH		624	194.4	20.4	62.2	79
PH+Cy+C		916.4	189	31.6	96.8	161
SHAM		51.6	45	43.9	45.2	75.4
SHAM+Cy+C		47.6	62	41.4	70	77.6
Mean	129					

ii) ALT (IU/L)

The changes in the serum alanine transferase (ALT) levels are shown in Figure 6.2. The changes in serum ALT levels were similar to the changes in the serum AST. The serum ALT levels remained unchanged after sham operation (Group 3). Statistically, there was no significant increase in serum ALT at 24 hours after partial hepatectomy (P=1.000). Thereafter there was a gradual decrease in ALT and a return to normal by 96 hours post-operatively. Serum ALT levels were significantly higher at 24 hours in the animals subjected to partial hepatectomy and cytosol infusion (P=0.008). The serum ALT levels in the animals subjected to sham operation with an injection of cyclosporine and cytosol (P=0.175) were also slightly higher than after sham operation only (Group 3). ALT increases after PH because of injury. ALT is higher in the groups receiving cytosol because cytosol contains ALT.

FIGURE 6.2: CHANGES IN SERUM ALANINE TRANSFERASE (ALT) AFTER STANDARD 2/3 PARTIAL HEPATECTOMY AND ADMINISTRATION OF CYCLOSPORIN AND LIVER CYTOSOL.

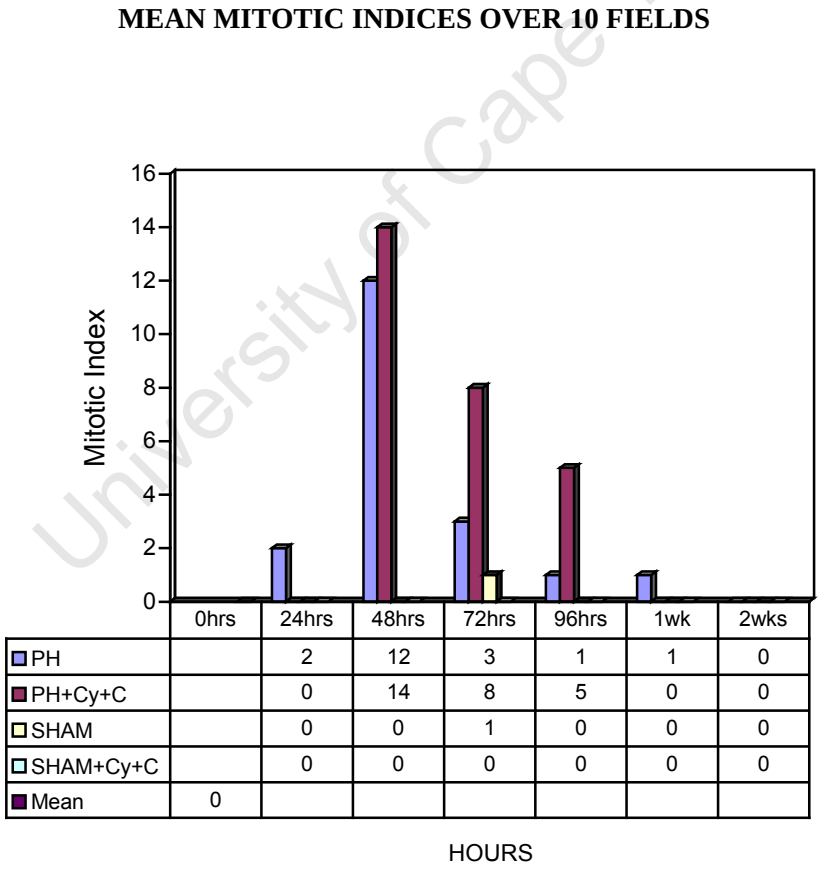


	0hrs	24hrs	48hrs	72hrs	96hrs	1wk	2wks
PH		295.4	178.6	64	19.8	30.2	24
PH+Cy+C		464.2	222	99.2	46.6	38.2	43.3
SHAM		9.2	26.8	27	24.6	23.6	27.8
SHAM+Cy+C		14.4	46	37.6	23	27.4	46.8
Mean	37						

B) MITOTIC INDEX

The changes in the mitotic indices in the four groups are shown in Figure 6.3. There was an increase in mitotic indices after partial hepatectomy at 24 hours and a further increase after 48 hours post-operatively. Thereafter the mitotic indices decreased to pre-operative levels by 2 weeks. The mitotic indices in the animals subjected to PH + Cy + C (Group 2) were higher than in the animals in group 1. There were no mitotic figures seen in the animals subjected to sham operation.

FIGURE 6.3: CHANGES IN MITOTIC INDEX OF HEPATOCYTES IN REMNANT LIVER AFTER STANDARD 2/3 PARTIAL HEPATECTOMY AND ADMINISTRATION OF CYCLOSPORIN AND LIVER CYTOSOL



	0hrs	24hrs	48hrs	72hrs	96hrs	1wk	2wks
PH		2	12	3	1	1	0
PH+Cy+C		0	14	8	5	0	0
SHAM		0	0	1	0	0	0
SHAM+Cy+C		0	0	0	0	0	0
Mean	0						

FIGURE 6.4 (a): Histological changes in the liver 24 hours after sham operation. Haematotoxylin and eosin (H&E) staining. X20

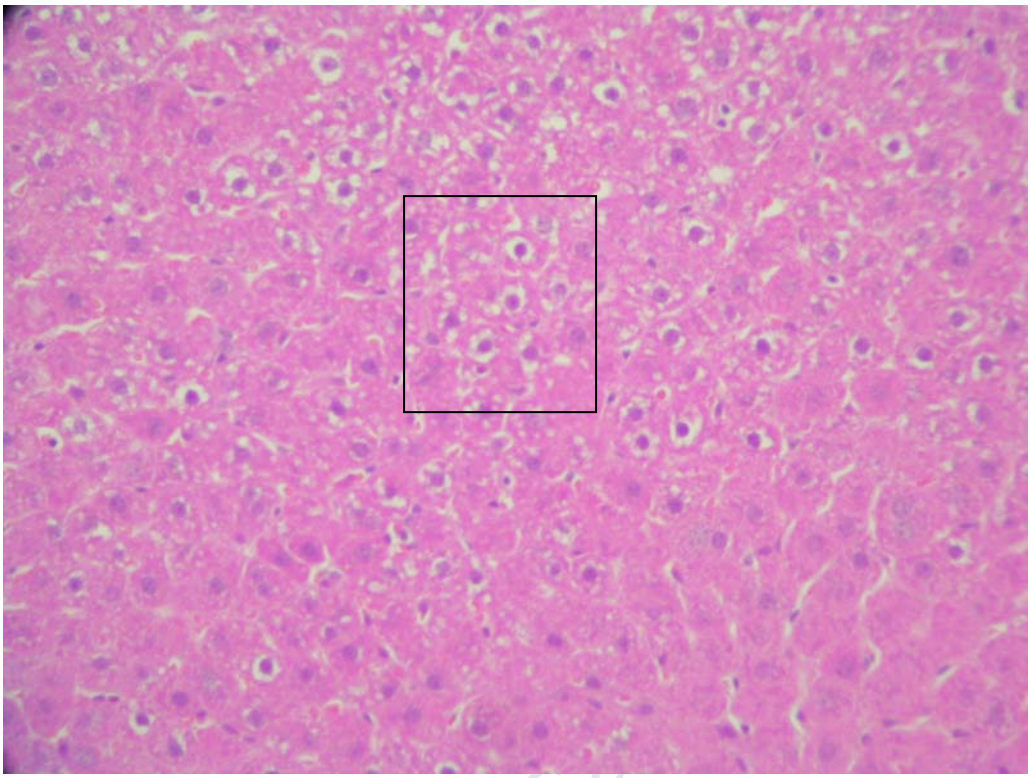


FIGURE 6.4 (b): Close-up of the boxed area in (a). No mitosis. H&E X40

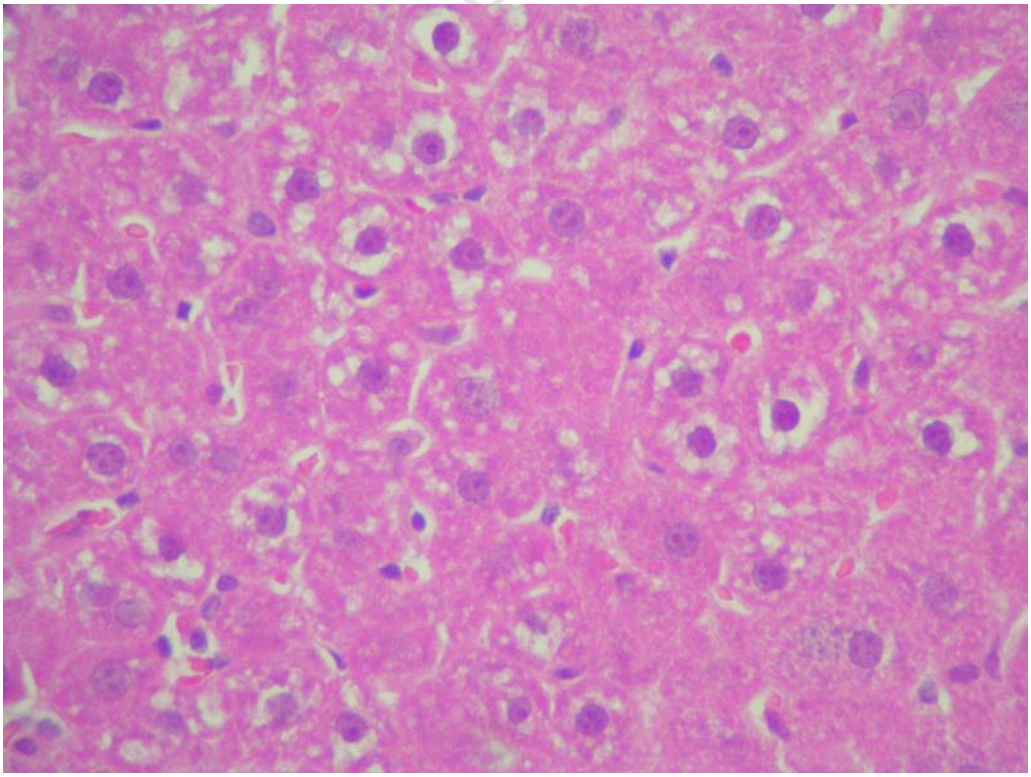


FIGURE 6.5 (a): Histological changes in the liver 24 hours after sham operation and administration of cytosol and cyclosporine. H&E X20

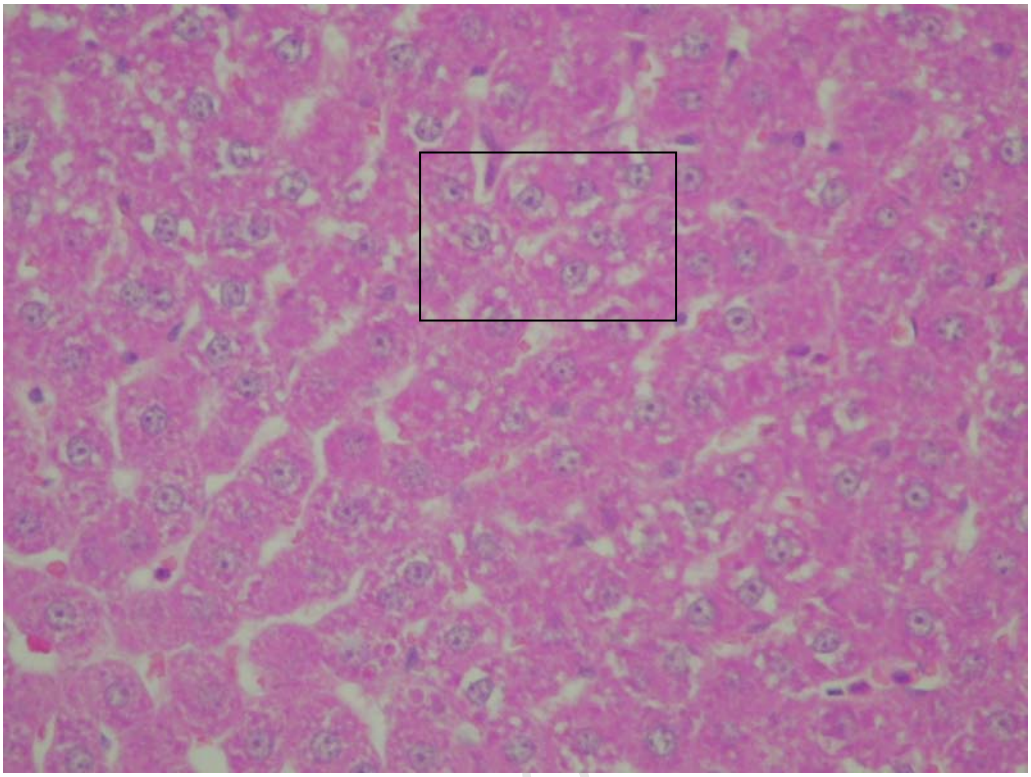


FIGURE 6.5 (b): Close-up of the boxed area in (a). No mitosis. H&E X40

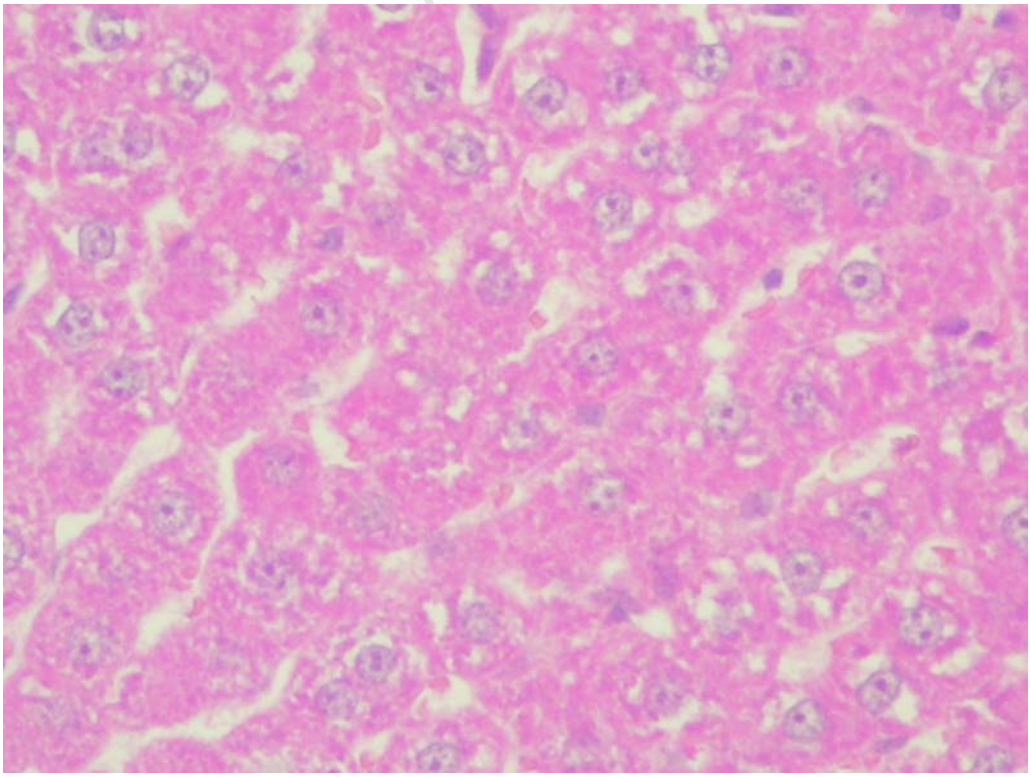


FIGURE 6.6 (a): Histological changes in the remnant liver 48 hours after standard 2/3 partial hepatectomy. H&E X20

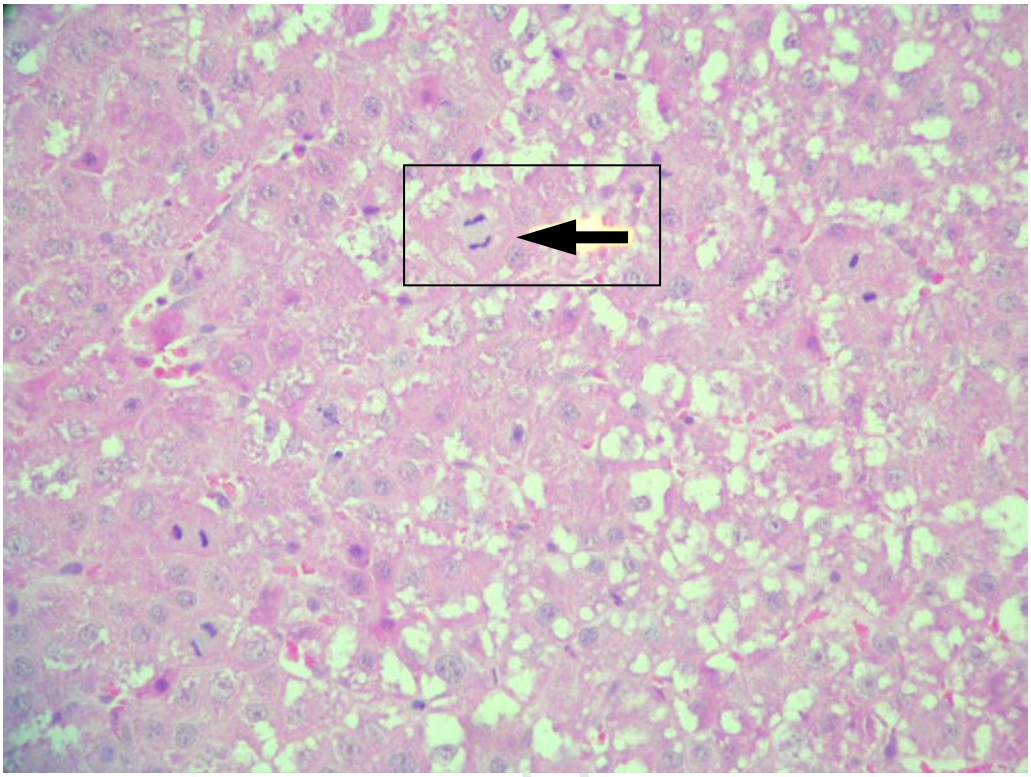


FIGURE 6.6 (b): Close-up of the boxed area in (a) showing mitosis (arrow). H&E X40.

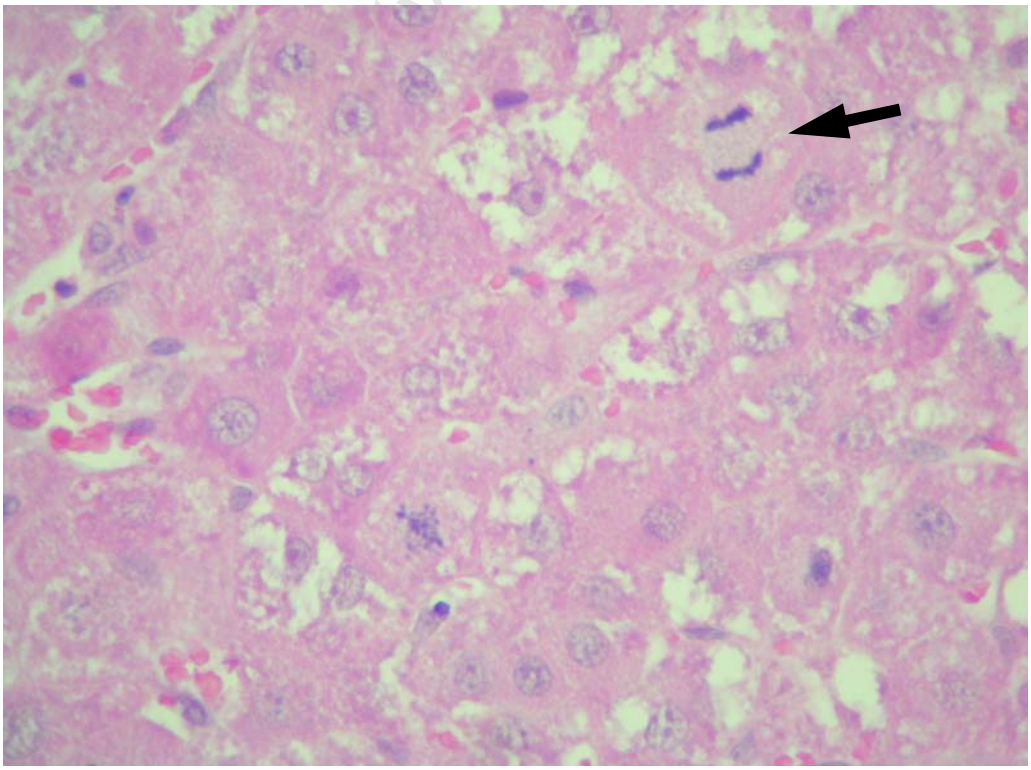


FIGURE 6.7 (a): Histological changes in the remnant liver 2 weeks after standard 2/3 partial hepatectomy. H&E X20

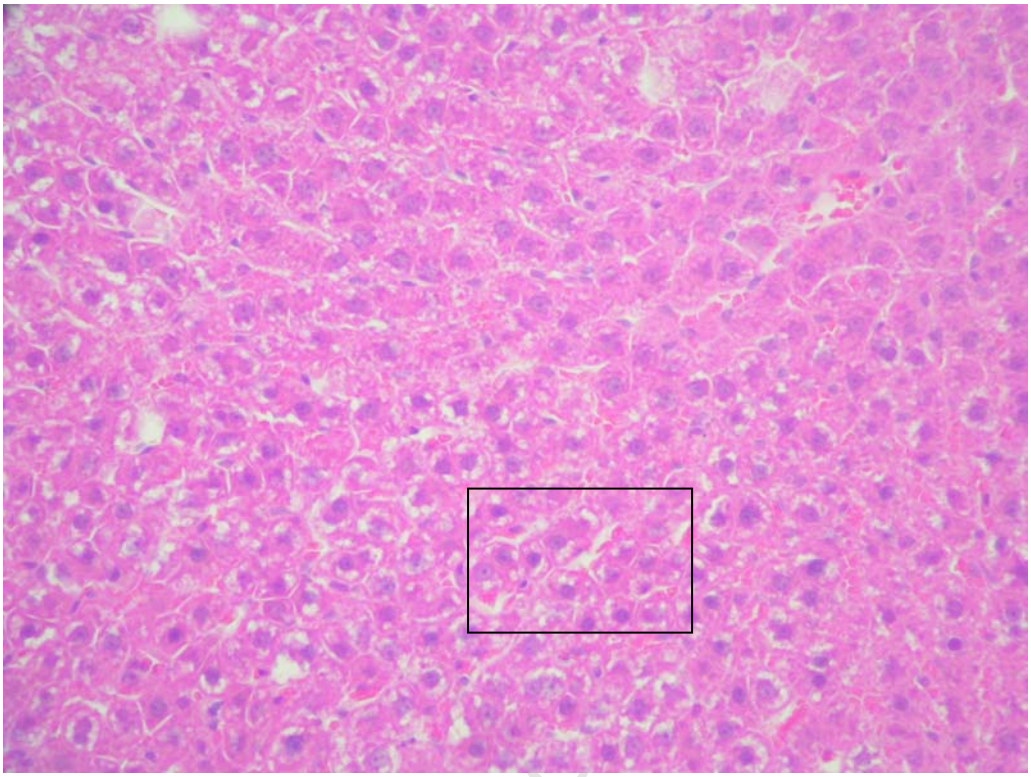


FIGURE 6.7 (b): Close-up of the boxed area in (a). No mitosis. H&E X40

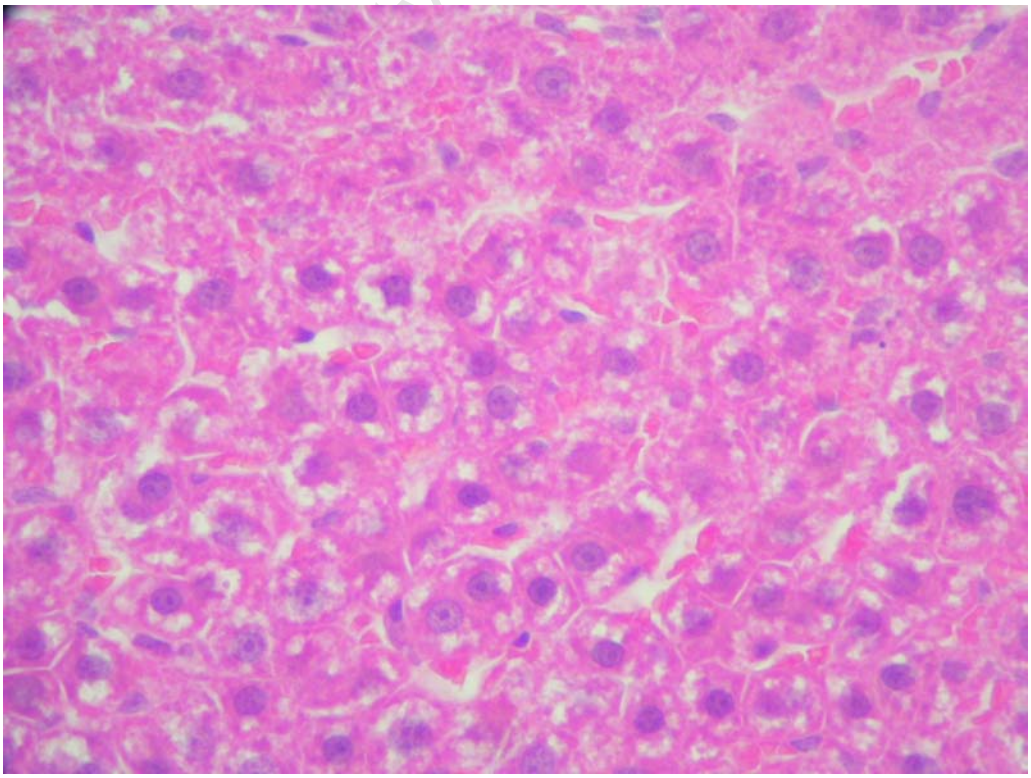


FIGURE 6.8 (a): Histological changes in the remnant liver 48 hours after standard 2/3 partial hepatectomy and administration of cyclosporine and cytosol. H&E X20

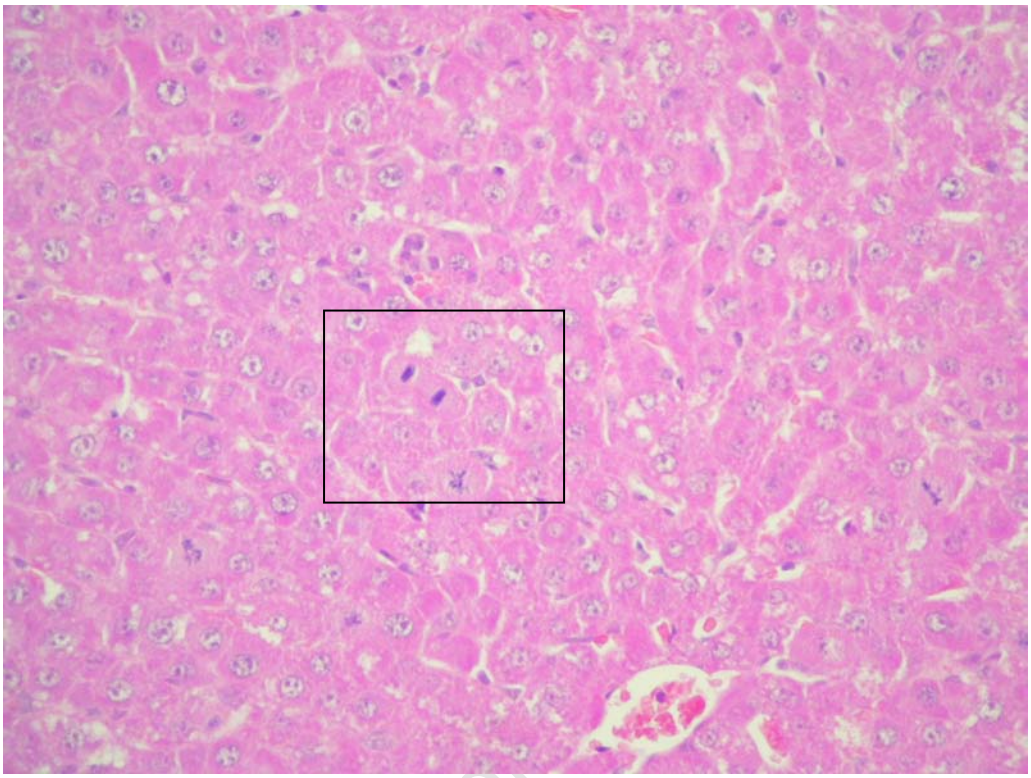


FIGURE 6.8 (b): Close-up of the boxed area in (a) showing mitosis (arrow). H&E X40.

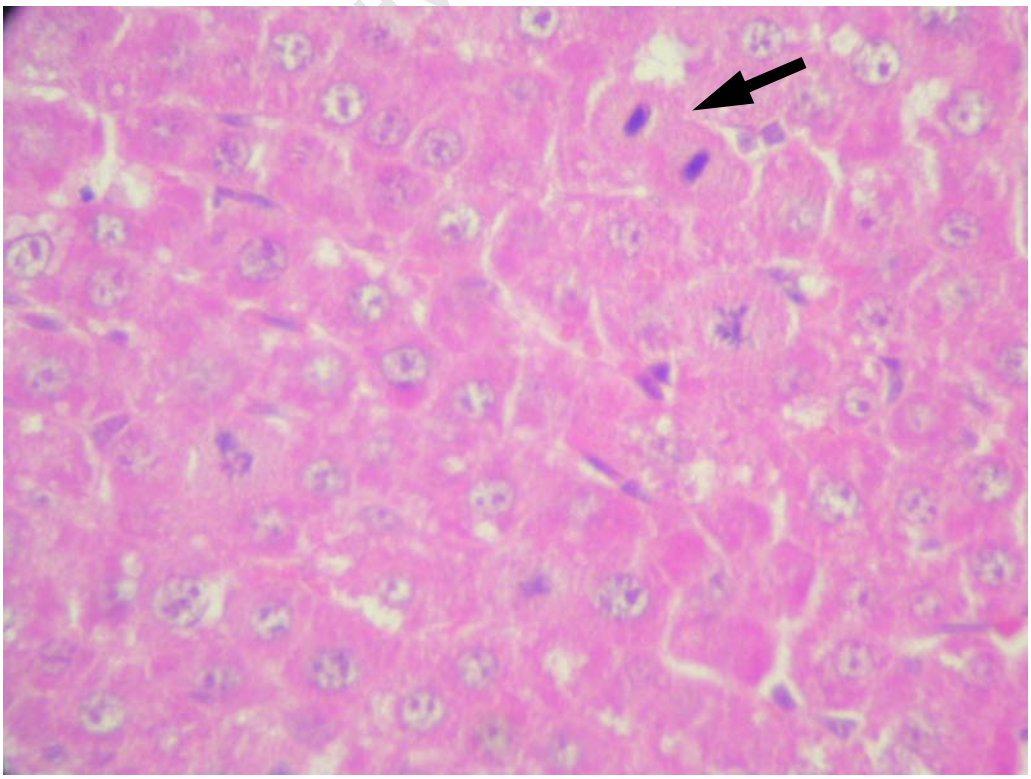


FIGURE 6.9 (a): Histological changes in the remnant liver 2 weeks after standard 2/3 partial hepatectomy and administration of cyclosporine and cytosol. H&E X20.

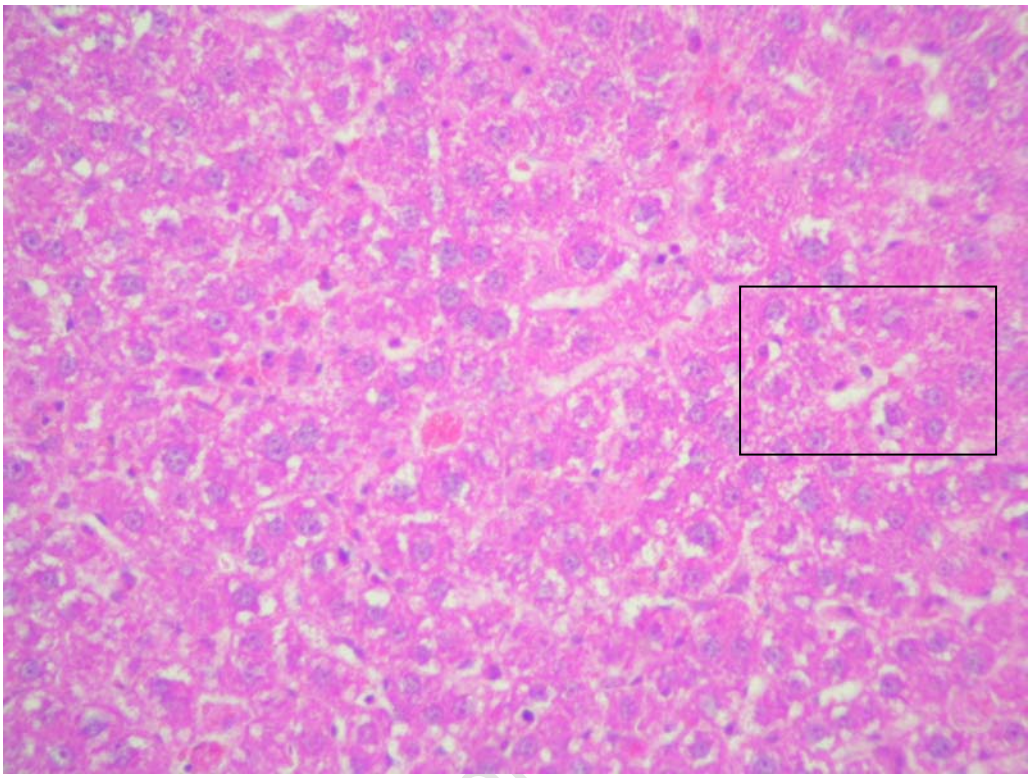
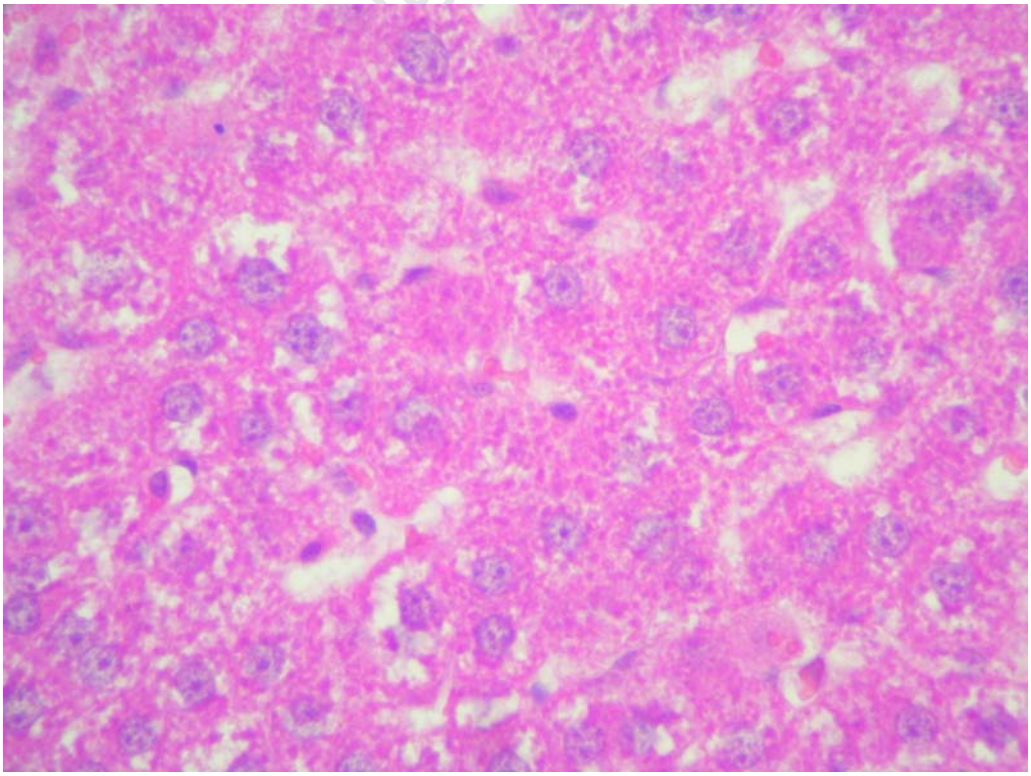


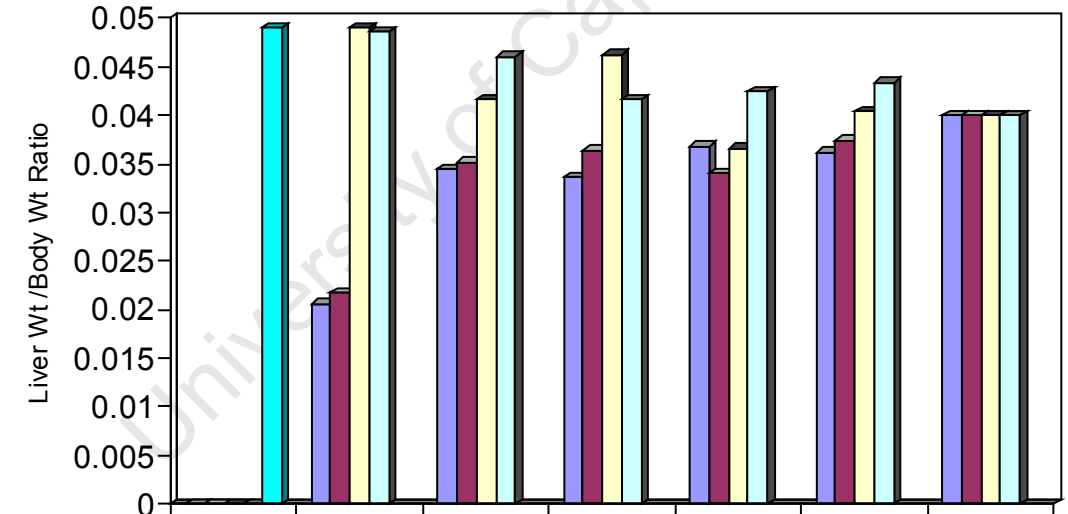
FIGURE 6.9 (b): Close-up of the boxed area in (a). No mitosis. H&E X40



C) LIVER WEIGHT BODY WEIGHT RATIO

The changes in the liver weight to body weight ratios in the animals studied are shown in Figure 6.10. There was a significant reduction in the liver weight to body weight ratio at 24 hours after partial hepatectomy (P= <0.001). This was compatible with a 2/3 partial hepatectomy. Thereafter the liver weight to body weight ratios in the animals in groups 1 and 2 increased steadily, but had still not reached pre-operative levels by 2 weeks after partial hepatectomy. Interestingly, the liver weight to body weight ratios in the animals subjected to sham operation decreased slightly over the 2 weeks. Treatment with liver cytosol or cyclosporine did not influence the liver weight to body weight ratio.

FIGURE 6.10: CHANGES IN LIVER WEIGHT BODY WEIGHT RATIO IN THE GROUPS COMPARED WITH BASELINE LIVER WEIGHT.



	0hr	24hrs	48hrs	72hrs	96hrs	1wk	2wks
PH	0	0.0206	0.03444	0.0336	0.0368	0.0362	0.04
PH+Cy+C	0	0.0217	0.03522	0.0364	0.034	0.0374	0.04
SHAM	0	0.04898	0.0416	0.0462	0.0366	0.0404	0.04
SHAM+Cy+C	0	0.0486	0.04608	0.0416	0.0424	0.0434	0.04
BLW	0.049						

In summary, AST and ALT unchanged after sham operation. AST and ALT increase after PH represents injury. The increase in AST and ALT in groups 2 and 4 related to liver enzymes in cytosol.

There were no mitotic figures seen in the animals subjected to sham operations. Increase mitotic index after PH reflect regeneration. Further increase in mitotic indices after PH + C + Cy represent increased regeneration over stimulus of PH related to hepatotrophic factors in cytosol and hepatotrophic effect of cyclosporine.

The liver weight/body weight (LW/BW) ratios increased after PH but had returned to preoperative levels by 2 weeks of which the changes were not modified by the cytosol or cyclosporine.

CHAPTER 7
DISCUSSION

Living donor liver transplantation is now performed routinely in most major centres throughout the world [1], with 1 and 5years survival rates between 85 and 75% respectively [2]. The improvement in the knowledge of liver anatomy, surgical technique, use of immunosuppressant drugs and the management of haemodynamics has resulted in an increased number of transplant centres and an increasing number of living donor liver transplantations. However, the regenerative response in the remnant liver in the donor and in the transplanted liver in the recipient has been studied previously.[89, 90] It has been noted that the restoration of liver mass in the donor takes longer than in the recipient, and that the liver volume does not return to pre-operative size by 12 months after the surgery. Several factors in the recipient may account for the discrepancy in the regenerative response compared to the donor.

Firstly, the recipient has high levels of circulating hepatotrophic factors because of the liver disease. Secondly, the recipient also receives cyclosporine, which is known to be hepatotrophic in the post transplant period. In contrast, the donor does not have any circulating hepatotrophic factors because of the normal liver preoperatively, and secondly does not receive cyclosporine post operatively.

Long Evans rats were subjected to either standard 2/3 partial hepatectomy (PH) or sham operation (SH) and were randomly allocated to the following treatment groups.

Group 1: partial hepatectomy (PH)

Group 2: partial hepatectomy and administration of cyclosporine and liver cytosol (PH + C + Cy)

Group 3: sham operation (SH)

Group 4: sham operation and administration of cyclosporine and liver cytosol (SH + Cy +C).

The AST and ALT levels were determined. The mitotic indices were obtained by counting the dividing cells using microscope and the results were given as cells per 10 fields. The liver weight/body weight ratio was calculated by dividing the liver weight by the body weight at death.

In this study we noted that the increase in AST and ALT in groups 2 and 4 are related to liver enzymes in cytosol. Moreover, liver mass had not yet returned to pre-operative levels by 2 weeks after partial hepatectomy in rats. The peak regenerative response, using DNA synthesis and mitotic index as markers of liver regeneration, is known to occur during the first 24 to 48 hours after partial hepatectomy. Most studies have been limited to the first post-operative week and the above markers have usually returned to normal by then. Restoration of liver mass is generally not used as an endpoint of liver regeneration.

Liver mass is obviously a very crude estimation and is influenced by water and fat content. These factors were not taken into consideration in these studies. However, the histological evaluation of the livers did not show any evidence of increased fat in the livers. In the clinical studies of liver transplant donors and recipients, liver mass has been estimated using CT scan calculations, [80] which are also relatively inaccurate.

The recipient presumably has high levels of hepatic growth factors in the circulation as a result of the liver disease. To simulate this, we infused liver cytosol from regenerating livers into animals after partial hepatectomy or sham operation. The liver cytosol did appear to modify the regenerative response after partial hepatectomy as indicated by the increased mitotic index. However, liver cytosol did not initiate a regenerative response after sham operation. Furthermore, liver cytosol did not modify the restoration of liver mass after partial hepatectomy.

Cyclosporine has also been shown to potentiate the regenerative response after partial hepatectomy. [84-87] In this study the animals receiving cyclosporine after partial hepatectomy had a greater mitotic index level compared to after partial hepatectomy. However, the restoration of liver mass was not modified by the addition of cyclosporine.

In summary, therefore, liver mass in this study was not restored by 2 weeks after partial hepatectomy even though the mitotic index had returned to normal after 48 to 72 hours. Hepatotrophic factors and cyclosporine, which are thought to be responsible for the more rapid growth of the liver in the recipient compared to the donor in living donor liver transplantation, did enhance regeneration but did not modify the restoration of liver mass.

CHAPTER 8

LEGENDS: PHOTOMICROGRAPHS

FIGURE 6.4 (a): Histological changes in the liver 24 hours after sham operation. Haematotoxylin and eosin (H&E) staining. X20.

(b) Close-up of the boxed area in (a). No mitosis. H&E X40.

FIGURE 6.5 (a): Histological changes in the liver 24 hours after sham operation and administration of cytosol and cyclosporine. H&E X20.

(b) Close-up of the boxed area in (a). No mitosis. H&E X40.

FIGURE 6.6 (a): Histological changes in the remnant liver 48 hours after standard 2/3 partial hepatectomy. H&E X20.

(b) Close-up of the boxed area in (a) showing mitosis (arrow). H&E X40.

FIGURE 6.7 (a): Histological changes in the remnant liver 2 weeks after standard 2/3 partial hepatectomy. H&E X20.

(b) Close-up of the boxed area in (a). No mitosis. H&E X40.

FIGURE 6.8 (a): Histological changes in the remnant liver 48 hours after standard 2/3 partial hepatectomy and administration of cyclosporine and cytosol. H&E X20.

(b) Close-up of the boxed area in (a) showing mitosis (arrow). H&E X40.

FIGURE 6.9 (a): Histological changes in the remnant liver 2 weeks after standard 2/3 partial hepatectomy and administration of cyclosporine and cytosol. H&E X20.

(b) Close-up of the boxed area in (a). No mitosis. H&E X40.

CHAPTER 9

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ADDENDUM

STATISTICAL ANALYSIS OF RESULTS

Though the sample size is small, the Shapiro-Wilk test revealed that the values are normally distributed.

The t-test shows no significant difference between PH and PH + C + Cy. (p=0.2448)

One-way analysis of variants was applied to test the significant difference between the four groups. This revealed an overall significant difference between the groups. (p=0.0001)

The Bonferroni for selected comparison was used to show which groups are significant. There is significant difference between:

- PH and Sham (p=<0.019)
- PH and Sham+ C+ Cy (p=<0.001)
- PH + C + Cy and Sham (p=<0.001)


```
 Oneway ast_ul24       procedure, tab bon

                    |        Summary of AST_UL24
          Procedure |        Mean   Std. Dev.       Freq.
--------------------+------------------------------------
                 PH |         624   312.63717           5
 PH + Cy + Cytos    |       916.4   416.60869           5
               Sham |        51.6   17.472836           5
Sham + Cy + Cyto    |        47.6   12.895736           5
--------------------+------------------------------------
              Total |       409.9   452.89779          20

                      Comparison of AST_UL24 by Procedure
                                (Bonferroni)
Row Mean-|
Col Mean |         PH   PH + Cy +Cytos         Sham
---------+--------------------------------
PH + Cs |      292.4
         |      0.571
         |
    Sham |     -572.4      -864.8
         |   P = 0.019       0.000
         |
Sham + C |     -576.4      -868.8             -4
         |      0.018       0.000          1.000
```

```
 oneway ast_ul48        procedure, tab bon

                |         Summary of AST_UL48
      Procedure |        Mean   Std. Dev.       Freq.
----------------+------------------------------------
             PH |       296.2   133.49607           5
 PH + Cy + Cytos |         486   133.33792           5
           Sham |        43.8   15.896541           5
Sham + Cy + Cyto |        76.2   18.660118           5
----------------+------------------------------------
          Total |      225.55   203.34375          20


                    Comparison of AST_UL48 by Procedure
                              (Bonferroni)
Row Mean-|
Col Mean |         PH   PH + Cy + Cytos        Sham
---------+---------------------------------
PH + Cs  |      189.8
         |      0.037
         |
    Sham |     -252.4     -442.2
         |      0.004      0.000
         |
Sham + C |       -220     -409.8         32.4
         |      0.013      0.000        1.000

Oneway ast_ul72        procedure, tab bon

                |         Summary of AST_UL72
      Procedure |        Mean   Std. Dev.       Freq.
----------------+------------------------------------
             PH |       194.4   87.503143           5
 PH + Cy + Cytos |       189.8   42.938328           5
           Sham |        47.8   4.0865633           5
Sham + Cy + Cyto |         62   20.518285           5
----------------+------------------------------------
          Total |       123.5   84.114271          20


                    Comparison of AST_UL72 by Procedure
                              (Bonferroni)
Row Mean-|
Col Mean |         PH   PH + Cy + Cytos        Sham
---------+---------------------------------
PH + Cy  |       -4.6
         |      1.000
         |
    Sham |     -146.6       -142
         |      0.002      0.002
         |
Sham + C |     -132.4     -127.8         14.2
         |      0.004      0.006        1.000
```

```
 Oneway ast_ul96        procedure, tab bon

                |         Summary of AST_UL96
      Procedure |        Mean   Std. Dev.       Freq.
----------------+------------------------------------
             PH |        32.4   8.8487287           5
 PH + Cy + Cytos |        64.8   9.1487704           5
           Sham |        29.6   14.909728           5
Sham + Cy + Cyto |        41.4   4.2190046           5
----------------+------------------------------------
          Total |       42.05   16.919235          20


                   Comparison of AST_UL96 by Procedure
                             (Bonferroni)
Row Mean-|
Col Mean |         PH   PH + Cy + Cytos        Sham
---------+---------------------------------
PH + Cy  |       32.4
         |      0.001
         |
    Sham |       -2.8      -35.2
         |      1.000      0.000
         |
Sham + C |          9      -23.4              11.8
         |      1.000      0.012             0.487

 Oneway ast_ul7days     procedure, tab bon

                |       Summary of AST_UL7days
      Procedure |        Mean   Std. Dev.       Freq.
----------------+------------------------------------
             PH |        62.2   8.5848704           5
 PH + Cy + Cytos |        96.8    45.72964           5
           Sham |        45.2   6.0580525           5
Sham + Cy + Cyto |          70   22.638463           5
----------------+------------------------------------
          Total |       68.55   30.594934          20

                   Comparison of AST_UL7days by Procedure
                             (Bonferroni)
Row Mean-|
Col Mean |         PH   PH + Cy + Cytos        Sham
---------+---------------------------------
PH + Cy  |       34.6
         |      0.312
         |
    Sham |        -17      -51.6
         |      1.000      0.039
         |
Sham + C |        7.8      -26.8              24.8
         |      1.000      0.740             0.910


 Oneway ast_ul14days    procedure, tab bon

                |       Summary of AST_UL14days
      Procedure |        Mean   Std. Dev.       Freq.
----------------+------------------------------------
             PH |          79   16.807736           5
 PH + Cy + Cytos |         161   59.556696           3
           Sham |        75.4   10.830512           5
Sham + Cy + Cyto |        77.6    11.28273           5
----------------+------------------------------------
          Total |   91.277778   39.657068          18
```

Comparison of AST_UL14days by Procedure
(Bonferroni)

Row Mean- Col Mean	PH	PH + Cy + Cytos	Sham
PH + Cy	82 0.004		
Sham	-3.6 1.000	-85.6 0.003	
Sham + C	-1.4 1.000	-83.4 0.003	2.2 1.000

Oneway alt_ul24 procedure, tab bon

Procedure	Summary of ALT_UL24 Mean	Std. Dev.	Freq.
PH	295.4	97.715403	5
PH + Cy + Cytos	464.2	357.73901	5
Sham	9.2	1.7888544	5
Sham + Cy + Cyto	14.4	6.7305275	5
Total	195.8	261.44509	20

Comparison of ALT_UL24 by Procedure
(Bonferroni)

Row Mean- Col Mean	PH	PH + Cy + Cytos	Sham
PH + Cy	168.8 1.000		
Sham	-286.2 0.160	-455 0.008	
Sham + C	-281 0.175	-449.8 0.009	5.2 1.000

Oneway alt_ul48 procedure, tab bon

Procedure	Summary of ALT_UL48 Mean	Std. Dev.	Freq.
PH	178.6	82.829946	5
PH + Cy + Cytos	222	69.188149	5
Sham	26.8	10.894953	5
Sham + Cy + Cyto	46	21.702534	5
Total	118.35	99.709196	20

Comparison of ALT_UL48 by Procedure
(Bonferroni)

Row Mean- Col Mean	PH	PH + Cy + Cytos	Sham
PH + Cy	43.4 1.000		
Sham	-151.8 0.003	-195.2 0.000	
Sham + C	-132.6 0.010	-176 0.001	19.2 1.000

```
 Oneway alt_ul72        procedure, tab bon

                |        Summary of ALT_UL72
      Procedure |        Mean   Std. Dev.       Freq.
----------------+------------------------------------
             PH |          64   30.659419           5
 PH + Cy + Cytos |        99.2   28.943048           5
           Sham |          27    9.486833           5
Sham + Cy + Cyto |        37.6   5.4129474           5
----------------+------------------------------------
          Total |       56.95   34.882472          20


                   Comparison of ALT_UL72 by Procedure
                                (Bonferroni)
Row Mean-|
Col Mean |         PH   PH + Cy + Cytos        Sham
---------+---------------------------------
PH + Cy  |       35.2
         |      0.127
         |
    Sham |        -37      -72.2
         |      0.097      0.000
         |
Sham + C |      -26.4      -61.6              10.6
         |      0.440      0.002              1.000


 Oneway alt_ul96        procedure, tab bon

                |        Summary of ALT_UL96
      Procedure |        Mean   Std. Dev.       Freq.
----------------+------------------------------------
             PH |        19.8   4.6043458           5
 PH + Cy + Cytos |        46.6   11.193748           5
           Sham |        24.6   9.9146356           5
Sham + Cy + Cyto |          23   2.7386128           5
----------------+------------------------------------
          Total |        28.5   13.084744          20

                   Comparison of ALT_UL96 by Procedure
                                (Bonferroni)
Row Mean-|
Col Mean |         PH   PH + Cy + Cytos        Sham
---------+---------------------------------
PH + Cy  |       26.8
         |      0.000
         |
    Sham |        4.8        -22
         |      1.000      0.003
         |
Sham + C |        3.2      -23.6              -1.6
         |      1.000      0.001              1.000


 Oneway alt_ul7days     procedure, tab bon

                |       Summary of ALT_UL7days
      Procedure |        Mean   Std. Dev.       Freq.
----------------+------------------------------------
             PH |        30.2   8.1363382           5
 PH + Cy + Cytos |        38.2   6.8702256           5
           Sham |        23.6   7.8930349           5
Sham + Cy + Cyto |        27.4   7.5033326           5
----------------+------------------------------------
          Total |       29.85   8.8927824          20
```

Comparison of ALT_UL7days by Procedure
(Bonferroni)

Row Mean- Col Mean	PH	PH + Cy + Cytos	Sham
PH + Cy	8 0.697		
Sham	-6.6 1.000	-14.6 0.048	
Sham + C	-2.8 1.000	-10.8 0.237	3.8 1.000

```
 Oneway alt_ul14days    procedure, tab bon
```

Procedure	Summary of ALT_UL14days Mean	Std. Dev.	Freq.
PH	24	3.5355339	5
PH + Cy + Cytos	43.333333	3.7859389	3
Sham	27.8	2.8635642	5
Sham + Cy + Cyto	46.8	12.00833	5
Total	34.611111	12.015377	18

Comparison of ALT_UL14days by Procedure
(Bonferroni)

Row Mean- Col Mean	PH	PH + Cy + Cytos	Sham
PH + Cy	19.3333 0.012		
Sham	3.8 1.000	-15.5333 0.054	
Sham + C	22.8 0.001	3.46667 1.000	19 0.005

```
 Oneway lwr_ul24        procedure, tab bon
```

Procedure	Summary of LWR_UL24 Mean	Std. Dev.	Freq.
PH	.0206	.00162173	5
PH + Cy + Cytos	.0217	.00257294	5
Sham	.04898	.00209809	5
Sham + Cy + Cyto	.04856	.00372062	5
Total	.03496	.01437792	20

Comparison of LWR_UL24 by Procedure
(Bonferroni)

Row Mean- Col Mean	PH	PH + Cy + Cytos	Sham
PH + Cy	.0011 1.000		
Sham	.02838 0.000	.02728 0.000	
Sham + C	.02796 0.000	.02686 0.000	-.00042 1.000

```
 Oneway lwr_ul48        procedure, tab bon

                  |        Summary of LWR_UL48
       Procedure |        Mean   Std. Dev.       Freq.
-----------------+------------------------------------
              PH |      .03444   .00286496           5
 PH + Cy + Cytos |      .03522   .00108028           5
            Sham |       .0416   .00294024           5
Sham + Cy + Cyto |      .04608   .00483032           5
-----------------+------------------------------------
           Total |     .039335   .00572624          20

                    Comparison of LWR_UL48 by Procedure
                                (Bonferroni)
Row Mean-|
Col Mean |         PH   PH + Cy + Cytos        Sham
---------+---------------------------------
PH + Cy  |     .00078
         |      1.000
         |
    Sham |     .00716      .00638
         |      0.017       0.038
         |
Sham + C |     .01164      .01086             .00448
         |      0.000       0.000             0.256

 Oneway lwr_ul72        procedure, tab bon

                  |        Summary of LWR_UL72
       Procedure |        Mean   Std. Dev.       Freq.
-----------------+------------------------------------
              PH |       .0356   .00879204           5
 PH + Cy + Cytos |       .0364   .00270185           5
            Sham |       .0462   .00535724           5
Sham + Cy + Cyto |       .0416   .00439318           5
-----------------+------------------------------------
           Total |      .03995    .0068708          20

Row Mean-|
Col Mean |         PH   PH + Cy + Cytos        Sham
---------+---------------------------------
PH + Cy  |      .0008
         |      1.000
         |
    Sham |      .0106      .0098
         |      0.061      0.096
         |
Sham + C |       .006      .0052             -.0046
         |      0.713      1.000              1.000


 Oneway lwr_ul96        procedure, tab bon

                  |        Summary of LWR_UL96
       Procedure |        Mean   Std. Dev.       Freq.
-----------------+------------------------------------
              PH |       .0368   .00228035           5
 PH + Cy + Cytos |        .034   .00223607           5
            Sham |       .0366   .00151657           5
Sham + Cy + Cyto |       .0424   .00391152           5
-----------------+------------------------------------
           Total |      .03745   .00396664          20
```

Comparison of LWR_UL96 by Procedure
(Bonferroni)

Row Mean- Col Mean	PH	PH + Cy + Cytos	Sham
PH + Cy	-.0028 0.675		
Sham	-.0002 1.000	.0026 0.831	
Sham + C	.0056 0.024	.0084 0.001	.0058 0.019

Oneway lwr_ul7days procedure, tab bon

Procedure	Summary of LWR_UL7days Mean	Std. Dev.	Freq.
PH	.0362	.00164317	5
PH + Cy + Cytos	.0374	.00250998	5
Sham	.0404	.00207364	5
Sham + Cy + Cyto	.0434	.00207364	5
Total	.03935	.00345307	20

Comparison of LWR_UL7days by Procedure
(Bonferroni)

Row Mean- Col Mean	PH	PH + Cy + Cytos	Sham
PH + Cy	.0012 1.000		
Sham	.0042 0.036	.003 0.228	
Sham + C	.0072 0.000	.006 0.002	.003 0.228

Oneway lwr_ul14days procedure, tab bon

Procedure	Summary of LWR_UL14days Mean	Std. Dev.	Freq.
PH	.04	.00291548	5
PH + Cy + Cytos	.04033333	.00585947	3
Sham	.0402	.00192354	5
Sham + Cy + Cyto	.0432	.00432435	5
Total	.041	.00364611	18

Comparison of LWR_UL14days by Procedure
(Bonferroni)

Row Mean- Col Mean	PH	PH + Cy + Cytos	Sham
PH + Cy	.000333 1.000		
Sham	.0002 1.000	-.000133 1.000	
Sham + C	.0032 1.000	.002867 1.000	.003 1.000