AUXILIARY LIVER ALLOTRANSPLANTATION

A HUMAN FEASIBILITY STUDY, AND AN EVALUATION

OF A NEW TECHNIQUE IN THE PIG.

BY

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T H E S I S

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THIS THESIS IS DEDICATED

TO

ANDY AND RICHARD

THEIR MOTHERS AND THEIR DOCTORS

PROLOGUE

This Thesis describes a series of basic investigations into a few important problems that have hampered the progress of liver transplantation in man. Three positive, original observations emerged from the studies:

(i) a promising new technique of auxiliary liver transplantation, previously developed in the pig, was shown to be technically feasible in man;

(ii) a new technique of biliary drainage was developed in a human cadaver study, and applied successfully in a series of live porcine auxiliary liver transplants;

(iii) the use of cerebrospinal fluid (CSF) glutamine as an index of donor liver quality in the postoperative period was investigated, and the values were shown to relate accurately to the histological quality of the donor liver in the auxiliary transplant model that was used.

Each of the three positive observations that arose from this experimental study, needs to be investigated in greater depth before they can be recommended for use in man.

This type of study has been the subject of adverse criticism. The main points have been the unsatisfactory cost/benefit ratio of liver transplantation in general, and the incongruity of undertaking this type of research in environments where the basic medical care of the populace falls short of the ideal. These points are well taken, but individual tragedy exists, and demands both compassion and vision on the part of the Medical Profession. The position has been put into perspective by Russell (150):

> "I am sure that all of us thinking about these things have serious concerns about their cost-effectiveness and perhaps even as to whether some of them are truly humane. Great efforts to benefit individual patients come naturally to surgeons, and I believe strongly that some champions of the individual are needed in medicine as they are needed elsewhere. Still it would be well if we have the foresight to develop a new "ethics of restraint" as we try to apply our knowledge with all the wisdom we possess. For there is no doubt that constructive surgery will flourish and that transplantation will be among its greatest achievements."

D E C L A R A T I O N

This experimental study was commenced in 1973. To the best of the author's knowledge, the transplantation technique had not been investigated or used in man prior to this study. The method of biliary drainage developed during this investigation appeared to be new. In addition, the author was unable to find any previous detailed study on the use of CSF glutamine as an index of donor liver quality in man or animal following liver transplantation.

All the operations, minor procedures and autopsies were carried out by the author himself. The analysis and interpretation of the liver biopsy material is that of the author, while the histopathological observations on the biliary tracts and other organs are those of Professor C.J. Uys and Dr. J. van den Ende of the Department of Pathology. The calculations and statistical analyses were all performed by the author, with the assistance of Miss. C. Vader, statistician to the Department of Obstetrics and Gynaecology. Diagrams, graphs and histograms were all handdrawn by the author prior to being completed by the artist. The biochemical and haematological estimations were not performed by the author.

The discussions, opinions and conclusions are those of the author.

The references have been taken to the end of 1974, and only important relevant references included after that.

The following publications and presentations arose directly or indirectly from the experimental studies (45, 46, 47, 48, 49, 86, 87, 88, 174).

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At the time of commencing this experimental study, no in-depth report existed on the details of the basic subhepatic transplantation technique, nor were detailed publications available about the local, regional and general effects of the techniques. The paucity of information demanded that the experimental study should be broadly-based and comprehensive, for academic purposes, to justify the tremendous outlay on time and money, and to prevent further live animal experimentation to detect facts that could have been detected in this study.

The project therefore required the advice, co-operation and assistance of many disciplines within the University of Cape Town Medical School. The author wishes to express his utmost gratitude to the following people, Departments and Institutions, all of whom made major contributions to the study and its presentation:

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ARRANGEMENT OF THE PRESENTATION

1. MAIN INTRODUCTION

2. PART I - THE HUMAN FEASIBILITY STUDY

3. PART II - THE COMPARATIVE LIVE PORCINE STUDY

4. PART III - STUDY OF THE RELATIONSHIP BETWEEN DONOR LIVER QUALITY, AND BIOCHEMICAL AND HAEMATOLOGICAL CHANGES IN THE RECIPIENT ANIMALS

5. APPENDIX

6. BIBLIOGRAPHY

7. STATISTICAL ADDENDUM

MAIN INDEX

* Supplementary Index at the beginning of the Section.

* Supplementary Index at the beginning of the Section.

* Supplementary Index at the beginning of the Section.

* Supplementary Index at the beginning of the Section.

INDEX OF TABLES

TABLES IN THE APPENDIX

* Mean levels and mean values are used synonymously in this presentation.

TABLES IN THE APPENDIX

INDEX OF FIGURES

MAIN INTRODUCTION

INDEX TO THE MAIN INTRODUCTION

MAIN INTRODUCTION

SUMMARY

The diseases for which liver transplantation has been advocated, are briefly reviewed, and the disappointing results of present day therapy of these conditions are noted. The results of clinical liver transplantation are presented, and the unacceptably high rate of failure due primarily to biliary tract complications is emphasised.

The theoretical advantages of auxiliary liver transplantation are briefly reviewed, the problems associated with the procedure are discussed, and criteria formulated for successful auxiliary transplantation.

The importance of, and the difficulties involved in the early diagnosis of rejection in the donor liver are outlined, and the absence of a simple, reliable test, specific for rejection is stressed.

Features of a promising, recently described auxiliary liver transplantation model are reviewed, and the rationale for further studies related to this technique is presented.

MAIN INTRODUCTION

Liver transplantation is an attractive and challenging concept in the treatment of life threatening liver disease that cannot be treated by conventional means, and has been used with gratifying success in a few patients. The procedure is, however, in its infancy, having been practised experimentally for only two decades, and clinically for barely one.

The overall clinical results have been unsatisfactory, and have led to serious doubts regarding the value of the procedure in man. In view of the prevailing pessimism on both experimental and clinical fronts, (116, 152,161), it is perhaps timely to review briefly some of the diseases for which liver transplantation has been advocated, and to reiterate the hopeless prognosis that exists with all other forms of therapy.

INDICATIONS FOR LIVER TRANSPLANTATION

Cirrhosis of the liver is a common condition and "during the past decade the incidence of cirrhosis has increased more than any other cause of death" (Orloff 140). The problem is worldwide, and the quoted death rates per 100 000 of population have ranged from 35,7 in France (217); 11,9 in the U.S.A. (44) to 2,8 in England and Wales (196). It has been estimated that there are 8 million chronic alcoholics in the U.S.A., most of whom have, or are potential candidates for cirrhosis, and its consequences of portal hypertension and variceal bleeding (140). Over the age of 40 years cirrhosis is the fourth commonest cause of death.

Once variceal bleeding commences, the prognosis is extremely grave. Orloff (140) quotes a mean hospital mortality of 73% in 1031 patients with the first variceal haemorrhage, and states that the prognosis has shown virtually no improvement over a twenty year period. From London, Sherlock (162) has reported an 80% mortality within one year in cirrhotic patients with variceal bleeding plus clinical evidence of liver failure.

The conventional treatment of these patients consists of specific medical measures, emergency surgical procedures to control life threatening haemorrhage, and a variety of elective portosystemic shunts to prevent further haemorrhage. The therapy is extremely costly in terms of bed occupancy, blood usage and staff time. Despite the most energetic therapy, most patients have a progressive downhill course, dying eventually from haemorrhage, liver failure or infection.

Present methods of therapy for end stage cirrhosis aim essentially at eliminating the precipitating causes, and treating the multiple effects of the disease. There is no known therapy that will transform a liver with severe established cirrhosis into a normal liver. On the basis of the present understanding of cirrhosis, it would appear that the only really effective treatment in established progessive disease would be the total replacement of the cirrhotic liver with a normal liver, or the implantation of a second liver into a heterotopic site with diversion of all or part of the portal blood into the healthy graft.

Cirrhosis has been a major indication for clinical liver transplantation, and by 1/1/74, 49 patients had been treated by transplantation, 36 by the orthotopic method and 13 by auxiliary transplantation - ACS/NIH Report (5). The results have been poor, only 3 of the 49 patients survived for more than 1 year.

Most of the operations have been performed on critically ill patients with end stage liver failure. The mortality and morbidity after any major surgery would probably have been high in these patients, and Williams (226) has made a plea for earlier operation in the cirrhotic patient with progressive disease.

Improvement in the results of clinical liver transplantation would provide the surgeon with a curative operation for the vast number of cirrhotic patients who cannot be cured by conventional means, and the operations could then justifiably be performed at an earlier stage.

Congenital biliary atresia has been estimated to occur in 1/20 000 to 1/30 000 births (158). Louw (117) has discussed the extremely complex problems inherent in the diagnosis and management of biliary atresia. The prognosis in these children is usually hopeless if there are no ducts available for biliary reconstruction. Survival is uncommon beyond 2 years, and survival in excess of 4 years is exceptional (106). The last months of life are an agony for both parents and child because of itching, ascites, haemorrhages, recurrent respiratory infections and finally coma (178). Surgically correctible lesions have been reported in 10% (37) to 38% (101) of patients but only 8% are cured by conventional surgical operations (158). Hepatic portoenterostomy has not lived up to earlier expectations (38).

Liver transplantation would appear to offer the only chance of survival for those children in whom the defects do not allow for conventional repair. Starzl (183) feels that these patients are ideal candidates for transplantation procedures because of their age, natural resiliency, and the indisputably tragic outcome of the disease with conservative treatment.

Biliary atresia had been the main indication for liver transplantation reported to the Registry of the ACS/NIH by 1/1/74. (5). Seventy-two out of 199 transplants had been performed for biliary atresia. Fifty-three children had orthotopic transplantation, and 19 had the heterotopic procedure. The overall survival rate after these heroic procedures has been extremely poor. Of the 72 patients, 8 survived for 1 year, 4 for 2 years, 2 for 3 years and 1 for 4 years. In individual large centres however, the results are more promising. Starzl (187) has had good short-term results using the orthotopic procedure, while Fortner (70) has revived interest in auxiliary transplantation due to his recent success with the technique in children with biliary atresia.

Practical and ethical reservations may exist with regard to doing such a major procedure in a small child. However, with our present state of knowledge, liver transplantation appears to offer the only chance of survival for the 92% of these patients who are destined to die.

Primary liver cell carcinoma is on the average the most rapidly lethal of all neoplasms (121). Once the diagnosis has been established, survival times have been quoted, in months, as 3,2 (Gustafson 79), 3,75 (Spatt 176), 4 (Berman 25), 6 (Bagshawe 17) and 7 (Lemmer 110). The disease is common, and is known to have a higher incidence in African and Asian countries than in Western countries, and in general there is a tendency for its incidence to increase throughout the world (114). The autopsy incidence varies from 5,7% (South Africa); 5,5% (Taiwan) to 0,21% in the U.S.A. (114).

The uniformly poor results of all types of therapy have been adequately reviewed by Lin (114). External radiotherapy, internal irradiation with intra-arterial radioactive isotopes, and chemotherapy by intravenous,

intraportal and intra-arterial routes, have all failed to increase the survival rates, although some palliation has been reported. Surgical measures, such as hepatic artery ligation, complete de-arterialisation of the liver, and selective hepatic portal venous ligation have likewise failed to produce any significant increase in survival.

Hepatic resection has provided a few long term survivors, but the overall mortality remains unacceptably high. Lin (114) analysed 271 cases of hepatoma. On preoperative assessment, 72,6% were considered "operable" but only 30% proved to have resectable lesions. Two-thirds of the resected patients died within one year, mainly as a result of local recurrence or distal metastases. In 1973 (115) he reported 9 patients who had survived more than 5 years, out of 97 hepatic lobectomies for primary liver cell cancer. Balasegaram (18) reported that less than 7% of 300 hepatomas, in his series, were considered suitable for resection, and that he had had only 3 patients with 5 year survival. Ong (139) reports equally dismal results.

The uniformly hopeless results of all forms of therapy, including hepatic resection, clearly indicate that some other form of therapy is required. By 1/1/74, 53 liver transplants had been performed for primary hepatic malignancy (5). Three patients survived for more than 1 year, and 1 for 4 years. Many of the transplanted patients developed local recurrence or distal metastases after transplantation, a distressing phenomenon that has caused many authorities to abandon liver transplantation as a form of treatment for hepatoma. Williams (109), however, reports a five year survival without recurrence in a patient in whom a massive hepatoma was the indication for transplantation. Her subsequent quality of life was such that she was able to go back to work, look after her family, and travel extensively (221)

Significant improvement in the overall mortality of liver transplantation may well make this procedure the treatment of choice for small hepatomas confined to the liver, as total extirpation of the liver would probably carry less risk of recurrence than hepatic resection. There appears to be no place for auxiliary transplantation in the management of hepatoma.

Cholangiocarcinoma has an entirely different malignant potential compared to hepatoma. With relief of the large duct obstruction, liver function can be preserved (163), and the patients may remain asymptomatic for 5 years or longer. Relief of the obstruction by means of a U-tube (Terblanche 200) is a lesser procedure than transplantation, and has none of the hazards of immunosuppression. Unless the results of liver transplantation can be drastically improved, there appears to be no indication for the procedure in cholangiocarcinoma (199).

The mortality rate from hepatic coma due to acute or subacute liver cell necrosis is one of the highest among liver diseases (1). The problems of fulminant hepatic failure have been thoroughly reviewed by Abouna (1), Breen and Schenker (30), Rueff (149) and Saunders (154), and critically aired at the 1973 Cape Town Liver Congress (153). Only 5-10% of patients with fulminant hepatic failure survive despite the most intensive treatment. Encephalopathy, hypoglycaemia and the haemorrhagic diathesis (225) have been the main causes of death in these patients. The use of steroids, exchange transfusions, cross circulation, plasmapheresis, extracorporeal liver perfusions, haemodialysis, L-Dopa therapy and other measures such as hyperbaric oxygenation have not added materially to the survival rate. Charcoal-column ion-exchange haemoperfusion has been reported (61), but it is too early to assess the results of this new development.

The rationale behind these forms of therapy is to keep the patient alive until the liver has regenerated, and to provide optimum conditions for regeneration. However, regeneration is not inevitable (154).

The methods of treatment outlined above are all temporary and intermittent, and in the event of failure of liver regeneration, the patient is doomed to die.

The treatment of acute liver failure by means of liver transplantation is theoretically attractive, especially the concept of using an auxiliary liver. The placement of a second liver would provide continuous support during the critical period, and if regeneration occurs in the host liver, the auxiliary liver could be removed. In the event of nonregeneration of the host's own liver, the graft could be retained.

The dangers of the donor liver being affected by the hepatotoxin or virus that afflicted the host's liver are self evident, but, in the experimental animal, auxiliary grafts have proved to be lifesustaining in animals in whom acute liver failure has been produced by a variety of toxins and surgical procedures (21, 107, 108, 113). By 1/1/74, 17 patients had undergone liver transplantation for hepatitis or hepatic necrosis (5), 4 of whom had survived for more than 1 year.

Patients with hepatic-based inborn errors of metabolism have been successfully treated by transplantation (58, 78), and as these diseases become more clearly understood and delineated, this may well become a fruitful field for the transplant surgeon. By 1/1/74, 2 patients with Wilson's Disease had been treated by orthotopic transplantation, and both were alive 4 years after transplantation (5).

Liver transplantation is possibly also indicated in patients with irreparable damage to the biliary ducts, and in irreparable trauma of the liver.

In all these diseases, it has been established that the presentday methods of medical, surgical and radio-therapeutic treatment are inadequate, and that some other form of therapy is required. There are strong theoretical indications for the removal and replacement of the damaged liver in some diseases, or for the implantation of a second liver to provide more adequate function on a temporary or permanent basis in others.

Clinical liver transplantation has not, to date, added significantly to the overall survival of these patients, but individual good results (192) even at this early stage in the development of transplantation, have indicated the rich potential of the procedures.

It appears imperative that further laboratory studies be undertaken to investigate the many technical and metabolic problems that have led to the poor results in liver transplantation. Improvement of the overall mortality may then enable us to offer the patients with these fatal liver diseases, a chance of survival sufficient in both quality and length.

RESULTS AND CAUSES OF FAILURE IN CLINICAL LIVER TRANSPLANTATION

1. International Results and Causes of Death in the First Week

By 1/1/74, the Organ Transplant Registry of the ACS/NIH (5) had listed 199 patients who had been treated by means of liver transplantation. The cases had been reported from 39 transplant teams in 14 countries. The accumulated survival figures are listed in the table below. The survival pattern is distressingly poor.

	Survival			
	1 Yr.	2 Yr.	3 Yr.	4 Yr.
Total number of patients 199	21	9	5	4
Number living 1/1/74 16	10	5	3	3

TABLE 1. INTERNATIONAL RESULTS OF LIVER TRANSPLANTATION BY 1/1/74 (5)

Included in these figures are the results from 1963 to 1970 when liver transplantation procedures were still in the early developmental stage, as evidenced by the high immediate mortality in these earlier cases. By 1970, 109 liver transplant operations on 107 recipients had been reported to the Registry (4). Details of 106 of the patients were made available. Analysis of the details in 106 patients revealed that 39 patients (37%) had died intra- or immediately postoperatively, or within the first week. These early deaths were due to, in order of frequency, haemorrhage, cardio-

Type of Biliary Reconstruction	No. of Cases	No. of Bile Leaks
End-to-end CBD-CBD	6	3
GB-CBD	6	3
GB-Jejunum	5	4
CBD = Common bile duct GB = Gallbladder		

TABLE 2 THE INCIDENCE OF BILE LEAKS IN THE KING'S COLLEGE/CAMBRIDGE SERIES (224)

respiratory complications, thrombotic or non-thrombotic vascular occlusion to the grafts, metabolic complications, hepatic failure, sepsis, peritonitis and prolonged hypotension. These complications could be anticipated when performing any new major surgical procedure on critically ill patients. Rejection was thought to be the cause of death in only two of the patients who died within the first week. Twenty three of the early deaths occurred in 75 patients subjected to orthotopic transplantation, while 16 early deaths occurred in 31 patients subjected to heterotopic transplantation. Since then, the immediate mortality has improved considerably in centres with wide experience (Starzl 183).

2. Complications and deaths related to the Biliary Tract

The causes of failure in clinical liver transplantation have recently been well documented by the King's College/Cambridge (224) and Denver (187) groups, who together have performed more than 50% of the transplants reported in the world. Their results, and those of others (pages 15 & 16) have shown that complications related to the reconstruction of the biliary drainage tract have been the major cause of death and morbidity in patients who have undergone liver transplantation and survived the immediate postoperative period.

(1) Complications of the extrahepatic biliary tract

Williams et al (224) reported that 9 (35%) out of 26 patients in the KING'S COLLEGE/CAMBRIDGE SERIES died intra-operatively, or in the immediate postoperative period. Of the 17 survivors, 9 subsequently developed external biliary fistulae, while 1 developed a localised anastomotic leak. Three types of biliary reconstruction were used, and leaks developed with all three methods, the incidence being shown in the Table on the opposite page.

Three of the patients in whom biliary leaks occurred, were also shown to have developed biliary calculi and biliary sludge. One patient who evidenced no leak, had developed biliary sludge and calculi. Thus 11 (64%) of the 17 survivors had some form of extra-hepatic biliary tract complication, and in 10 patients (58%) biliary tract complications were directly responsible for death.

In addition, it has recently been reported that the longest survivor in the series died more than 5 years after transplantation from biliary tract obstruction and sludge (109). She had been well since the transplant, performed as treatment for primary hepatoma, and showed no evidence of tumour recurrence.

Rejection accounted for only 3 (18%) deaths in the King's College/ Cambridge series, infection for 3 (18%) and tumour recurrences for 2 (11%) deaths.

Starzl et al (187) reported an equally high number of potentially fatal extrahepatic biliary tract complications from the large DENVER SERIES. In 82 cases of orthotopic transplantation, the initial biliary reconstruction was eventually shown to be unsatisfactory, leading either to death or early re-operation in 25 cases (an incidence of 30%). The potential frequency may have been even higher, since many patients died so early postoperatively that biliary tract problems would not yet have been manifest. Attempts at secondary repair aided only 4 of the 13 patients in whom repair of the biliary tract complications were attempted.

The Denver group used 5 different methods of biliary tract reconstruction, only 1 of which proved to be trouble-free, as shown in Table 3 on the next page.

	Type of Biliary Reconstruction				
	Biliary-biliary	Biliary-enteric			
	CBD-CBD	GB-DUOD	ROUX-Y GB-JEJ	CBD-DUOD	CBD-LOOP -JEJ
Number	9	59	8	4	2
Obstruction	0	15	2	0	0
Fistula	5	2	0	1	0

GB = Gallbladder
DUOD = Duodenum
CBD = Common bile duct
JEJ = Jejunum

TABLE 3. COMPLICATIONS FROM THE BILIARY TRACT RECONSTRUCTION

(DENVER SERIES 187)

Common bile duct to common bile duct anastomosis resulted in a leakage rate of 55% while direct biliary enteric anastomosis showed a 23% rate of obstruction. In three patients who died from obstructive biliary complications, the obstruction was clearly iatrogenic, and in retrospect, could have been avoided.

Overall the Denver group reported 18 recipients who survived more than 1 year, 9 for more than 2 years, and 2 for more than 4 years.

Of the 64 patients who survived for less than 1 year, the single most important factor leading to death was a series of technical misadventures, of which complications from biliary tract reconstruction head the list. Poor control of rejection and systemic infection were the next most important causes of early death. Ten late deaths occurred from infection, recurrent cancer, and rejection.

Extrahepatic biliary tract complications in human transplantation have been reported from OTHER CENTRES. Najarian (4) reported infarction of the gallbladder in one patient, and necrosis of the gallbladder with a bile leak at the cholecystoduodenostomy in another. Absolon (2) reported common bile duct necrosis with bile peritonitis following heterotopic transplantation in a child. Fortner (68, 69) has reported bile leaks in both orthotopic and heterotopic transplantation of the liver. Aune (15) reported evidence of obstruction when using a cholecystocholedochostomy and Fonkalsrud (67) reported disruption of a cholecystogastric anastomosis in a heterotopic transplant.

(2) Other Complications associated with the Biliary Drainage Tract

The spectrum of serious and fatal complications related to the reconstruction of the biliary tract, extends far beyond the obvious mechanical and technical complications of the extrahepatic biliary tract, which have been discussed in the foregoing pages. Intrahepatic sepsis, generalised septicaemia and intrahepatic cholestasis, have frequently been encountered in clinical liver transplantation in different centres - Denver (71, 120, 128, 179, 186, 187, 193), New York (68), England (32, 135, 222, 224, 226), and in Scandinavia (15), and are thought to be due to, in part, the type of biliary drainage used.

The intrahepatic sepsis that has been reported in man, has ranged through clinical evidence of ascending cholangitis, histologically and bacteriologically proven cholangitis, liver abscesses, and septic infarction of the liver. Liver sepsis has occurred both with, and without evidence of obstruction to the donor biliary drainage tract. The problem of intrahepatic sepsis was investigated in the experimental laboratory by the Denver group (31). They defined three factors which appeared to play a part in the development of intrahepatic sepsis following liver transplantation. These factors were:-

(a) Ischaemic injury to the liver

(b) Immunological injury to the liver - "Rejection Injury"

(c) Attenuation of the host's capacity to resist infection (due to immunosuppression)

In addition, they showed that the portal vein and biliary drainage tract, were the sources of entry for the organisms

from the gastro-intestinal tract and duodenum respectively. This is in line with Calne's experience (32) in which he found a high incidence of cholangitis in pigs and monkeys following transplantation with biliary drainage via cholecystoduodenostomy, and a low incidence when bile duct to bile duct anastomosis was used.

Both the Denver (71) and King's College/Cambridge (135) groups have encountered bacterial septicaemia and systemic infections as common and fatal complications following human liver transplantation. In a high proportion of patients the bacteraemia or focal sepsis was due to endogenous bowel-based organisms (E. coli, B. fragilis, Aerobacter klebsiella, Pseudomonas sp.), and the incidence and severity of the infections due to bowel-based organisms was greater than that seen following renal transplantation (71). With further experience the Denver group (187), have encountered bacteraemia both early and months after liver transplantation, often without the detection of a focus of infection. These findings have led to the proposal that the donor liver was the site of entry, and the portal vein and/or the extrahepatic biliary tract the portal of entry (187). Starzl feels that the latter portal of entry is of more importance than the former. The Denver group suggests that the donor liver becomes bacteriologically "porous", and that bacterial "leak" occurs into the systemic circulation, with or without evidence of cholangitis. Starzl further proposes that the biliary drainage should be removed as far as possible away from the mainstream of the gastrointestinal tract, which he

achieves by the use of cholecystenterostomy-en-y, and Calne (32) by the use of choledochocholedochostomy or cholecystocholedochostomy.

Intrahepatic cholestasis has frequently been encountered in clinical liver transplantation, with and without evidence of extrahepatic biliary tract obstruction. The ischaemically damaged liver immediately after the transplantation operation cannot produce normal bile in normal volumes, and there is a tendency for precipitates to form (32). The cholestasis seen in immunosuppressed, allografted livers is accompanied by elevated bile viscosity and diminished biliary output. "In addition to the operative ischaemic damage, biliary secretion may be unfavourably influenced by immunosuppressive treatment itself, especially the drug azathioprine which causes cholestasis; by immunological rejection and by ascending cholangitis. Analysis of the contributions of each of these factors in an individual case can be extremely difficult" (32). Waldram (215) suggests that the effect of infection on bile composition may be important in producing precipitates.

The Achilles heel of clinical liver transplantation has thus been shown in many centres to be reconstruction of the biliary tract. It is a distressing fact that the apparently simplest part of a major procedure should cause the death of so many patients who have survived the immediate postoperative period, and in whom adequate immunosuppression seemed to have been achieved.

Clearly, the problems relating to biliary tract reconstruction need to be investigated intensively before any further advance in survival can be achieved.

3. Complications and failure related to Rejection

Starzl (192), Calne and Williams (224) have all listed systemic infection and rejection as causes of death in several of their patients. Both the Denver and King's College/Cambridge groups have emphasised the extreme difficulty in the early diagnosis of rejection, and its differentiation from sepsis, biliary tract leaks and obstruction, and other complications.

Injudicious use of immunosuppressive therapy may unnecessarily depress the body's defence mechanisms and predispose to further infection, while failure to recognise and treat rejection may result in irreversible graft rejection. The problem of the diagnosis of rejection requires further investigation, and will be discussed in more detail on pages 30-32.

ADVANTAGES OF, AND PROBLEMS ASSOCIATED WITH, AUXILIARY HETEROTOPIC LIVER TRANSPLANTATION

1. Theoretical Advantages

Auxiliary heterotopic liver transplantation is the transplantation of an additional healthy liver into a subject without removal of the recipient's own diseased liver. The concept of auxiliary liver transplantation in non-neoplastic diseases is attractive, and has many theoretical advantages.

(1) Transplantation without hepatectomy would appear to be simpler technically, less time-consuming and less stressful to an already critically ill patient, than hepatectomy plus orthotopic transplantation.

(2) In potentially reversible liver disease it could fulfil several roles. Auxiliary transplantation would help to keep the patient alive during the time required for the patients own liver to regenerate (all other types of hepatic support having been shown to be inadequate). In the event of adequate regeneration and recovery of function, the auxiliary graft could be removed with subsequent benefit to the patient who would no longer be subjected to immunosuppressive therapy. Should the host's liver not recover function, the graft would serve as a permanent replacement, while the patient's own damaged liver may be retained or removed.

(3) In chronic irreversible liver disease, the auxiliary graft could help to maintain more adequate liver function. The patient's own liver would provide lifesaving protection for the patient during the period of establishment of graft function, and during rejection episodes. Should the graft fail to function, it could be removed leaving the patient little worse off than he was prior to transplantation.

(4) In critically ill patients who would be unable to withstand hepatectomy plus transplantation, an auxiliary graft would assist in improving the patient's general condition to the extent where he would be fit enough to undergo removal of his own liver.

(5) In end stage cirrhosis with portal hypertension and variceal haemorrhage, the portal pressure could be lowered by diverting the portal blood into the healthy auxiliary graft with immediate decrease in bleeding, and eventual improvement in the patient's coagulation and nutritional status, whereafter the cirrhotic liver could be removed as a precaution against the development or spread of hepatoma.

(6) Auxiliary transplantation would permit the use of small donor livers, for example from anencephalic monsters (68), in older patients for whom a suitably sized donor liver for orthotopic transplantation is not available.

(7) The auxiliary technique has been advocated as a palliative procedure (69) in patients with irresectable slow-growing malignancy which obstructs biliary outflow, or portal inflow, although this indication is debatable.

(8) The theoretical concept of using auxiliary liver xenografts from abbatoir animals (7) or primates, on a temporary or permanent basis, is extremely attractive, although at present the immunological problems appear insurmountable.

Experimental and clinical practice has, to date, exposed problems which have almost nullified all the theoretical advantages of heterotopic auxiliary transplantation. In clinical transplantation, only 39 auxiliary transplants had been performed by 1/1/74, of whom only 1 patient (2,5%) had survived for 1 year or longer (5). Twenty out of

160 patients who had undergone orthotopic transplantation had survived for 1 year or longer (12,5%). The problems encountered in clinical and experimental auxiliary liver transplantation have led many people to abandon the concept as a practical one.

Fortner (70) has recently revived interest in heterotopic transplantation by reporting successful transplants in 2 children.

2. Problems associated with Auxiliary Transplantation

The theoretical and practical problems associated with auxiliary transplantation will now be discussed.

(1) The problem of space

It has been argued that the placement of a large additional organ such as the liver, into the abdominal cavity may totally prevent closure. This problem has been encountered clinically by Fortner (69) and by Hume (cited by Starzl 187). Forced closure may result in respiratory complications, compression of intra-abdominal organs, kinking of vasculature with subsequent haemodynamic problems, and poor wound healing with eventual dehiscence.

Solutions to this problem would appear to be the gradual preoperative enlargement of the abdominal cavity, the transplantation of small or partial grafts (209), or the removal of other organs such as the spleen or kidney to create space (2, 43). In patients with gross ascites, space will have been gradually increased. The same result has been achieved by the use of

repeated pre-operative pneumoperitoneum (69). The terminal stage cirrhotic liver will already have decreased in size, partially solving the problem of space.

The provision of small grafts is a logistic problem limited at present by our inability to store livers for prolonged periods, and by public disinclination to donate organs from children.

The liver is extremely sensitive to hypotension, anoxia and handling, and the time and trauma involved in preparing partial grafts, combined with the potential complications of haemorrhage and bile leaks, would tend to militate against this procedure.

The problem of space has been investigated in a cadaver study in Part I of the presentation.

(2) The problem of position

The finding of a bed suitable for an additional liver in heterotopic liver transplantation has taxed the ingenuity of many workers (23). In orthotopic liver transplantation, the donor liver is placed in a preformed bed with relatively simple anatomical and physiological vascular reconstruction. The only variant open to contention is the method of biliary drainage.

The heterotopic graft must of necessity find different residence. Through the period of experimental development of the technique, partial and whole liver grafts have been placed in the right (24, 204) and left (80) upper abdomen, right (76, 126, 155, 159) and left (129, 164, 201) lower abdomen, the thoracic cavity (129, cited 201, 207) the groin (189) and the neck (166, 173, 189).

With the liver in the orthotopic position, the hepatic veins drain into an area of negative pressure which fluctuates with respiration (85, 133). This benefits circulation within the liver. In addition, the diaphragm exerts a massaging action on the liver, further aiding its circulation (63).

Jerusalem (100) has shown that pressure in the inferior vena cava increases proportionately to the distance from the right atrium, while the fluctuations in pressure are inversely related to the distance from the right atrium. These findings have been substantiated by Novak (138), Van der Heyde (207) and Hess (85), and it has been shown that pressure in the inferior vena cava distal to the renal veins is high enough to cause hepatic venous outflow obstruction and graft damage.

These findings suggest that a heterotopic graft should drain into the inferior vena cava as close to the diaphragm as possible to avoid damage due to outflow obstruction, and to benefit from the massaging action of the diaphragm and the intermittent fluctuation in vena caval pressure. This will preclude extra-abdominal auxiliary grafting, and limit intra-abdominal sites to the upper abdomen.

In addition, it has been shown that a long donor vena caval segment increases the hepatic venous outflow pressure (85, 100) and predisposes to outflow obstruction by virtue of kinking and twisting (137, 138, 206, 208). It has been suggested that the vena caval segment used for anastomosis to the inferior vena cava should be cut flush with the liver to avoid these hazards.

Thus high implantation plus a minimal donor vena caval cuff would appear to offer the best combination for optimal drainage of venous blood from the liver. In addition, Immelman has shown that outflow via the suprahepatic vena cava is preferable to that via the infrahepatic cava (personal communication).

(3) The problem of creating satisfactory vascular circuitry

The creation of anatomically and physiologically satisfactory vascular circuits in auxiliary transplanation has been fraught with problems, many of which are of contentious nature (85, 138, 148, 156, 171, 185, 210, 211,213, 218). The requirements for satisfactory hepatic venous drainage have already been discussed.

In its normal position, the liver has a dual blood supply - hepatic arterial and portal venous. It is known that ligation of the hepatic artery in man may lead to massive necrosis or infection of the liver (104, 122), but that these sequelae are not inevitable because of collateral arterial blood supply to the normal liver. In the transplant situation, no collaterals exist ab initio, and transplantation without a hepatic arterial inflow may result in both hepatic infection and infarction, and may seriously compromise the blood supply to the gallbladder, cystic and common bile ducts. These problems may well negate the efforts of those who have attempted auxiliary transplantation using a portal venous blood supply only (85, 105, 132, 166, 228).

Depriving the liver of direct access to portal blood by means of a portacaval shunt has been shown in animals (27, 168, 175) to

cause shrinkage of the liver. Child (41) has shown, by means of experimental portacaval transposition, that the shrinkage in livers following portacaval shunting is due to a decrease in the volume of blood flowing through the liver, and suggested that lack of "regenerative factors" contained in portal blood may play an additional role in inducing the shrinkage. Marchioro (125, 127) showed conclusively that non-hepatic splanchnic venous blood had a hepatotrophic influence, and that lack of portal blood itself caused liver shrinkage, even if an equivalent volume of systemic venous blood was directed through the liver.

These observations in non-transplanted normal livers would tend to indicate that a whole or partial liver requires both an arterial and portal venous blood flow for prevention of ischaemic complications and atrophy.

These principles have been shown to be applicable in experimental auxiliary liver transplantation. Starzl (191) and others (43, 50, 51) have shown that atrophy rapidly develops in auxiliary liver transplants which do not have this dual blood supply.

It has been shown repeatedly that transplantation of an auxiliary graft without any disturbance to the host's own liver, results in rapid atrophy of the donor liver, even if the donor liver is supplied with an equivalent volume of systemic venous blood through its portal system (146, 191, 201, 202, 203). Others have shown that a host portacaval shunt (81, 166) prevents some of this graft atrophy. Marchioro (126) and others

(39) have shown that this atrophy can be prevented by passing all the portal blood through the graft. Ranson (147, 148) has further proved that an important factor or factors are present in the portal blood derived from the gastro-pancreatic-splenic bed, while Orloff (141) has suggested that the pancreas is the site of origin and Starzl (185) suggests that insulin and glucagon are the main hepatotrophic factors.

All these studies have indicated that portal venous blood contains a substance or substances essential for the maintenance of size and function in a liver, or a liver graft, and that most or all of this "hepatotrophic factor" appears to be extracted by the liver that has primary access to the portal blood.

It thus appears that the creation of optimal conditions for success in auxiliary liver transplantation demands that the donor liver should have an adequate hepatic arterial inflow, plus an adequate inflow of portal venous blood. Anatomically these anastomoses can be constructed more readily in the upper abdomen than in the pelvis or any extra-abdominal position.

This subject is further discussed in Part I of this presentation.

(4) <u>The problem of biliary drainage</u>

The construction of biliary drainage can be divided into two basic methods in both heterotopic and orthotopic liver transplantation. The first method affords direct bile drainage into the bowel (biliary-enteric), while the second utilises the recipient's own distal biliary tract (biliary-biliary), thus permitting final drainage through the sphincter of Oddi.

The unacceptably high rate of fatal biliary tract complications in human liver transplantation has been outlined on pages 13-17.

Serious or fatal biliary tract complications have not been limited to human experience. The same spectrum of complications has been seen in many centres where experimental liver transplantation has been practised. The biliary tract complications reported may be divided into two types - macroscopically obvious complications of the extrahepatic biliary apparatus, and microscopic complications of the intrahepatic biliary tract seen on histological sections.

For the purposes of this discussion, the extra-hepatic biliary apparatus consists of the gallbladder, cystic duct, common duct and extra-hepatic hepatic ducts.

Of the extra-hepatic biliary tract complications, anastomotic leaks or complete anastomotic breakdown with bile peritonitis, have been reported in both dogs (165, 171, 172) and pigs (113) in heterotopic transplantation, and in dogs (42, 191, 195) and pigs (19,42, 130) in orthotopic transplantation. Necrosis of the gallbladder or bile duct has been reported with both transplant techniques in the dog (120, 201) and in pigs (42, 111).

Anastomotic strictures have been reported in pigs (95) and dogs (124, 146, 172). Biliary sludge, gallstones and suture concretions have also been reported (19, 42).

The intrahepatic biliary tract consists of the canaliculi, bile ducts and ductules for the purposes of this presentation.

The intrahepatic biliary tract complications have been cholestasis, cholangitis and cholangitic abscesses. These complications have been reported in heterotopically transplanted dogs (21, 81, 100,108, 119, 124, 134, 146, 160, 171, 172, 208, 212, 217) and pigs (97) as well as orthotopically transplanted dogs and pigs (3, 19, 31, 33, 34, 35, 36, 42, 55, 72, 94, 95, 120, 131, 190, 191, 195). The development of cholestasis, cholangitis and cholangitic abscesses appears to be related to the type of biliary tract drainage that is used. This relationship has been demonstrated clearly by Dent (55). Using an orthotopic porcine model, he showed that direct biliary-enteric anastomosis by means of a cholecystoduodenostomy led to a high incidence of cholestasis and cholangitis in both allo- and autografts. These complications were significantly less when using a direct bile duct to bile duct anastomosis in similar groups of animals. Similar findings have been reported by Calne (32), and MacSween (120), while Lempinen (112) reports 2 completely satisfactory long term results with choledochocholedochostomy.

Immelman (99), working in the same laboratory, has reported results similar in principle to those of Dent, when using a heterotopic porcine model. In Immelman's animals, a gallbladder to gallbladder anastomosis produced significantly less cholestasis and cholangitis than biliary drainage via a Roux-Y cholecystojejunostomy or external biliary drainage.

There appears to be little doubt that direct biliary enteric anastomoses predispose to cholestasis and cholangitis, and that the incidence of these complications is less when utilising the

recipient's distal biliary tract for final bile drainage. The exact role played by the sphincter of Oddi in preventing these complications is uncertain.

The upper abdomen is best suited to biliary-biliary anastomosis in auxiliary liver transplantation and provides a further indication for placement of grafts as close to the diaphragm as possible. The problems associated with biliary tract reconstruction form a major part of this presentation and have been investigated in the human cadaver study in Part I, and in a heterotopic porcine transplant model in Part II.

(5) The problem of the diagnosis of rejection

All major surgical procedures on the liver or biliary tract have a significant incidence of complications which tax the diagnostic and management skill of the surgeon. In liver transplantation these postoperative problems are further complicated by the occurrence of varying degrees of rejection, and by the use of immunosuppresive drugs that are known to have wide-ranging malevolent side effects.

In all organ transplantation, the continued viability of the graft is largely dependent on the early identification of rejection and the prompt institution of correct treatment. In both clinical and experimental liver transplantation the early detection of rejection per se, as well as its differentiation from cholangitis, cholestasis and other complications, has remained a major problem. (6, 15, 32, 204, 223).

The presence of two livers in auxiliary transplantation compounds the problem of the early diagnosis of rejection, as function, or dysfunction of the recipient's own liver (75) may modify clinical, biochemical, haematological and other factors, and render them invalid as an index of graft status. Tests based on immunological changes, such as the leucocyte migration test (60) are in their infancy, and have not proved to be of consistent value in the diagnosis of rejection (19, 59).

Angiographic and radio-isotopic (66, 204, 217) methods are expensive, and repeated investigations expose the patients to excessive irradiation and add to the already costly therapeutic regime. In the presence of two closely apposed livers, percutaneous needle biopsies are unreliable, and repeated laparatomies for diagnostic purposes are impractical.

An urgent need exists to develop a method of diagnosing the onset and degree of rejection at an early stage. Ideally, a single easily reproducible test is required that can be done on a daily basis, and that reflects the status of the graft only.

At present, no such test exists, and rejection and other complications are identified by the combination of clinical features, a battery of biochemical and haematological investigations, immunological studies, radiographic investigations, radio-isotopic studies and often culminate in percutaneous needle biopsy or possibly even laparotomy (180). The whole process of elimination is onerous and dangerous to the patient, and costly in terms of staff time and money.

Immelman (97) has suggested that the cerebrospinal fluid (CSF) glutamine levels may be a useful index of donor liver function in auxiliary liver transplantation. A controlled, comparative study of the relationship between donor liver quality and CSF glutamine will be presented in Part III of this experimental work.

CRITERIA FOR SUCCESSFUL HETEROTOPIC TRANSPLANTATION

The foregoing resume of the clinical and experimental experience in both orthotopic and heterotopic liver transplantation enables one to propose a set of theoretical criteria essential for optimum function and maintenance of graft size in auxiliary transplantation.

The donor liver should have:-

(1) Adequate space for its accommodation

(2) Adequate low pressure hepatic venous outflow

(3) An adequate arterial inflow to both liver and biliary tract

(4) An adequate portal venous inflow

(5) Adequate, secure, non-obstructive biliary drainage with utilisation of the host's own biliary tract where possible

(6) Adequate fixation to prevent kinking of blood vessels and biliary tract.

In addition, the transplant team should have at its disposal, a simple, reliable test for distinguishing between rejection in the donor liver, and other complications.

SOLUTION OF SOME OF THE PROBLEMS BY A NEW TECHNIQUE

In 1972 Immelman (97) reported a new technique of auxiliary liver transplantation in the pig, which closely approaches the ideal criteria mentioned on page 32. The technique provides a near-physiological environment for the donor liver, while at the same time providing a handicap to the host liver function.

Immelman's description of the technique is as follows:-

> "The liver is inserted immediately caudal to the host liver. Venous drainage is via the suprahepatic cava with anastomosis to the host suprarenal vena cava. Venous inflow is via end-to-end portal vein anastomosis, thus depriving the host liver of direct access to portal blood. An arterial supply is provided from the donor aorta"

In 25 animals biliary drainage was by direct gallbladder to gallbladder anastomosis.

Using this technique, Immelman reported good quality grafts with avoidance of donor liver atrophy in 9 animals. Histological quality was excellent with no evidence of cholestasis or cholangitis (99). Thrombotic vascular complications occurred in 6, mild to moderate donor atrophy occurred in 4 and severe rejection in 2 animals. Four animals survived less than 3 days.

Using the same basic model in 10 additional experiments, he reported a high incidence of cholestasis and cholangitis when cholecystenteric or external biliary drainage was used (99). His results stressed the importance of the type of biliary anastomosis in heterotopic liver transplantation. Dent (55), working in the same laboratory, has made similar observations on biliary drainage using an orthotopic pig model.

In addition, Immelman reported that the study of cerebrospinal fluid glutamine levels in a few of the transplant and control animals suggested that this biochemical estimation may offer an index of donor liver function during life (97).

RATIONALE FOR FURTHER STUDIES WITH THE HETEROTOPIC TECHNIQUE

The indisputable need for improved forms of therapy for patients with fatal liver diseases has been briefly reviewed. Liver transplantation, on a temporary or permanent basis, would, in theory, be an adequate curative procedure for many of these patients. In practice, orthotopic, and in particular, auxiliary liver transplantation, have not added significantly to the survival or quality of life in the group of patients who have undergone this form of treatment.

Major reasons for the poor results have been the problem of donor liver procurement and preservation, selection of the optimal stage of the disease to perform the transplant, the technical problems associated with the operations, and the inability to recognise and treat rejection adequately in the postoperative period. These problems are common to both the liver transplantation techniques, and indeed, to all forms of organ transplantation.

In both orthotopic and heterotopic liver transplantation, significant clinical and experimental failure has been due to complications related to the biliary tract reconstruction.

In addition to these common problems, the development and application of auxiliary liver transplantation has been hampered by the inability of research workers to derive technique which is satisfactory both anatomically and physiologically.

It is clear that the results of liver transplantation cannot improve unless the technical and management problems can be overcome by comprehensive laboratory and clinical investigation. Wide clinical application of the technique demands prior solutions to these problems. Immelman's heterotopic porcine model appeared to be an advance in auxiliary transplantation technique, as it embodied desirable anatomical and physiological principles, avoided direct biliary enteric anastomosis, and the results were promising. The feasibility of the technique in the human, however, remained in question.

From the management point of view, Immelman suggested that CSF glutamine might be a valuable index of donor liver function in the heterotopic model. The relationship of CSF glutamine to donor liver quality and function had, however, not been established decisively. The lack of a simple test specific for donor liver function in the heterotopic model has been stressed, and demands further investigation of any simple test that appears promising.

In view of the promising theoretical and practical implications of Immelman's preliminary report, it was decided to undertake further experimental studies relating to the transplantation technique, and the observations regarding CSF glutamine. The experimental studies were designed and undertaken with three major objectives in view, and will be presented in three parts.

The first objective was to assess the clinical feasibility of Immelman's heterotopic technique by means of a human cadaver study. All the technical aspects of the technique would be assessed, with special emphasis on the biliary tract reconstruction. Should any modifications to his

original technique be deemed necessary, these would be developed and assessed in the cadaver study. The human feasibility study is detailed in Part I of this presentation.

The second objective was to apply any modifications found to be necessary in the human cadaver study, to a series of live porcine transplants. The effects of these modifications would be assessed, and compared with those seen in a parallel series of transplants using Immelman's unmodified technique. Pigs subjected to portacaval shunts, and sham laparotomies, would serve as additional controls. The comparative study is presented in Part II of this report.

The third objective was to assess fully the value of CSF glutamine as an index of donor liver quality in this heterotopic model, and to compare its value with the information provided by other biochemical and haematological factors. Cerebrospinal fluid, blood and liver biopsies from the animals used in Part II, would be used in this part of the study. The comparative evaluation of CSF glutamine is detailed in Part III of this presentation.

PART I

THE HUMAN FEASIBILITY STUDY.

INDEX TO PART I

THE HUMAN FEASIBILITY STUDY

PART I

SUMMARY

The design and conduct of the human feasibility study is described in detail. The basic subhepatic liver transplantation technique, as described by Immelman, is shown to be feasible in most of the cadaver transplants that were studied. Reconstruction of the biliary drainage tract by means of cholecystocholecystostomy was, however, only possible in 50% of the cases.

A new technique is described, whereby the gap between two widely separated gallbladders can be bridged by the interposition of an isolated, vascularised, isoperistaltic jejunal loop. Cholecystojejunocholecystostomy could be performed in all the cadaver transplants. The merits and demerits of different types of biliary reconstruction are briefly discussed.

PART I

CHAPTER 1

INTRODUCTION AND AIMS OF THE HUMAN STUDY

INTRODUCTION

The problems specifically associated with auxiliary liver transplantation in man and experimental animals, have been discussed in the Main Introduction. The spectrum of problems ranged through the provision of space for the donor liver, the optimal situation for the graft, the creation of physiologically satisfactory vascular in- and outflow tracts, and the institution of adequate bile drainage. The review of these problems led to the formulation of a set of criteria (page 32) that, theoretically, would provide the donor liver with optimal conditions for adequate survival and function.

The features of a new technique of liver transplantation, described by Immelman, were reviewed. In principle, Immelman's technique embodied most of the desired criteria. Immelman had developed the technique in pigs, and in his hands the technique had provided highly satisfactory results compared to results he achieved using other heterotopic models in the same laboratory.

Immelman's experience with the technique had been confined to pigs. In view of the promising results he reported, a study was designed and under-

taken to assess the feasibility of the technique in a human cadaver study, and to develop any modifications that appeared necessary.

AIMS OF THE HUMAN STUDY

The study was designed to answer the following questions:

1. Is the basic technique described by Immelman possible in humans with regard to:
   (a) The Subhepatic position?
   (b) Linking up the vascular circuits?
   (c) Constructing the biliary drainage by means of a gall-bladder to gallbladder anastomosis?
   (d) Providing physical stability for the donor liver?
   (e) Accommodating the donor liver within the abdominal cavity?

2. What modifications would be necessary in the human?

An early pilot study in human cadavers suggested that the donor and recipient gallbladders could not be apposed easily in all cases. Consequently the idea was entertained of bridging the gap between the donor and recipient gallbladders by the interposition of an isolated, vascularised isoperistaltic loop of jejunum. In addition, the feasibility of an end-to-side bile duct to bile duct anastomosis would be investigated.

PART I

CHAPTER 2

MATERIALS AND METHODS

The study was undertaken in a mortuary. Twenty-six human cadaver transplants were performed.

DONOR LIVER PROCUREMENT

Donor hepatectomy was performed according to the method described by Starzl (181), and was basically similar to the technique used in the porcine donor hepatectomies, to be described in Part II.

The suspensory ligaments were incised close to the diaphragm leaving an adequate length attached to the liver. The common bile duct and portal vein were isolated, mobilised and transected immediately above the second part of the duodenum. The hepatic artery was mobilised from hilum to coeliac axis. A length of thoraco-abdominal aorta bearing the coeliac axis was mobilised, and excised in continuity with the hepatic artery and liver. The suprahepatic and infrahepatic vena cavae were transected.

The liver with attached vessels was removed from the cadaver, examined and weighed. The suprahepatic vena cava was trimmed flush with the liver. The length, body build and age or approximate age, of the

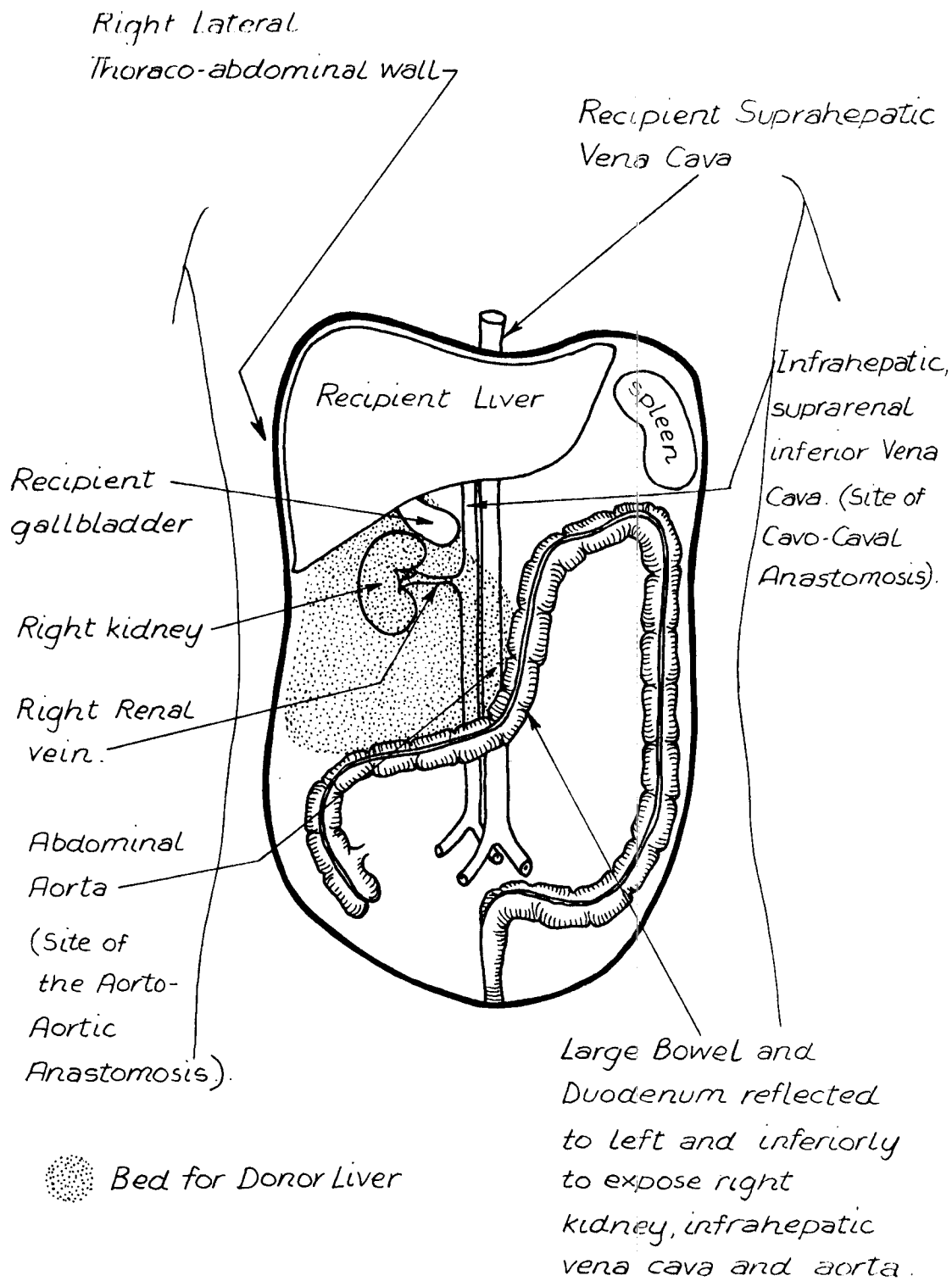


FIGURE 1 DIAGRAM OF THE BED WHICH HAS BEEN PREPARED FOR THE DONOR LIVER IN MAN

(Study this diagram in conjunction with the photograph in Figure 10, page 78, which shows the bed in the recipient pig)

donor was noted. The excised liver was transplanted into as many cadavers as time and availability would permit on that day.

TRANSPLANTATION PROCEDURE

The recipient's length, build and age were noted.

1. Preparation of the graft bed

Where possible, implantation was performed through a long midline incision prior to the chest being opened, or the abdominal contents being disturbed by the pathologist.

The ascending colon, right hepatic flexure, right transverse colon and duodenum were mobilised and reflected to the left and inferiorly, to expose the vena cava and aorta, and to provide a bed for the graft, as shown in Figure 1 opposite.
The recipient's portal vein was mobilised and transected close to the hilum of the liver.

2. Positioning of the donor liver

The donor liver was placed in the right paravertebral gutter immediately caudal and posterior to the recipient's liver, with the hilum and gallbladder facing anteriorly; the suprahepatic vena cava directed towards the host's subhepatic suprarenal inferior vena cava, and the right lobe directed towards the right inguinal region. The left lobe of the donor liver, especially if abnormally long, created an immediate problem for high implantation. It could be placed either between the host liver and posterolateral thoracoabdominal wall, as shown in Figure 5a on page 50F, or folded medially across the hilum, as shown in Figure 5b , page 50F,

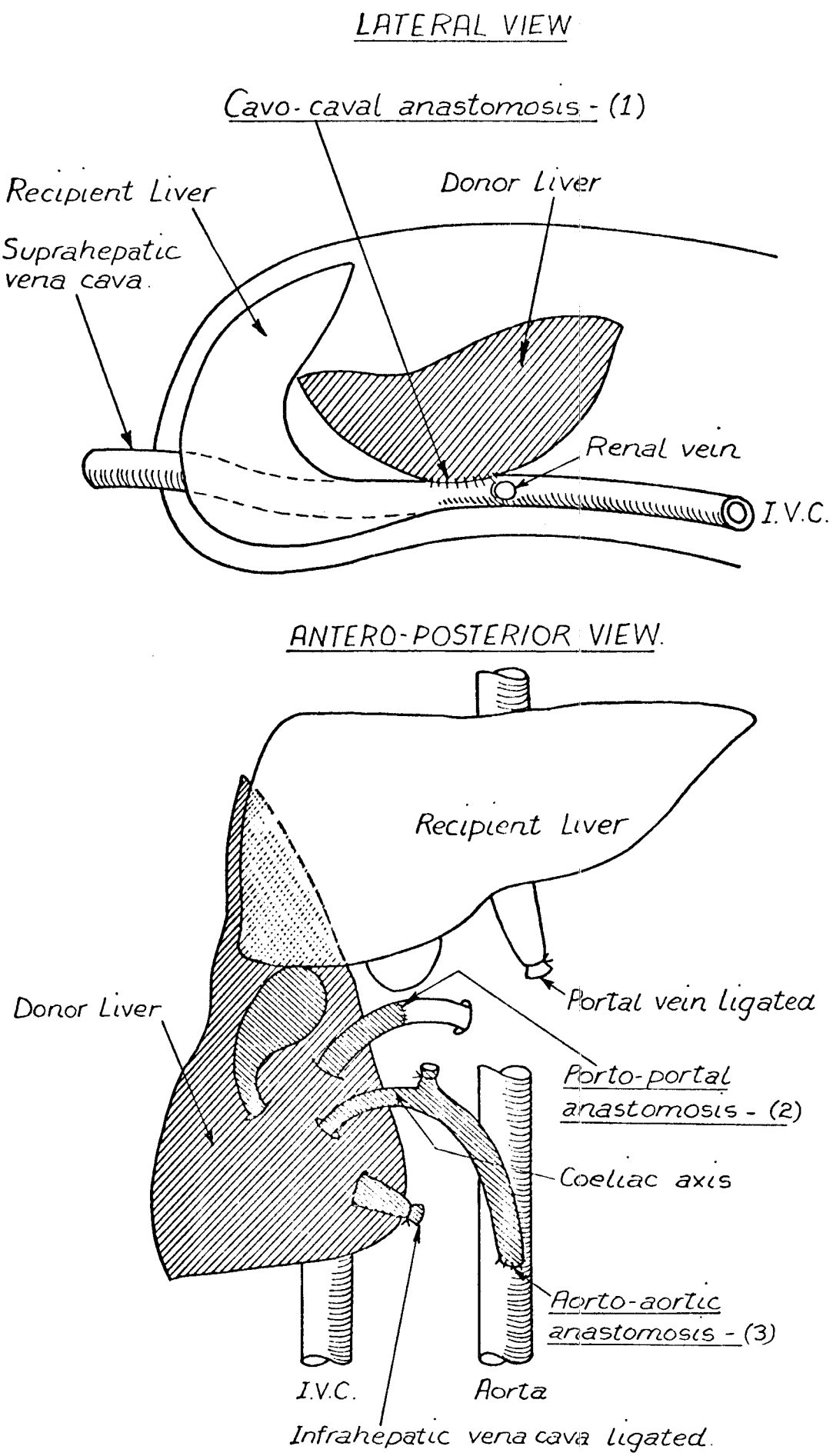


FIGURE 2 DIAGRAM OF THE BASIC TRANSPLANTATION TECHNIQUE IN MAN

in which case the liver tended to rotate away from the position described by Immelman. The livers were assessed in both positions.

3. Reconstruction of the vascular circuits

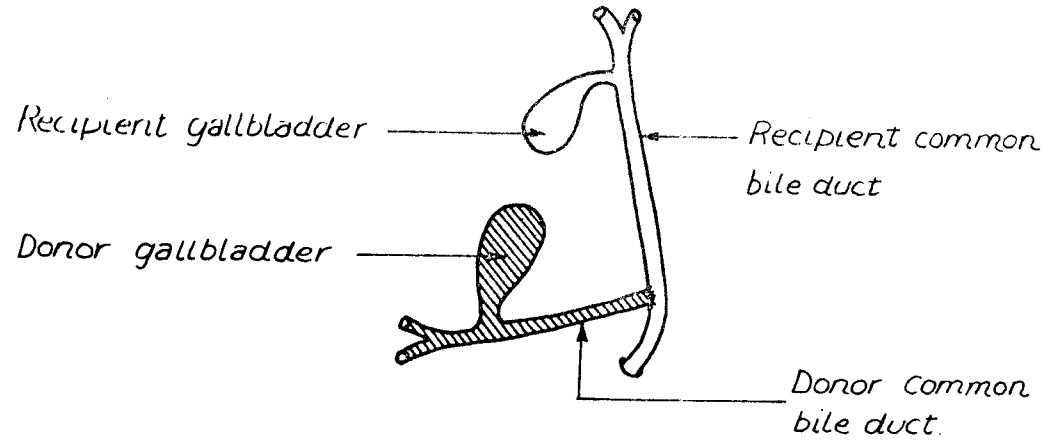
The short suprahepatic donor vena caval cuff was sutured end-to-side to the infrahepatic host vena cava as cephalad as possible above the renal veins using 000 silk sutures. The host and donor portal veins were anastomosed end-to-end with 000 silk sutures after vessel lengths had been trimmed to avoid kinking and tortuosity. The donor aortic cuff to which the coeliac axis and hepatic artery were attached, was trimmed and anastomosed end-to-side to the host aorta immediately distal to the renal arteries, using 000 silk. Vessel positions and anastomotic problems were noted. A sketch of the basic technique is illustrated in Figure 2 opposite.

4. Reconstruction of the biliary drainage tract

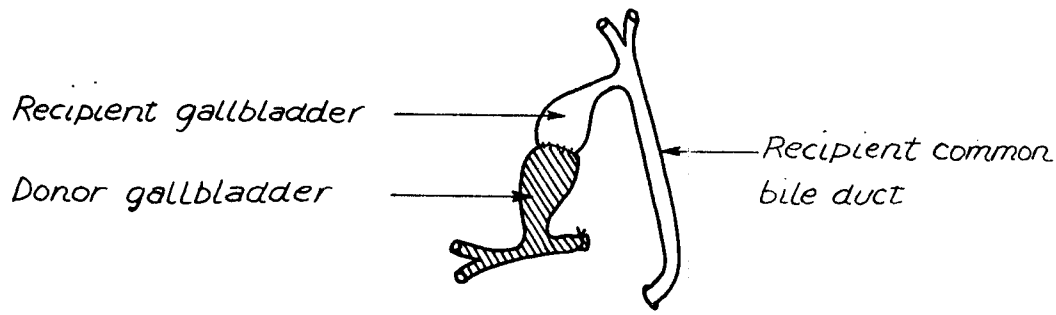
On completion of the vascular anastomoses, three methods of biliary anastomosis were attempted in each case. The three methods are illustrated in Figure 3 on page 46F.

(i) Choledochocholedochostomy (CBD-CBD)

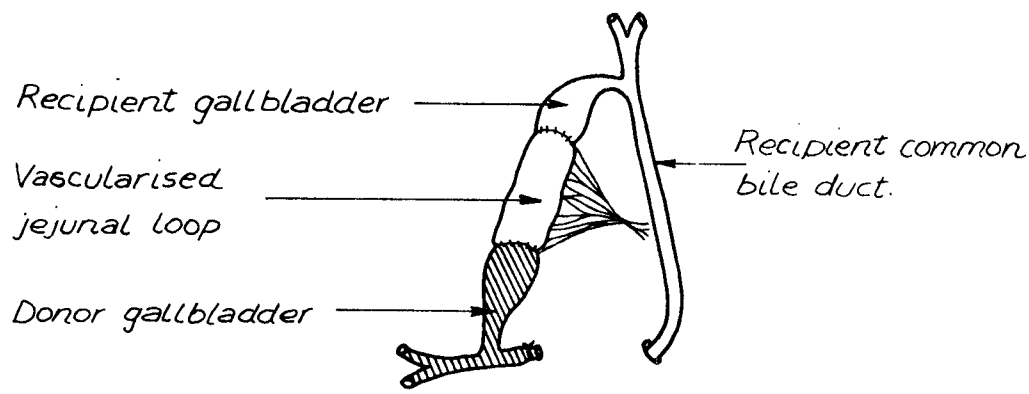
An end-to-side bile duct to bile duct anastomosis was created between the distal donor common bile duct and the host common bile duct, using four 000 silk sutures. After assessing the result the anastomosis was taken down.



(a) CHOLEDOCHOCHOLEDOCHOSTOMY (CBD-CBD)



(b) CHOLECYSTOCHOLECYSTOSTOMY (GB-GB)



(c) CHOLECYSTOJEJUNOCHOLECYSTOSTOMY (GB JEJ GB)

FIGURE 3 THE THREE TECHNIQUES OF BILIARY DRAINAGE ASSESSED IN THE HUMAN CADAVER STUDY

(ii) Cholecystocholecystostomy (GB-GB)

The fundi of the donor and recipient gallbladders were anastomosed end-to-end using four 000 silk sutures. No attempt was made to mobilise the gallbladders because it was felt that the blood supply from the gallbladder beds should be left undisturbed, and the possibility of kinking and tension on the cystic ducts should be avoided. The result was assessed, and the anastomosis taken down.

(iii) Cholecystojejunocholecystostomy

(Interposition of an isolated, vascularised, isoperistaltic jejunal loop). (GB-JEJ-GB)

A loop of jejunum 20-30 cms. distal to the ligament of Treitz was brought up through a window made in the mesocolon lateral to the middle colic artery. A suitable length was selected and transected at both ends and the mesentery divided, as shown in Figure 4 on page 47F.

The vessels supplying the segment were kept intact according to the method of Roux (123). The loop was rotated through 180^{o} to ensure isoperistaltic flow, and the proximal end of the loop was anastomosed to the donor gallbladder fundus and the distal end to the recipient fundus. The loop would thus act as a conduit between the donor and recipient gallbladders. After re-establishing jejunal continuity, the jejunum was replaced, and the hole in the transverse mesocolon closed. The result was assessed, with special attention to tension in the conjoined biliary

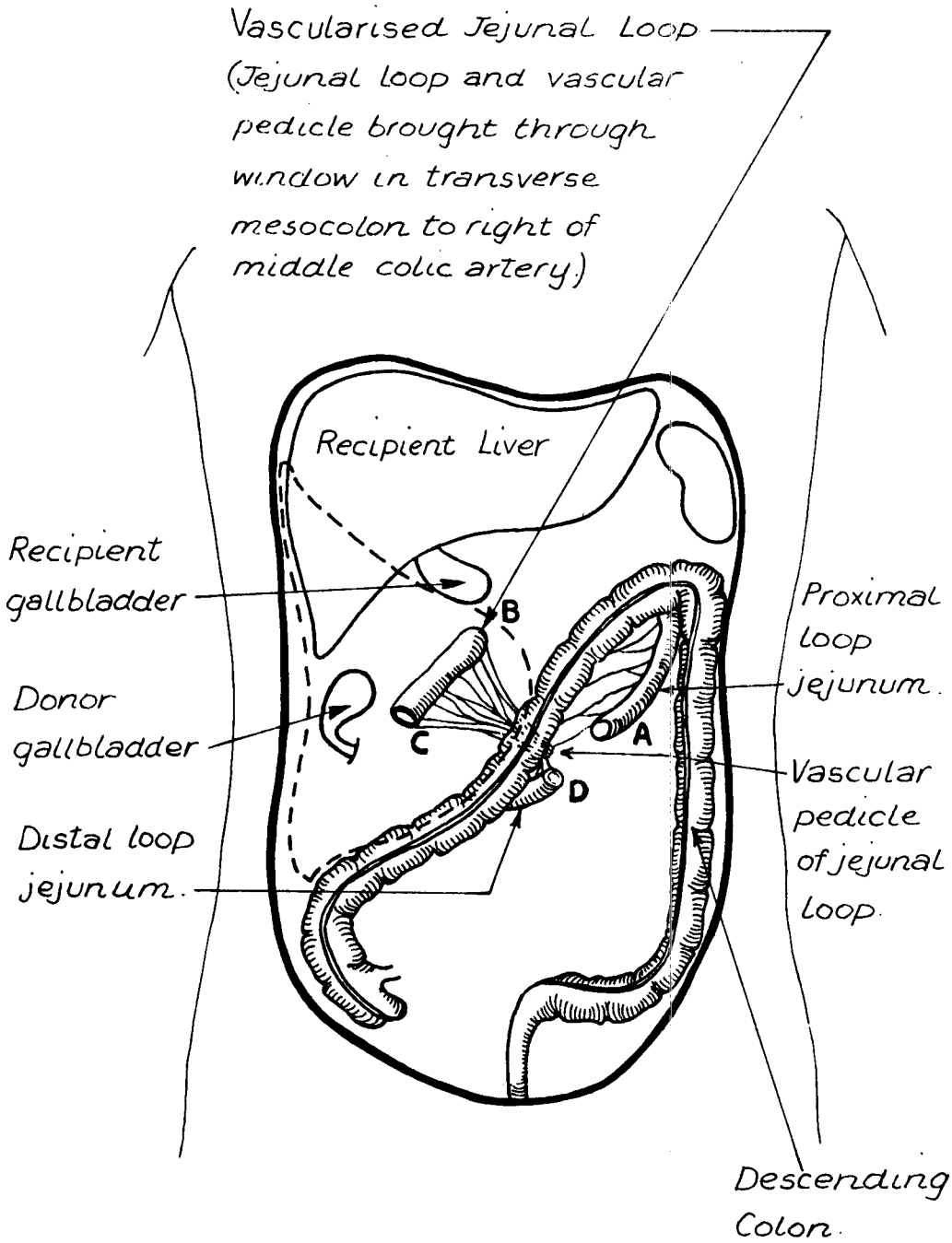


FIGURE 4 THE TECHNIQUE OF JEJUNAL INTERPOSITION IN MAN

The vascularised jujunal loop (B-C) is brought up through the transverse mesocolon, rotated through 180°, and end C is anastomosed to the recipient gallbladder, and end B to the donor gallbladder. Jejunal continuity is re-established by anastomosis of loop A to loop D. Study this diagram in conjunction with Figures 18 and 19 on pages 85 and 86.

apparatus, and tension in the mesentery of the isolated loop.

5. Additional donor liver stabilisation

The need for additional graft stabilisation was assessed by manipulating the graft, and by estimating possible movements that would occur with changes in posture. The feasibility of creating additional stability for the graft by tethering the donor falciform ligament and the ligamentum teres to the diaphragm, lateral abdominal wall and suspensory ligaments of the recipient, was assessed.

6. Accommodation for the donor liver

The feasibility of accommodating the graft in the abdominal cavity was roughly estimated by attempting to close the abdomen in those cases where the normal anatomy had not been unduly disturbed by the autopsy procedure. Closure was attempted using 1/0 linen suture. No omentectomy was performed prior to attempted closure.

At the end of the investigations, the donor liver was removed and returned to the donor body.

PART I

CHAPTER 3

LIMITATIONS AND RESULTS OF THE HUMAN STUDY

LIMITATIONS OF THE STUDY

At the outset of the study, defects and limitations of this type of study were recognised.

1. Limitations due to mortuary procedure

   The transplants had to be carried out swiftly, and without disturbance to the daily routine of the mortuary. Of necessity, several studies had to be performed after the chests had been opened, but with the lungs in situ and diaphragm intact. Photography could not be performed for permanent record. Cadaver lengths were measured, but the cadavers could not be weighed, and in a few cases ages had to be estimated. Livers were weighed on the mortuary scale.

2. Limitations due to the cadaver state

   Attempts were made to use livers and cadavers that had not been cooled in a refrigerator, but this was not possible in all cases. Bodies and organs were often firm and inflexible, making extrapolation to the living state difficult, and in addition, organ

DONOR CADAVER DETAILS				RECIPIENT CADAVER DETAILS						TRANSPLANTATION DETAILS								
											Vascular Anastomoses			Biliary Anastomoses				
Age	Length	Liver weight	Liver condition	Cadaver No.	Age	Sex	Length	Build	Host Liver Condition	Positioning of graft	IVC-IVC	PV-PV	Aorta-Aorta	GB-GB	CBD-CBD	GB-JEJ-GB	Closure	Problems and further comments
5	1,06	NR	Soft	1	A	M	1,65	m	Large. CCF	+	+	+	ND	TF	ND	TF	TF	Nil
				2	A	M	1,74	o	Normal	+	+	+	ND	TF	ND	TF	TF	Nil
				3	A	F	1,60	m	Normal	+	+	+	ND	NP	ND	TF	TF	Recipient GB. deeply embedded in liver.
				4	A	F	1,64	m	Normal	+	+	+	ND	TF	ND	TF	TF	Slight folding of donor liver required for GB-GB anastomosis.
35	1,64	1900	Firm	5	45	M	1,75	l	Normal	ϕ +	+	+	+	TF	* TF	TF	TF	* Anastomotic angle acute. ϕ Only possible if left lobe between liver and diaphragm.
				6	55	M	NR	o	Large. Fatty liver. Cirrhosis.	D	+	+	+	NP	TF	TF	TF	Peritoneal adhesions ++. Large right kidney obstructed implantation. Chest unopened.
New-born	NR	NR	Soft	7	2	M	0,74	-	Normal	+	+	+	ND	TF	TF	TF	TF	Nil. Chest unopened.
A	1,60	1080	Normal	8	A	M	1,64	s	Normal	+	+	+	+	TF	TF	TF	NR	Nil
				9	A	M	1,64	s	Normal	+	+	+	+	TF	TF	TF	TF	Nil. Chest unopened.
A	1,80	1750	Stones in GB. Entire GB firmly adherent to liver	10	A	M	1,60	m	Normal	+	+	+	+	* TF	TF	TF	TF	* Only if left lobe tucked in under right diaphragm. Chest unopened. Easy suspension.
				11	A	F	1,62	o	Large fatty liver	D	+	+	+	NP	MT	TF	ET	Large perinephric fat pad pushed liver anteriorly.
50	NR	1350	Normal	12	50	M	1,75	o	Normal	+	+	+	+	* ET	TF	TF	TF	* Possible with contortion. Chest unopened.
				13	50	M	NR	s	Normal	+	+	+	+	* ET	MT	TF	NR	* Only by tension and twisting liver. Suspension easy FL-FL.
				14	35	M	NR	m	Normal	+	+	+	+	NP	TF	TF	NP	Chest unopened. Omentum easily covers GB anastomosis.
10	NR	900	Normal	15	45	M	1,80	l	Normal	+	+	+	+	* ET	TF	TF	TF	* Possible with contortion. Suspension easy. Chest unopened.
				16	45	M	1,80	o	Normal	+	+	+	+	* ET	TF	TF	TF	* Possible with contortion. Chest unopened.
				17	15	M	1,62	s	Normal	+	+	+	+	MT	MT	TF	TF	Easy implantation and closure in apparently small abdominal cavity. Chest unopened.
20	1,73	1430	Normal	18	45	M	1,80	l	Normal	+	+	+	ND	* ET	TF	TF	TF	* Possible with contortion. Suspension easy. Chest unopened.
				19	45	M	1,80	o	Normal	+	+	+	ND	MT	MT	TF	TF	Chest unopened.
				20	15	M	1,62	s	Normal	D	+	+	ND	MT	MT	TF	NP	Large, lengthy left donor lobe difficult to place. Transplant appears impossible. Chest unopened.
30	1,45	1380	Normal	21	60	M	1,65	s	Normal	+	+	+	+	MT	TF	TF	TF	Suspension easy. Chest unopened.
				22	40	M	1,71	s	GB adhesions ++	+	+	+	+	MT	ET	TF	TF	Oblique suspension. Chest opened, not disturbed.
				23	30	M	1,63	m	Normal	+	+	+	+	* ET	ET	TF	TF	* Only with contortion. Suspension easy. Chest opened, not disturbed.
				24	50	F	1,52	o	Adhesions++	D	+	+	+	NP	TF	TF	ET	Very large omentum. Suspension easy. Chest opened, not disturbed.
				25	A	M	1,83	l	CCF. Ascites. Firm. Large.	+	+	+	+	NP	ET	TF	TF	Space no problem despite huge firm liver.
50	1,80	1580	Normal	26	A	M	NR	s	Normal	+	+	+	+	* ET	ND	TF	ET	* Possible with contortion. Chest unopened.

Key:
A = Adult, M = Male, F = Female, s = slender, m = medium, l = large, o = obese

+ = Problem free
TF = Tension free
MT = Moderate tension
ET = Excessive tension with contortion
NP = Not possible
NR = Not recorded
ND = Not done
D = Difficult

IVC-IVC = Vena caval anastomosis
PV - PV = Portal venous anastomosis
GB-GB = Cholecystocholecystostomy
CBD-CBD = Choledochocholedochostomy
GB-JEJ-GB = Cholecystojejunocholecystostomy
FL = Falciform ligament
CCF = Congestive cardiac failure

TABLE 4 SUMMARISED DETAILS OF THE HUMAN FEASIBILITY STUDY

positions and shapes in the live state may vary from those seen in the cadaver state (77). Potential haemodynamic problems could not be assessed accurately, nor could the actual effect of postural changes be assessed. Attempts at abdominal closure might not reflect the true in vivo state. Bearing these defects in mind, it was nevertheless felt that useful information had been obtained from the cadaver study.

RESULTS OF THE STUDY

Twenty-six transplants were studied. The results are summarised in Table 4, opposite. The basic technique described by Immelman in his porcine studies, was found to be feasible in most of the human cadaver transplants performed.

1. Preparation of the graft bed

This proved to be a relatively simple dissection in most cases. In obese cadavers with a large perinephric fat pad, the paravertebral gutter was shallow, and satisfactory positioning of the graft was difficult. In two such cases (Cadavers Nos. 6 and 11), the graft appeared unstable and projected anteriorly sufficiently to hamper abdominal closure. The question of right nephrectomy to provide a more spacious bed for the donor liver may be pertinent to such obese subjects.

2. The basic subhepatic position

The subhepatic placement of small grafts in large recipients was technically simple. The larger grafts, and especially those with long left lobes, presented a problem. By placing the left

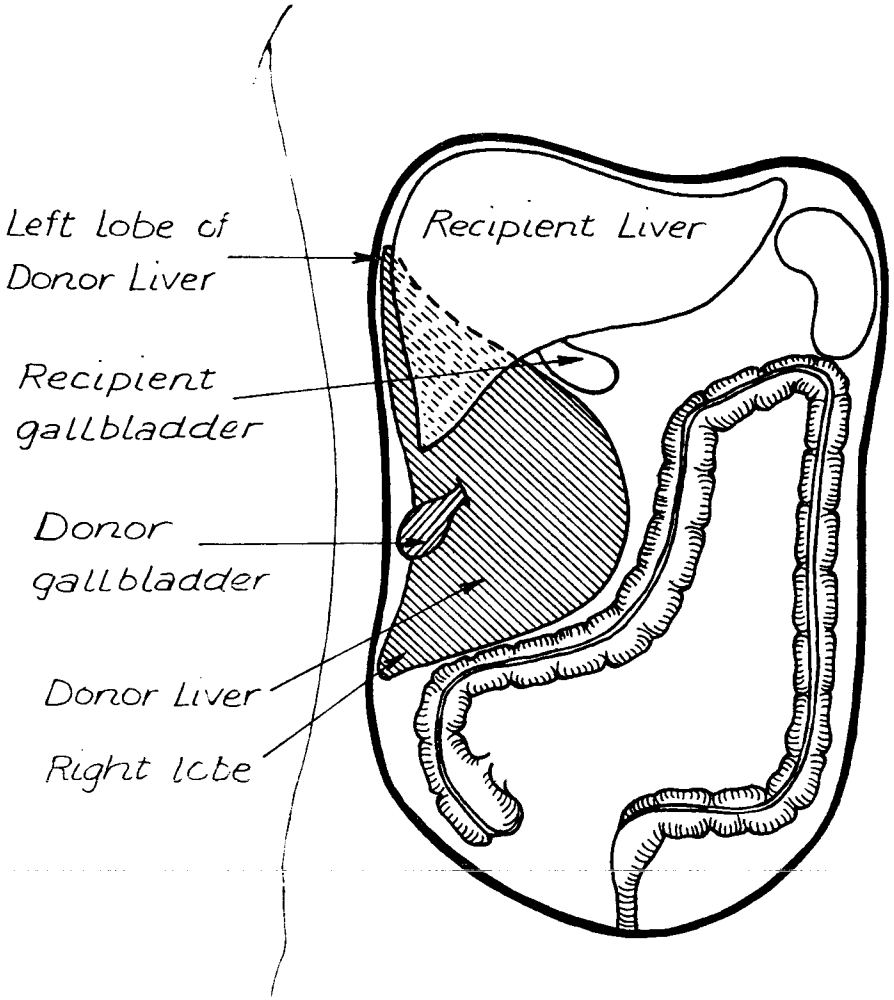


Figure 5a The left lobe of the donor liver placed between the right lobe of the recipient liver and posterolateral thoracoabdominal wall.

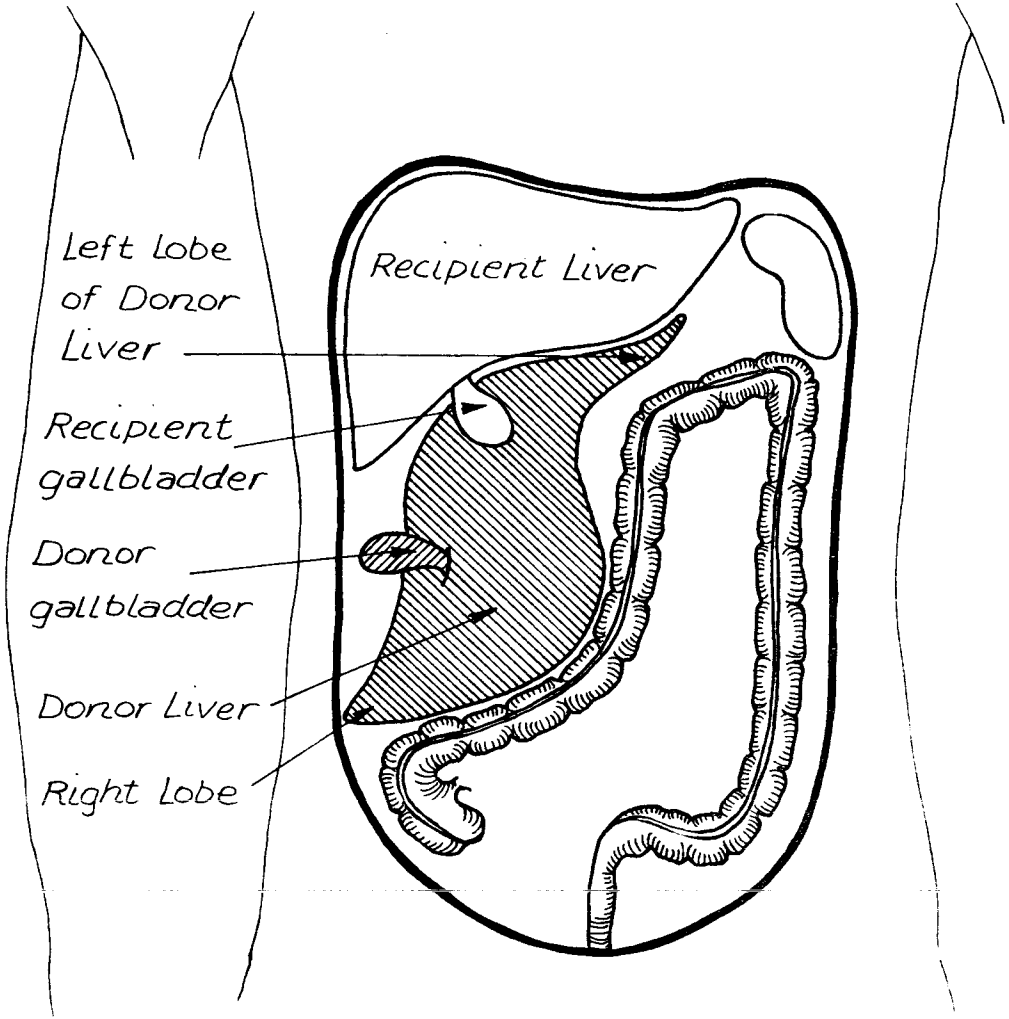


Figure 5b The left lobe of the donor liver folded across the hilum of the recipient liver.

FIGURE 5 DIAGRAM OF THE TWO POSITIONS FOR THE LEFT LOBE OF THE DONOR LIVER

lobe of the donor liver between the recipient's liver and the posterolateral thoracoabdominal wall as shown in Figure 5a on page 50F the grafts were provided with additional support, but in some cases the grafts appeared liable to compression, especially in the face of a host liver of firmer consistency.

Folding the left lobe of the graft medially across the hilum as shown in Figure 5b on page 50F appeared satisfactory until the biliary anastomosis was constructed. Any tension of the conjoined biliary apparatus tended to rotate some of the grafts clockwise around an axis running through the gallbladder and suprahepatic vena cava, as shown in Figure 6 on page 51F, thus twisting the vena caval anastomosis and potentially kinking the portal vein and biliary anastomosis. The former method appeared more satisfactory, because it appeared to provide additional stability, and additional suspension could more readily be constructed.

3. Reconstruction of the vascular circuits

Vascular circuitry could be established in all cases. The end-to-side cavo-caval anastomoses could readily be performed and appeared technically no more difficult than in the live porcine experiments. The possibility of kinking appeared less when using a short donor vena caval cuff. The anastomosis could easily be placed more caudally if operative circumstances dictated this. The portal venous anastomoses posed no problem, but this reconstruction would appear to present potential complications of kinking and tortuosity once all the abdominal contents

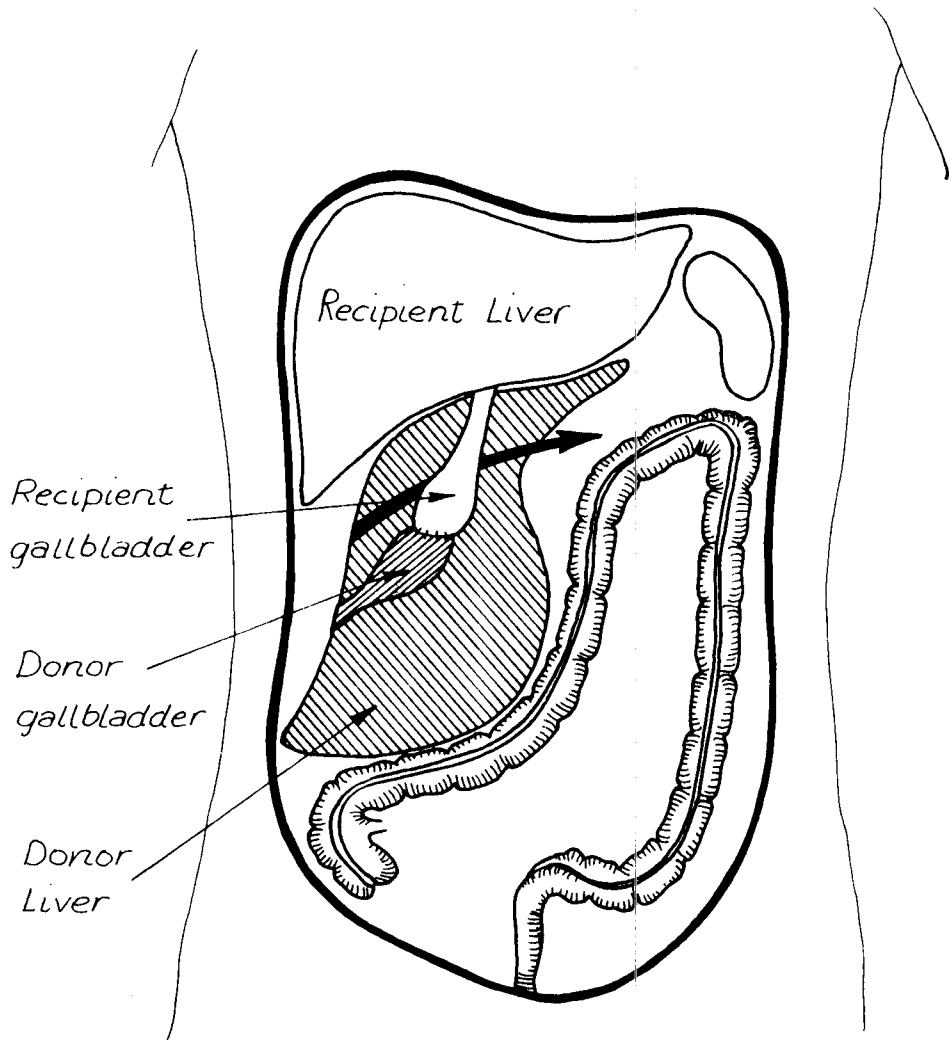


FIGURE 6 ROTATION OF THE DONOR LIVER

(With the left lobe of the donor liver folded across the hilum, tension on the biliary anastomosis caused the donor liver to rotate in the direction shown by the arrow).

are returned and closure effected. Optimum vessel lengths would call for fine judgement. The aortic anastomoses would appear to pose few problems in view of the easy accessibility, and readily variable lengths of the donor aortic cuffs.

In conclusion, construction of the vascular circuitry appears technically feasible from this cadaver study, but no assessment of haemodynamic efficiency could be made.

4. Reconstruction of the biliary drainage tracts

(i) Choledochocholedochostomy (CBD-CBD) (Table 5, page 52F)

End-to-side common bile duct to common bile duct anastomosis appeared possible and tension-free in 13 cases. In 5 cases the tension was moderate and in 3 cases excessive, and caused moderate to acute angulation of the host's common bile duct respectively. This procedure would theoretically be possible without tension in all cases, if the donor common bile duct dissection were carried out more distally in the direction of the head of the pancreas. The reported problems of bile duct to bile duct anastomosis in the human (discussed in the Main Introduction) may militate against this form of anastomosis as a first choice in adults.

(ii) Cholecystocholecystostomy (GB-GB)

Direct gallbladder to gallbladder anastomosis without tension was possible in only 8 cases. Moderate tension was seen in 5 cases. Seven anastomoses could only be constructed with undue anastomotic tension or contor-

DRAINAGE PROCEDURE	ASSESSMENT				
	TF	MT	ET	NP	NR
End-to-side Choledochocholedochostomy (CBD-CBD)	13	5	3	0	5
Cholecystocholecystostomy (GB-GB)	8	5	* 7	6	0
Cholecystojejunocholecystostomy (GB-JEJ-GB)	26	0	0	0	0
Donor Cholecystojejunostomy (GB-JEJ)	"26"	-	-	-	-

TF = Tension Free
MT = Moderate Tension
ET = Excessive Tension
NP = Not Possible
NR = Not Recorded
* = Anastomoses only possible with twisting of the liver and contortion of the anatomy.

TABLE 5 ANALYSIS OF BILIARY DRAINAGE TECHNIQUES IN THE HUMAN CADAVER STUDY

tion of the position of the liver. In six cases apposition of the donor and recipient gallbladders was not possible. The limitations imposed by tense cadaveric gallbladders and firm livers in this survey, however, do not allow for accurate extrapolation to the living subject, as livers in the live state are flexible. The evidence, however, strongly suggests that gallbladder to gallbladder anastomosis would not be possible in every case.

(iii) Cholecystojejunocholecystostomy (GB-JEJ-GB)

The interposition of a loop of jejunum was technically feasible in all cases (Table 5, opposite) and permitted tension-free anastomoses with large stomata. No undue tension was noted in the vascular mesentery of the interposed jejunal loop.

(iv) Cholecystojejunostomy (GB-JEJ)

From the interposed loop findings, it can be extrapolated that a direct anastomosis of donor gallbladder to host jejunum would also be possible in all cases.

The human study demonstrates that at least four different types of biliary drainage can conveniently be constructed with this transplantation technique, and that three of the drainage procedures would satisfy the principle of utilising the host biliary apparatus for ultimate drainage, and thus predispose to less cholangitis and cholestasis in the graft as discussed in the Main

DONOR			RECIPIENTS					
Age		Liver Weight	Abdominal Closure					
Actual	Class	g	No	Class	TF	ET	NP	NR
Newborn	Infant	NR	1	2 year child	1	0	0	0
5	Child	NR	4	Adults	4	0	0	0
10	Child	900	3	Adults	3	0	0	0
NR	Adult	1080	2	Adults	1	0	0	1
50	Adult	1350	3	Adults	1	0	1	1
±30	Adult	1380	5	Adults	4	1	0	0
±20	Adult	1430	3	Adults	2	0	1	0
±50	Adult	1580	1	Adult	0	1	0	0
NR	Adult	1750	2	Adults	1	1	0	0
35	Adult	1900	2	Adults	2	0	0	0

TF = Tension Free
ET = Excessive Tension
NP = Not Possible
NR = Not Recorded

TABLE 6 THE EFFECT OF DONOR LIVER SIZE ON ABDOMINAL CLOSURE IN THE HUMAN CADAVER STUDY

Introduction. The technique to be selected could be tailored to the circumstances encountered at operation.

5. Stabilisation of the donor liver

Basic stability was provided by the cavo-caval anastomosis, and placing the left lobe of the donor liver between the recipient's liver and the lateral thoracoabdominal wall.

Additional lateral stability appeared desirable, and could readily be obtained by suturing the falciform ligament and ligamentum teres to the right lateral abdominal wall at the level of the costophrenic angle. Such tethering appeared to provide good additional support for the graft, although the effects of postural changes could not be assessed.

In light of Starzl's earlier experience (186), the additional suspension would appear desirable.

6. Accommodation within the abdominal cavity

The new-born child's liver was easily accommodated in the 2 year old's abdomen. Using donor livers weighing up to 1 080 g no problems of space or closure were recorded in the adult cadavers, see Table 6, opposite. From 1 350 g upwards a number of cases were found in which closure was difficult or impossible because of lack of space to accommodate the extra organ.

The results thus indicate that the auxiliary liver can be accommodated in most cases, and that a relatively smaller donor liver would provide fewer problems of space and closure.

PART I

CHAPTER 4

DISCUSSION AND CONCLUSIONS

PART I

CHAPTER 4

DISCUSSION AND CONCLUSIONS

DISCUSSION

Bearing in mind the limitations of this human study, the Immelman technique appeared feasible in most of the 26 human cadaver transplants that were performed.

Various aspects of the technique were studied. The preparation of a bed for the donor liver utilises simple, established surgical techniques, and could readily be performed. Placement of the donor liver in the subhepatic position was possible, and in most cases there appeared to be sufficient space in the abdominal cavity to accommodate the additional organ in the heterotopic site. The donor liver appeared to be stable in the subhepatic position by virtue of the cava-caval anastomosis, placing the left lobe between the recipient liver and the lateral thoraco-abdominal wall, and tethering the donor hepatic ligaments to the right lateral thoraco-abdominal wall. The vascular anastomoses could readily be constructed using standard vascular surgical techniques.

The technique allowed for the construction of several different methods of bile drainage. A gallbladder to gallbladder anastomosis could be constructed with ease in 50% of the cases studied. A technique of interposing a vascularised, isoperistaltic loop of jejunum

between the donor and recipient gallbladders was developed, and shown to be possible in all cases. The technique of jejunal interposition involved several additional surgical steps, and it is suggested that the technique be used only when operative circumstances do not permit a gallbladder to gallbladder anastomosis.

Biliary drainage by means of end-to-side common bile duct to common bile duct anastomosis was shown to be feasible, but presented the hazards of anastomotic tension and angulation of the recipient's common bile duct in a significant number of cases.

The interposed jejunal loop could be brought up in all cases, indicating that a standard cholecystojejunostomy could have been constructed in all cases.

The subhepatic position described by Immelman thus allows for at least four alternative methods of biliary drainage, all of which can readily be constructed. The choice of method would be determined, largely, by the operative circumstances, and to a lesser extent by the philosophy of the surgeon. In adults all four methods could be used, while in children with biliary atresia, the choice would be limited to cholecystojejunostomy in most cases.

The subject of choice of biliary drainage procedure will be discussed in more detail in Part II of this presentation.

CONCLUSIONS

The technique of heterotopic liver transplantation which was developed by Immelman in his porcine studies, was investigated in a human cadaver study. The basic technique was found to be feasible. Biliary drainage by means of a gallbladder to gallbladder anastomosis could only be performed in 50% of the cases studied. A means of bridging the gap between two gallbladders which could not be apposed was developed and studied. It was shown that an isolated, vascularised, isoperistaltic loop of jejunum could be used as a conduit in all cases. Two other methods of biliary drainage, namely cholecystojejunostomy and end-to-side choledochocholedochostomy were also shown to be possible with the subhepatic technique.

The positive results obtained from the human study, provided motivation for further experimental studies on the pig. These studies will be detailed in Part II of this presentation.

PART II

THE COMPARATIVE LIVE PORCINE STUDY

GENERAL INDEX TO PART II

THE COMPARATIVE LIVE PORCINE STUDY

* Supplementary Index at the beginning of the Section.

PART II

SUMMARY

The comprehensive details of a live porcine study are presented, analysed and discussed in Part II.

The operations, pre- and postoperative care, and methods of investigation, are presented in detail.

The survival, quality of life, general, regional and local effects are presented.

It will be shown that the technique of jejunal interposition was feasible in the pig and that the general, regional and local effects were similar and comparable to those seen with cholecystocholecystostomy.

PART II

CHAPTER 1

INTRODUCTION AND OBJECTIVES

The review of the literature in the Main Introduction has revealed that complications arising from the reconstruction of the biliary tract have been the major cause of morbidity and mortality in patients who have undergone liver transplantation and survived the immediate postoperative period. The complications ranged through anastomotic leaks and strictures, biliary sludge, calculus formation, necrosis of the gallbladder or common bile duct, and intrahepatic cholestasis, cholangitis and cholangitic abscesses. The same spectrum of complications has been seen in experimental liver transplantation, with both the orthotopic and heterotopic techniques.

Immelman (99) reported a low incidence of biliary tract complications with a newly developed technique of heterotopic transplantation, in which biliary drainage was afforded by a gallbladder to gallbladder anastomosis. The cadaver study in Part I of this presentation has shown that the basic subhepatic transplantation technique described by Immelman in the pig, would be feasible in the human, but that a gallbladder to gallbladder anastomosis would not be possible in all cases. It was further demonstrated that an isolated, vascularised, isoperistaltic loop of jejunum could be interposed between the donor and recipient gallbladders in all the cases where gallbladder to gallbladder

anastomosis was not possible. This modification would allow ultimate drainage of the donor bile through the recipient's sphincter of Oddi, thereby decreasing the risks of cholestasis and cholangitis as discussed in the Main Introduction.

Part II of this study was designed and undertaken firstly to develop the technique of jejunal interposition (cholecystojejunocholecystostomy) in a series of live porcine hepatic allografts, in which Immelman's basic subhepatic technique would be used. This group of allografted recipients, with biliary drainage by means of cholecystojejunocholecystostomy, comprise group 1(b) in the text.

Secondly it was designed to compare the local, regional and general effects of this modification in biliary drainage technique, with the effects seen in a parallel series of heterotopic porcine allografts using the unmodified Immelman technique (bile drainage by means of cholecystocholecystostomy) - group 1(a) in the text.

Two groups of control animals would be established and studied to eliminate local, regional and general effects which could be due to non-biliary causes. The first group of control animals would undergo a sham laparotomy, (group 2(b) in the text) and then be subjected to exactly the same postoperative regime as used for the two allografted groups. The use of a sham-operated control group, would enable one to assess the effects of an operation, the medications used, the housing conditions and repeated anaesthetic procedures, on normal pigs, and to compare the effects with those seen in the two allografted groups.

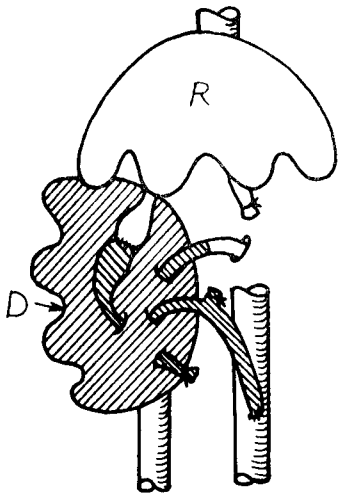
The second control group would undergo end-to-side portacaval shunts, (group 2(a) in the text) and then be subjected to exactly the same

postoperative regime as the allografted groups 1(a) and 1(b) and the sham-operated control group 2(b). The recipient livers in both groups 1(a) and 1(b) would, with the transplantation technique to be used, be deprived of primary access to portal blood, and the use of a control portacaval-shunted group would enable one to establish and compare the local, regional and general effects which could be attributed to porto-systemic shunting in the normal pig.

Bearing in mind the requirements of Part III of this presentation, simultaneous serial samples of liver, CSF, and blood would be obtained from all the animals used in Part II. These samples would be subjected to histological, biochemical and haematological analyses, and used both in the comparative study in Part II, and in the evaluation of CSF glutamine in Part III.

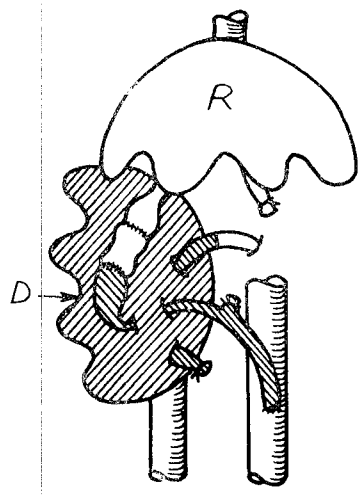
In Part II it will be shown that cholecystojejunocholecystostomy was a successful modification of bile drainage in the porcine model used, and that the local, regional and general effects were comparable to those seen in the group of pigs with bile drainage via a cholecystocholecystostomy. In addition, it will be shown that the incidence of complications involving, or attributable to, the biliary drainage tract, were minimal and comparable in the two transplant groups.

INDEX TO PART II, CHAPTER 2



GROUP 1(a).

Auxiliary transplantation plus cholecystocholecystostomy.

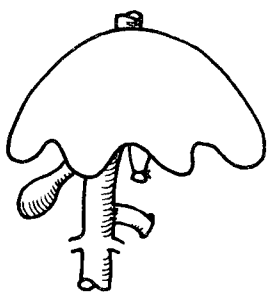


GROUP 1(b)

Auxiliary transplantation plus cholecysto-jejuno-cholecystostomy.

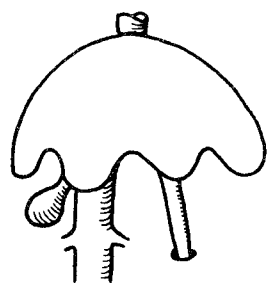
D = DONOR

R = RECIPIENT



GROUP 2(a).

End-to-side portacaval shunt.



GROUP 2(b)

Sham laparotomy

FIGURE 7 THE FOUR EXPERIMENTAL GROUPS

PART II

CHAPTER 2

MATERIALS AND METHODS

THE FOUR EXPERIMENTAL GROUPS

In this chapter, the design and conduct of the experiments is described. Four groups of experimental animals were established, as shown in Figure 7 opposite.

Two groups of pigs were subjected to auxiliary heterotopic liver allotransplantation, the groups differing in the type of biliary drainage used:

Group 1(a) Heterotopic allograft plus cholecystocholecystostomy (GB-GB).

Group 1(b) Heterotopic allograft plus cholecystojejunocholecystostomy (GB-JEJ-GB)

Two groups of pigs underwent control operations:

Group 2(a) End-to-side portacaval shunt (PCS).

Group 2(b) Sham laparotomy (Shams).

Sixty-nine liver transplants, 19 portacaval shunts and 5 sham laparotomies were attempted. The immediate mortality was high in the two allograft groups, as described in Part II, Chapter 3, Section 1. Fourteen animals in each of groups 1(a), 1(b) and 2(a) and 5 in group 2(b), survived in excess of 7 days. The operative details and times

taken to perform the various procedures, are derived from the animals which survived in excess of 7 days.

All animals received the same pre-, intra- and postoperative care, and were subjected to the same clinical, biochemical, haematological, gross, and histopathological analyses.

The pre-operative care, anaesthesia, operative techniques and post-operative care for the groups is described in detail. The methods and frequency of blood, CSF, bacteriological and histological sampling are described.

Finally, the autopsy technique used for all animals is presented.

SELECTION OF ANIMALS

The pigs all originated from farms in the Western Cape. They were supplied by G. Engelbrecht - Philadelphia, and from Champagne Estates, Franschoek. The animals comprised White Landrace, Black Landrace, Large White, and some obviously mixed breeds. On occasions, the suppliers informed us that the pigs had not been bred on their own farms, but had been obtained from other farmers to supply us. The genealogy of the pigs used in these studies could therefore not be established.

Selection of pigs for a particular experiment was random, the only requirement being that the donors should be smaller than the recipients. At operation a crude attempt was made to establish the breed of the pigs. This was done by looking at the ears; pigs with large broad dependent ears were classified as Landrace; those with peaked upright ears, as Large White. In addition, the colour was recorded. Some animals

OPERATION	ANIMAL WEIGHTS (Kg)					
Heterotopic Allotransplantation	Recipient			Donor		
	Mean ± 1 SD	Range	No	Average	Range	No
Group 1(a) (GB-GB)	39,07 ± 3,26	35-46	14	19,6	14-26	14
Group 1(b) (GB-JEJ-GB)	31,64 ± 5,94	22-46	14	17,0	12-25	14
Control Operations	ANIMAL WEIGHTS (Kg)					
	Mean ± 1 SD	Range	No			
Group 2(a) (PCS)	16,2 ± 2,52	13-21	10*			
Group 2(b) (Sham)	18,4 ± 2,80	15-21	5			

TABLE 7 ANIMAL WEIGHTS IN THE FOUR GROUPS.

Group 1(a) - Heterotopic allograft plus cholecystocholecystostomy
Group 1(b) - Heterotopic allograft plus cholecystojejunocholecystostomy
Group 2(a) - End-to-side portacaval shunt
Group 2(b) - Sham laparotomy

* The pre-operative weights of 4 animals were not recorded.

used were of mixed breed and did not fall clearly into either Landrace or Large White categories. The sex of the animals was not considered in their selection.

The weight profiles of the animals used in the experiments are tabulated on the opposite page. The mean weights of the groups were fortuitous and dictated by the logistics of supply.

PRE-OPERATIVE MANAGEMENT

After delivery from the farms, the animals were kept in an enclosed sty, and allowed free access to water and commercial pig food. The pigs were routinely dewormed twice after birth, and once in our sty after delivery from the farms.

The animals were starved for 24 hours preceeding operation, but had free access to water. No pre-operative antibiotics were administered, nor were bowel preparations performed.

ANAESTHETIC PROCEDURES

The techniques of anaesthesia and intubation were basically similar to those described by Bowes (28) and Terblanche (197).

Two methods of anaesthesia were used:

1. Definitive operations - Closed Circuit Administration of Halothane and Nitrous Oxide

   Anaesthesia was induced with Sodium Pentothal (1-1,5 gm) injected into an ear vein. Animals were then weighed, endotracheal intubation performed and anaesthesia maintained with Oxygen,

Nitrous Oxide and Halothane. Intermittent positive pressure respiration was instituted using either a "Bird" or "Manley" respirator, with a closed Magill-type circuit. The gases were warmed and humidified, and the percentage of Halothane kept as low as possible. The stomach was decompressed by the insertion of a large bore stomach tube, which was kept in situ throughout the operation. This method of anaesthesia was used for the initial operation in all animals.

2. Serial minor procedures - Halothane and Nitrous Oxide administered via a nose cone.

For all serial sampling procedures and at sacrifices, anaesthesia was induced and maintained with Halothane, Nitrous Oxide and Oxygen administered through a nose cone.

OPERATIVE PROCEDURES

1. AUXILIARY HETEROTOPIC LIVER ALLOTRANSPLANTATION: (GROUPS 1(a) and 1(b))

The basic technique used was that described by Immelman (97), and the operations were organised along the lines described by Dent (53). The operative techniques on both donor and recipient were adapted from those described by Terblanche (197). Porcine anatomy was studied from Sisson and Grossman's textbook (169). In essence the operations were carried out in 4 stages - (i) the donor liver was skeletalised, (ii) the recipient prepared, (iii) the donor liver perfused and excised, and (iv) implanted into the right upper quadrant immediately caudal to the pig's own liver. The sequence of the procedures was organised so as to ensure minimal graft ischaemia and short portal bypass times.

(1) STAGE 1 - Preparation of the donor animal

An initial lumbar puncture was performed. The anaesthetised pig was placed on the operating table in the left lateral position and held in slight spinal flexion. A disposable 20 gauge spinal needle was inserted, in the midline, into the lower lumbar vertebral canal and 3 ml of cerebrospinal fluid (CSF) collected into a clean tube. The CSF sample was immediately spun down in a centrifuge, the supernatant decanted and placed in a refrigerator. The animals were then turned onto their backs and secured in the operative position.

Cannulation of the cervical vessels was performed next. A vertical incision was made on the right side of the neck to expose the jugular vein and carotid artery. Both vessels were cannulated with sterile polythene infant feeding cannulae (French Size 8). The arterial cannula was used for blood sampling and blood pressure monitoring, while the venous cannula served as an access to venous blood and an inlet for intravenous fluids, antibiotics and other medication. Venous and arterial blood samples were taken as soon as the cannulae had been placed.

Skeletalisation of the donor liver was then carried out.

(i) With the use of a cautery knife a long midline incision was made from xiphisternum to pubis, deflecting laterally to avoid the urethra in males. The peritoneum was opened, the abdomen explored and any pathology noted. A wedge liver biopsy was taken and immediately placed in formol saline. The gallbladder was inspected, and the cystic and common ducts palpated.

(ii) With retraction of the bowel inferiorly and to the left, the right lobe of the liver was elevated to expose the peritoneal attachments of the liver and inferior vena cava to the posterior abdominal wall. Vessels in the ligament were coagulated, and the ligament incised from the adrenal below to the suprahepatic vena cava above.

(iii) Dissection of the portal tract followed. With the stomach retracted downwards, and the liver upwards, the anterior layer of the gastro-hepatic omentum was incised from the foramen of Winslow to the oesophagus as described by Dent (53). A further incision was made in line with the hepatic artery in the direction of the coeliac axis. Dissection was carried out to the left of the hepatic artery, and the gastroduodenal artery and coronary veins were ligated. The portal vein was dissected clear of peritoneum and attached lymph nodes, from pancreas to hilum, and haemostasis secured.

(iv) The hepatic ligaments were next incised. The liver was retracted down, and left triangular ligament incised to the suprahepatic vena cava, and the small falciform ligament divided.

(v) Isolation of the coeliac axis and suprarenal aorta followed. The bowel and spleen were retracted inferiorly and to the right. The splenic artery was isolated, and the overlying peritoneum incised down to the coeliac

axis and aorta. The coeliac axis was cleaned of adventitia; and the hepatic artery was carefully isolated. The origins of the splenic, unnamed gastric, pancreatic and phrenic arteries were undermined and individually clamped and ligated. The bowel was replaced and the arterial tree adjacent to the liver checked for pulsation. Skeletalisation was thus complete, leaving the donor liver attached only to the major hepatic vessels, inferior vena cava and common bile duct.

A segment of the infrarenal aorta was then isolated in preparation for subsequent exsanguination in Stage 3. The bowel was retracted to the left and a segment of the infrarenal aorta cleaned of adventitia and undermined. Two tapes were passed under the isolated vessel and left in situ. The contents of the abdominal cavity were then replaced, and the contents covered with moist warm swabs.

Ringers Lactate with 10% Invert Sugar was administered throughout the procedure, and the flow rate adjusted to correct hypotension.

Donor preparation required an average time of 43 minutes in Group 1(b) (GB-JEJ-GB), and 52 minutes in Group 1(a) (GB-GB) (Appendix-Tables 49 - 51). The difference was significant ($p < 0,05$), and was due to the fact that some of the operations performed early on in the project were

according to the GB-GB technique. As surgical and organisational skill developed, the time taken to prepare the donor decreased.

(2) STAGE 2 - Preparation of the recipient animal

Lumber puncture was performed as previously described on page 70.

Cannulation of the cervical vessels differed slightly from that performed in the donor animals. A more extensive vertical neck incision was made to allow for the subsequent insertion of a splenojugular bypass cannula. The carotid artery was cannulated for blood pressure monitoring and arterial blood sampling. The right subclavian vein was cannulated with a sterile polythene infant feeding cannula to serve as an access for venous blood samples and intravenous fluid administration. Arterial and venous blood samples were taken. The jugular vein was dissected free for later insertion of the bypass line.

Preparation of the bed and vessels for reception of the graft was carried out next. A midline incision was made as described in the donor operation on page 70. Exploratory laparotomy was performed to detect any disease or abnormality, and a wedge liver biopsy performed. The gallbladder was inspected, and cystic and common ducts palpated. The major portion of the bowel was then placed in a clean plastic bag and lightly constricted at the root of the mesentery.

(i) The infrahepatic vena cava was examined for accessibility, and the peritoneum between vein and right adrenal gently

dissected away when it appeared that the vascular clamp would include the adrenal.

(ii) With the stomach retracted downwards and the liver upwards, the portal vein was carefully cleaned of enveloping peritoneum and isolated from the hilum to the duodenum. An attempt was made to preserve the coronary vein, but this was not possible in many cases. Care was taken not to damage nor disturb the hepatic arterial tree during dissection.

(iii) The bowel was retracted to the left, and the infrarenal aorta cleaned of adventitia for approximately 2 cms., in preparation for arterial reconstruction.

Insertion of the splenojugular bypass lines. The spleen was retracted gently downwards, and the short gastric vessels ligated. Omental attachments of the spleen to the stomach, left crus and posterior abdominal wall were cut, leaving the spleen attached only at its hilum. The splenic vein and artery were undermined for about 2 cms.

The pig was heparinised (1mg/Kg) and bypass lines prepared by attaching two 0,6 mm silastic catheters to a T-piece, the third limb of which was connected to a sterile fluid administration set. The bypass lines were primed with heparinised saline, and clamped at each end. A bulldog clamp was placed across the splenic pedicle to distend the splenic vein, and distally the splenic vein and artery were clamped and ligated. One

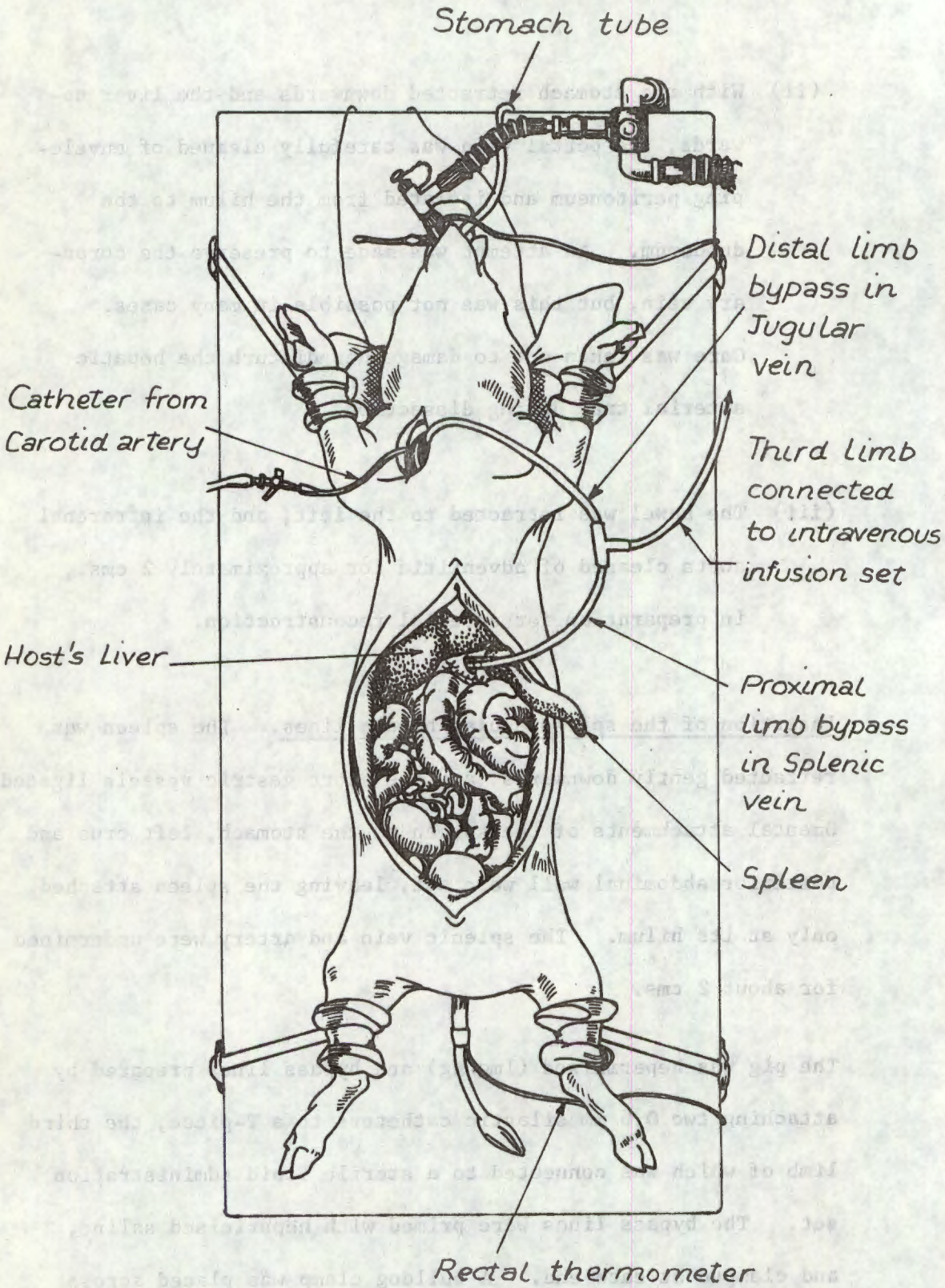


FIGURE 8 THE ARRANGEMENT OF THE SPLENOJUGULAR BYPASS

(Acknowledgement to Professor E. Immelman for permission to use this diagram)

end of the bypass catheter was inserted into the splenic vein through a venotomy, the clamp released and the catheter advanced about 5 cms. along the posterior abdominal wall to prevent obstruction and kinking. A double linen suture was tied around the hilum and catheter, thus occluding the splenic artery and holding the catheter in place. A second anchoring suture was tied to the catheter to prevent accidental removal with haemorrhage. The second end of the primed bypass catheter was inserted through a venotomy into the exposed right jugular vein, and doubly secured. The arrangement of the splenojugular bypass is illustrated in Figure 8 on the opposite page. The function of the bypass was checked and adjustments made in the positions of the catheters until a satisfactory flow rate was seen. Proximal and distal limbs were then flushed with heparinised saline, and both ends clamped.

The abdominal contents were returned, and large, warm, moist swabs placed over the bowel and liver.

The recipient preparation required an average time of 43 minutes in both transplant groups. (Tables 49-51 in the Appendix).

(3) STAGE 3 - Donor liver perfusion and excision

Exsanguination of the donor was performed by cannulating the exposed infrarenal donor aorta with a plastic tube. After donor heparinisation, exsanguination into a sterile bottle was commenced. The blood was kept for subsequent transfusion into the recipient.

Cold perfusion of the donor liver was commenced while exsanguination was proceeding. A primed cannula was inserted into the portal vein through a venotomy and directed towards the hilum. The proximal portal vein was ligated, and cold perfusion commenced, using 3ℓ cold perfusate. The perfusate was made up of 90 ml Rheomacrodex, 10% m/v in Dextrose (Baxter) and 3000 ml Physiosol (Abbott). This procedure is discussed in more detail on pages 93-94 and 194.

Removal and preservation of the graft. With the perfusion in progress, and the heart still beating, the bowel was retracted to the right. The left chest was opened by incising through the cartilagenous ribs, and the incision continued along the diaphragm to the left crus. The pleura was incised along the length of the thoracic aorta, and the intercostal and upper lumbar vessels were divided from the aorta. A clamp was placed below the aortic arch, and the aorta transected and pulled into the abdominal cavity. The aorta was further transected 2 cms distal to the coeliac axis, and with attached vessels was rapidly freed up to the portal tract. The suprahepatic vena cava was transected at its junction with the diaphragm, and the infrahepatic cava at the level of the adrenals. The portal vein was divided below the perfusion catheter, and the common bile duct at the level of the duodenum.

The whole liver preparation with perfusion catheter in situ was placed in a sterile bowl, and perfusion continued until the cold perfusate covered the liver, and the liver had reached a homogeneous grey colour. During this time the intercostal and

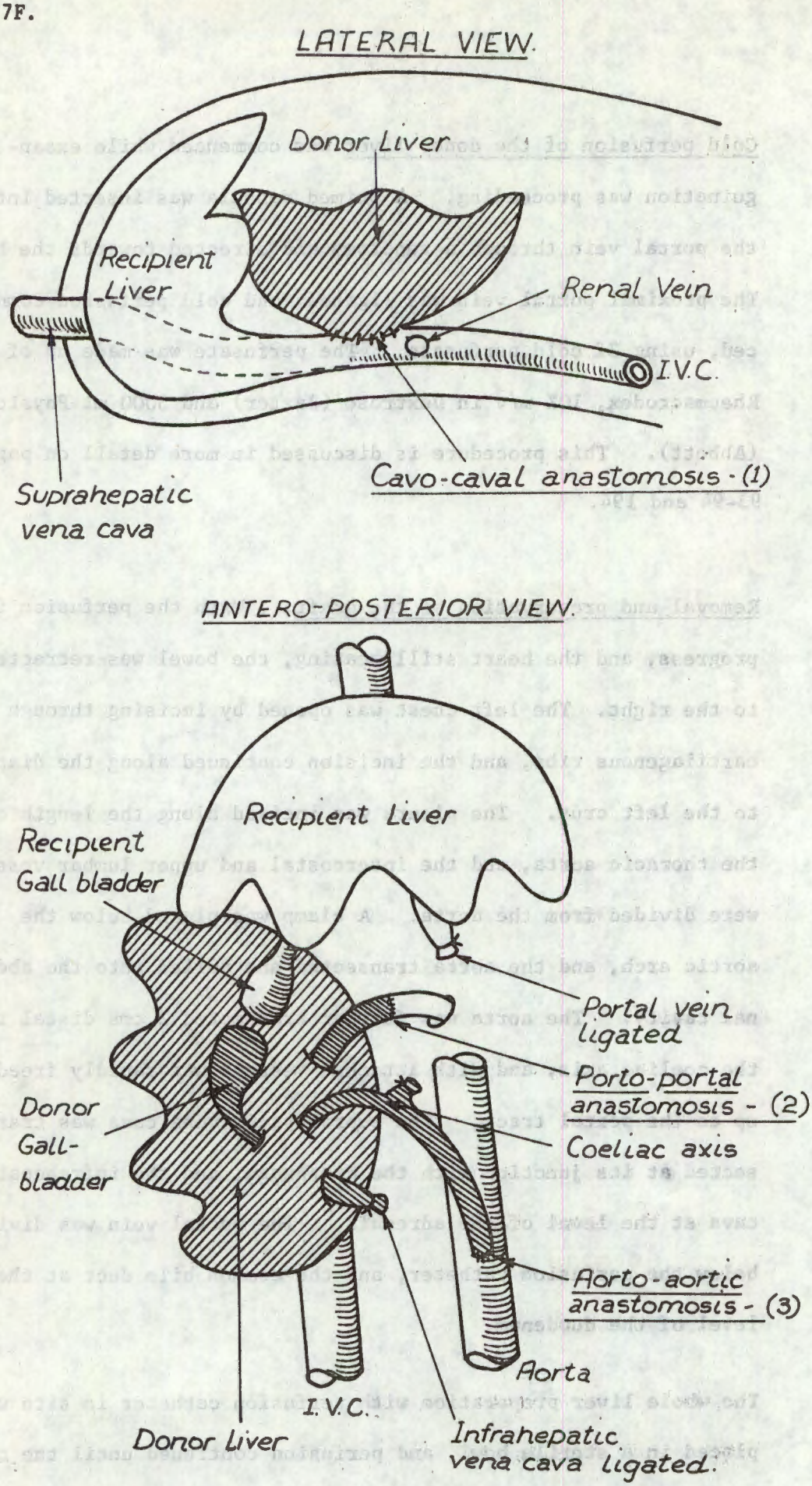


FIGURE 9 THE BASIC TRANSPLANTATION TECHNIQUE

lumbar vessels on the aortic segment were ligated. Thereafter the aortic segment was cannulated and filled with heparinised saline under pressure, thus perfusing the hepatic arterial tree and permitting detection and ligation of leaking vessels. The suprahepatic vena cava was trimmed flush with the liver. The liver was then weighed. The time from completion of perfusion to weighing was approximately 15 minutes.

In both groups 1(a) and 1(b), exsanguination and donor hepatectomy required an average of 15 minutes. The average total anaesthetic period for the donor pigs up to completion of the hepatectomy, was 113 minutes for group 1(b) (GB-JEJ-GB) and 118 minutes for group 1(a) (GB-GB) (Tables 49 - 51 in the Appendix) Of this period, only 60 minutes was spent usefully operating on the donor, the excess anaesthetic period being that required to prepare the recipient. With two surgical teams, the donor animal and liver could be spared approximately 50 minutes of anaesthetic exposure.

(4) STAGE 4 - Implantation of the donor liver

THE BASIC TECHNIQUE

With the exception of the methods of biliary drainage, the transplantation technique was the same in both group 1(a) and group 1(b) animals. The basic technique is diagrammatically shown on the opposite page (Figure 9) and photographically on the following page (Figures 10 - 13)

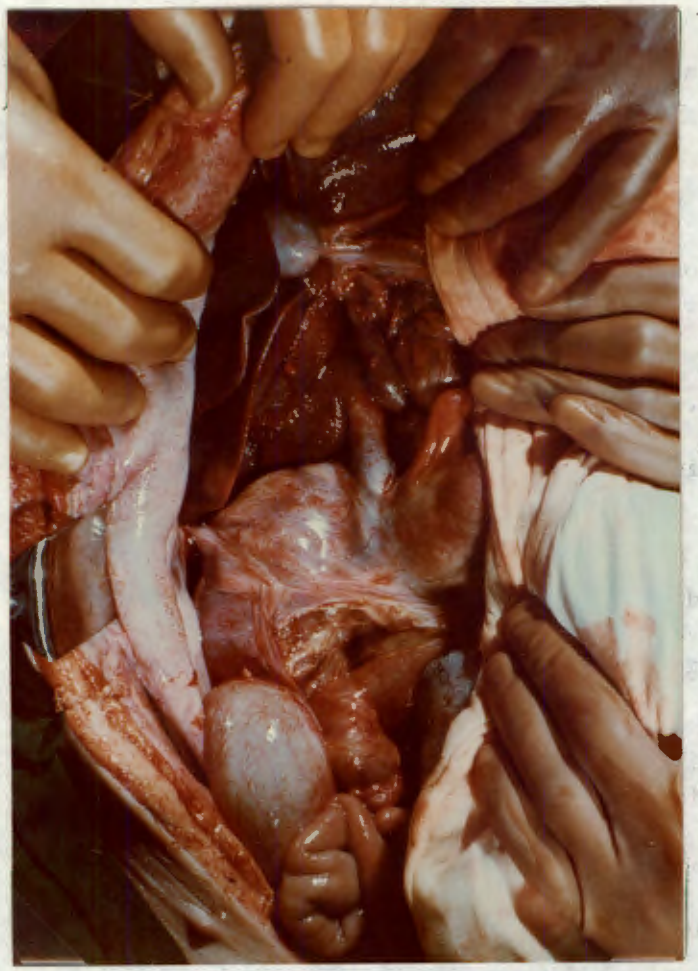


FIGURE 10

Shows the bed into which the graft will be placed. The recipient's infrahepatic vena cava is clearly shown, with its enveloping sleeve of liver

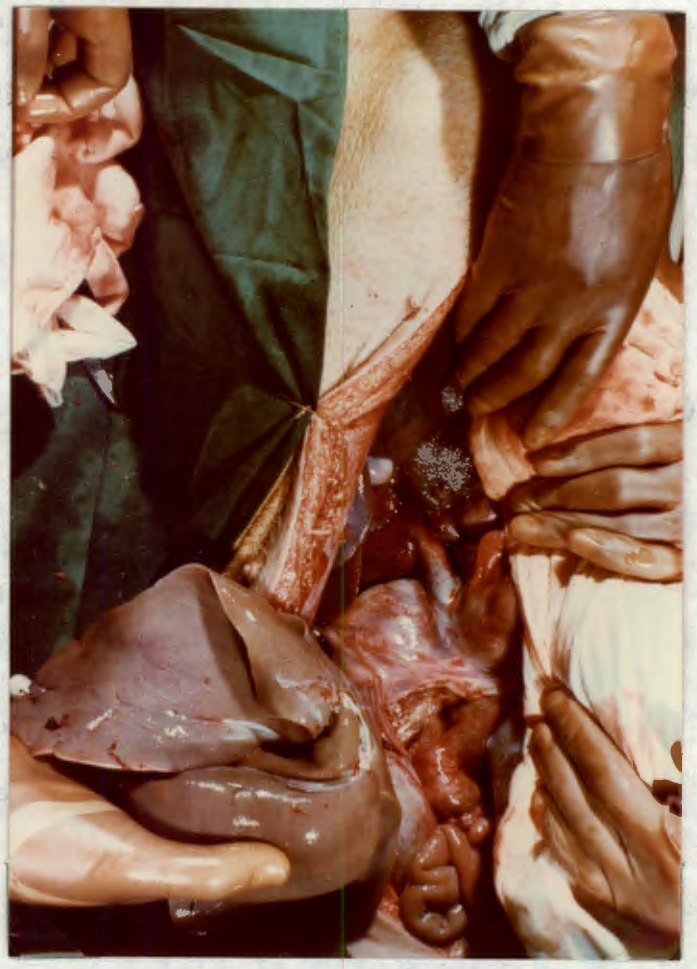


FIGURE 11

The pale, perfused donor liver being correctly aligned prior to being placed in the recipient

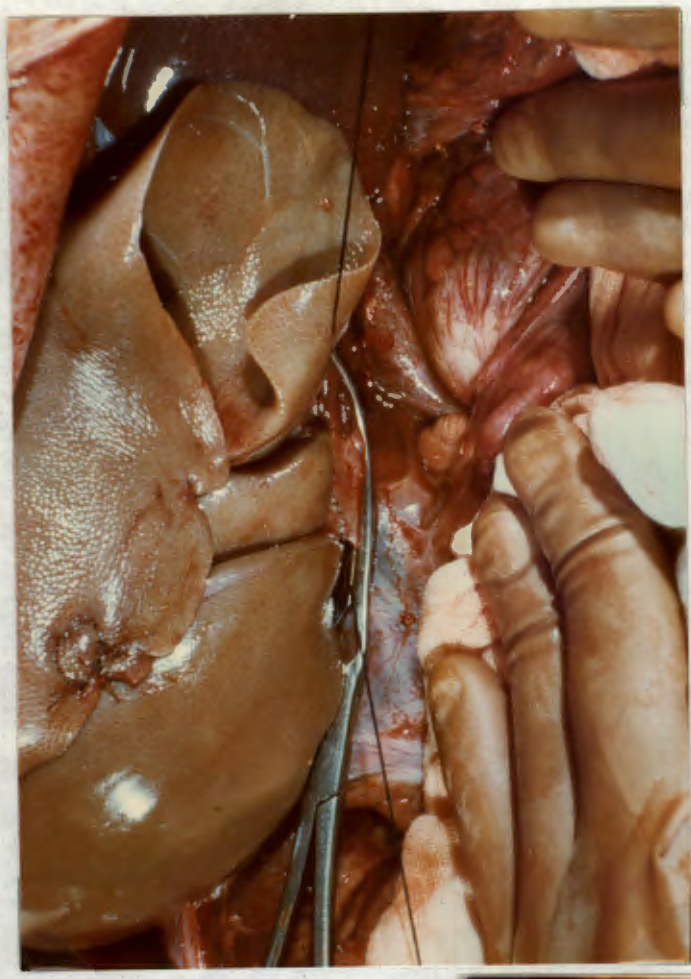


FIGURE 12

Construction of the cavocaval anastomosis. Part of the sleeve of recipient liver enveloping the recipient's infrahepatic vena cava is included in the Potts-Satinsky clamp

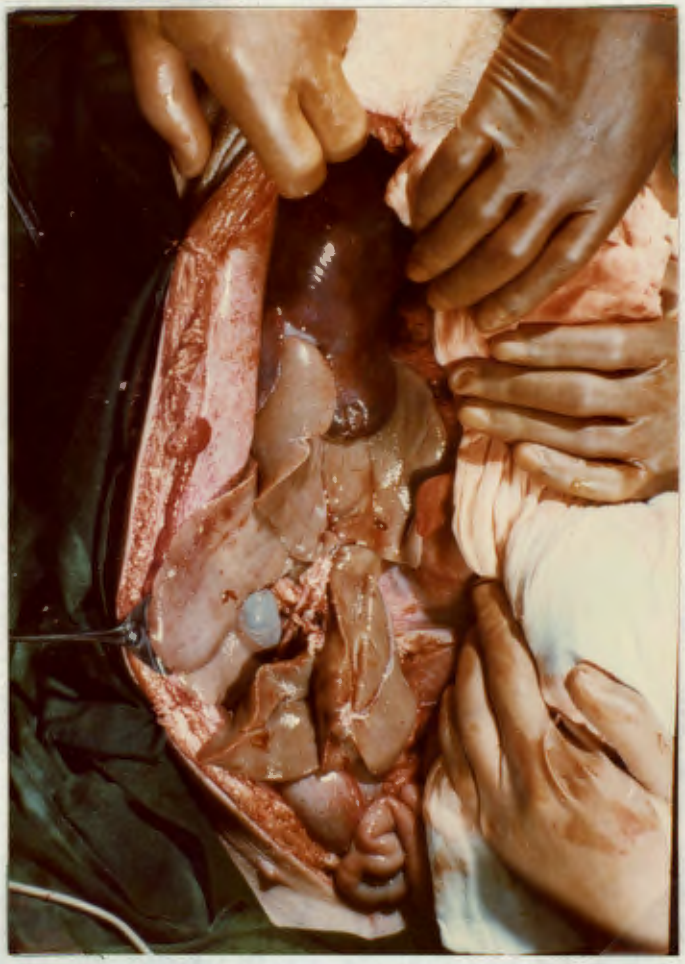


FIGURE 13

The donor liver in the subhepatic position on completion of the cavocaval anastomosis

(a) Positioning of the donor liver

The recipient's bowel was placed in a plastic bag and thus the more easily retracted downward in the midline. The right lobes of the recipient's liver were retracted upwards and to the left to expose the infrahepatic vena cava.

The graft was placed in the right upper quadrant, with its diaphragmatic surface on the posterior abdominal wall, the suprahepatic vena cava on the infrahepatic recipient vena cava, and the right lobe directed to the pelvis. With the graft suitably positioned cold saline swabs were placed around the graft, and the vascular anastomoses performed.

(b) Construction of the vascular anastomoses

(i) End-to-side cavo-caval anastomosis A curved semi-occlusive Potts Satinsky vascular clamp was placed on the recipient's infrahepatic suprarenal vena cava. In most cases part of the enveloping sleeve of liver tissue was included in the clamp, but care was taken to avoid the right adrenal. A longitudinal venotomy was made, curving the incision to create an oval window. Two 0000 silk sutures were inserted through the distal ends of the venotomy, and through the appropriate parts of the short donor suprahepatic vena caval cuff. The liver was gently manipulated and the corner sutures tied, knots being on the outside. The lower suture was brought into the lumen, and the right layer com-

pleted with a simple over and over suture, the sutures being placed 2-3 mm apart. After completion and checking of the right layer, the left layer was completed using the simple over and over technique from the outside as shown in Figure 12 on page 78. On completion, the two suture arms were tied at the lower corner.

The cavo-caval anastomotic time was approximately 14 minutes, with no significant time differences between the two transplant groups. (Tables 49 - 51 in the Appendix).

(ii) End-to-end porto-portal anastomosis. The spleno-jugular bypass was opened at this stage and rechecked for flow and leakage.

The recipient's portal vein was ligated distally as close to the hilum as possible. A bulldog clamp was applied across the portal vein at the level of the duodenum, and the vein transected proximal to the ligature. Lateral retraction on the right abdominal wall was released and the correct length of portal vein assessed to avoid kinking and tortuosity. The donor portal vein was trimmed to exclude the segment damaged by the cannula. Graft position was maintained by swabs placed between the graft and posterior abdominal wall. The anastomosis

was performed using 3 triangulating stay sutures and completed using 00000 silk sutures. Just before completion of the anastomosis the clamp on the recipient portal vein was opened momentarily to allow escape of air. On completion of the anastomosis, the portal venous clamp was removed allowing perfusion of the liver. The initial effluent through the donor infrahepatic vena cava was discarded - usually 200 - 300 ml blood.

The semi-occlusive vena caval clamp was then removed, thus establishing a portal flow through the donor liver into the general circulation. The infrahepatic vena cava was ligated. The bypass line was clamped, but left in situ.

At this stage haemostasis was checked and secured where necessary. If any tortuosity or kinking of the portal vein was seen, the anastomosis was taken down and reconstructed.

The average portal venous anastomotic time was approximately 10 minutes for each group. The average total ischaemic period up to re-institution of portal flow was about 47 minutes. Of this period, 22 minutes were true cold ischaemia, and for 25 minutes the graft lay in the recipient abdominal cavity covered with cold insulating swabs. There was no significant difference in the time

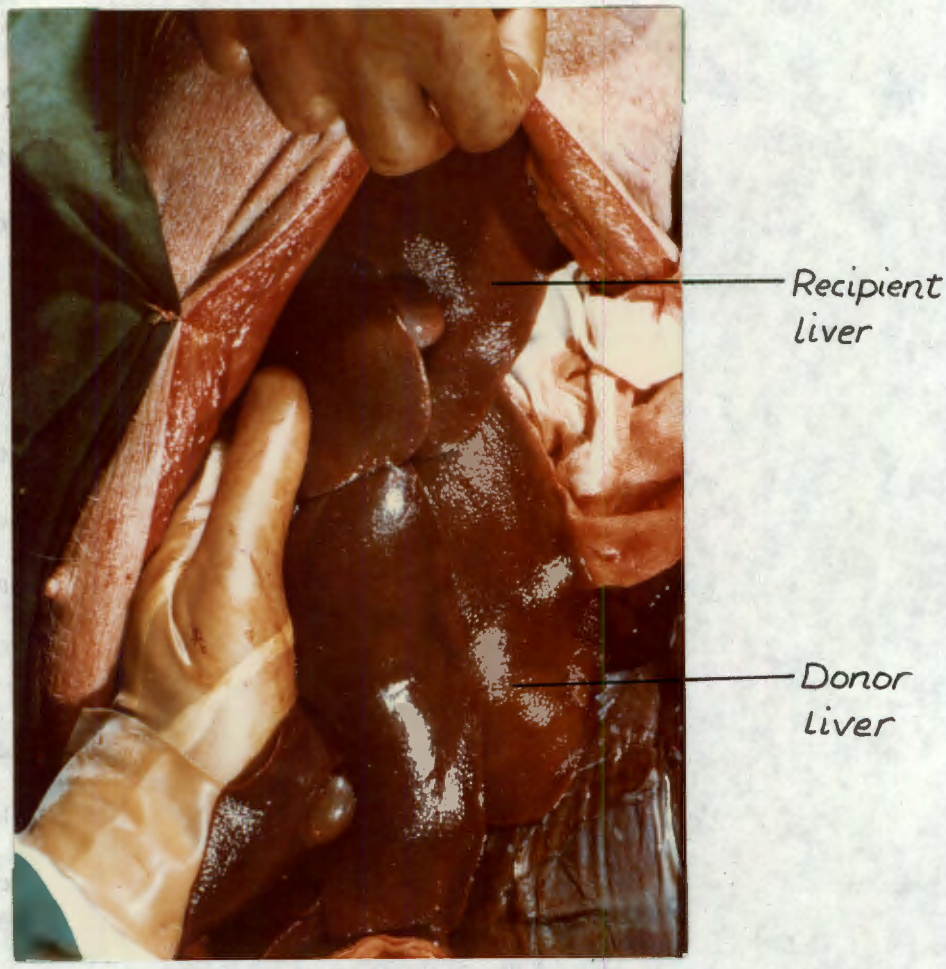


FIGURE 14 THE DONOR LIVER ON COMPLETION OF THE THREE VASCULAR ANASTOMOSES

Note: (1) The similarity in appearance of the donor and recipient livers
(2) The wide gap between the donor and recipient gallbladders

between the two transplant groups. (Tables 49 - 51 in the Appendix).

(iii) End-to-side aorto-aortic anastomosis. With the bowel retracted to the left, a curved Satinsky vascular clamp was applied to the prepared 2 cms of recipient infrarenal aorta. An anterior longitudinal arteriotomy was made and the vessel wall trimmed on either side to create an oval window. The donor aortic segment was positioned and trimmed to the correct length; and the hepatic artery inspected to exclude any twisting. The anastomosis was performed with double armed 0000 silk sutures using a simple over and over technique, with all knots being tied on the outside.

On completion of the anastomosis, a bulldog clamp was placed on the donor coeliac axis, and the occlusive aortic clamp released to exclude air from the segment of donor aorta. The clamp was reapplied, and the distal donor aorta ligated adjacent to the coeliac artery. Both clamps were then removed, allowing arterial perfusion of the graft.

On completion of the three vascular anastomoses, the donor and recipient livers usually had an identical colour and texture, as illustrated in Figure 14 on the opposite page. The operative techniques in both groups 1(a) and 1(b) were identical up to this stage.

serosal layer. The anastomosis was moderately inverted. The size of the resultant anastomoses were 1-2 cm in diameter. On completion of the anastomosis the donor common bile duct was ligated distally. The gallbladder to gallbladder anastomoses required approximately 11 minutes on average.

The photograph on this page (Figure 16) demonstrates a GB-GB anastomosis in the process of being constructed.

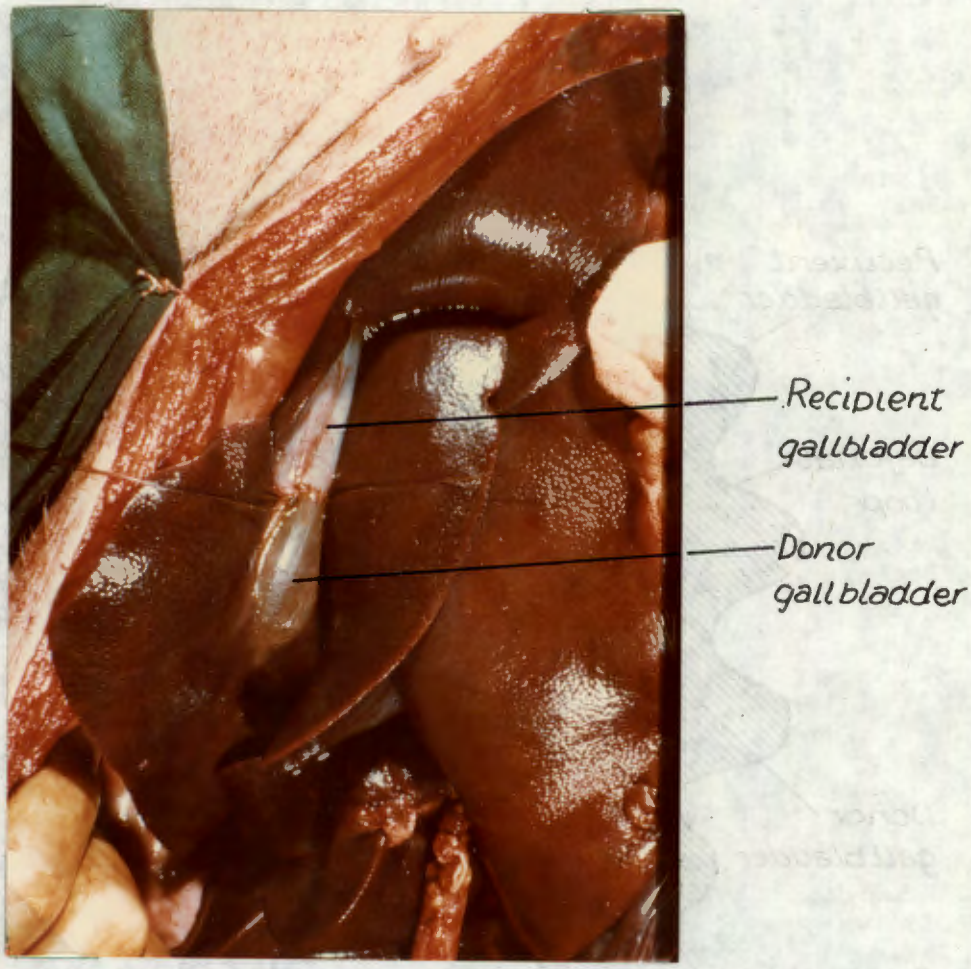
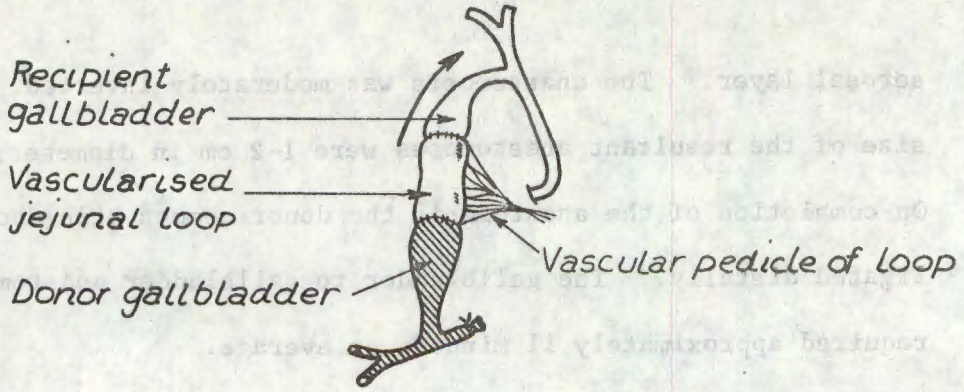
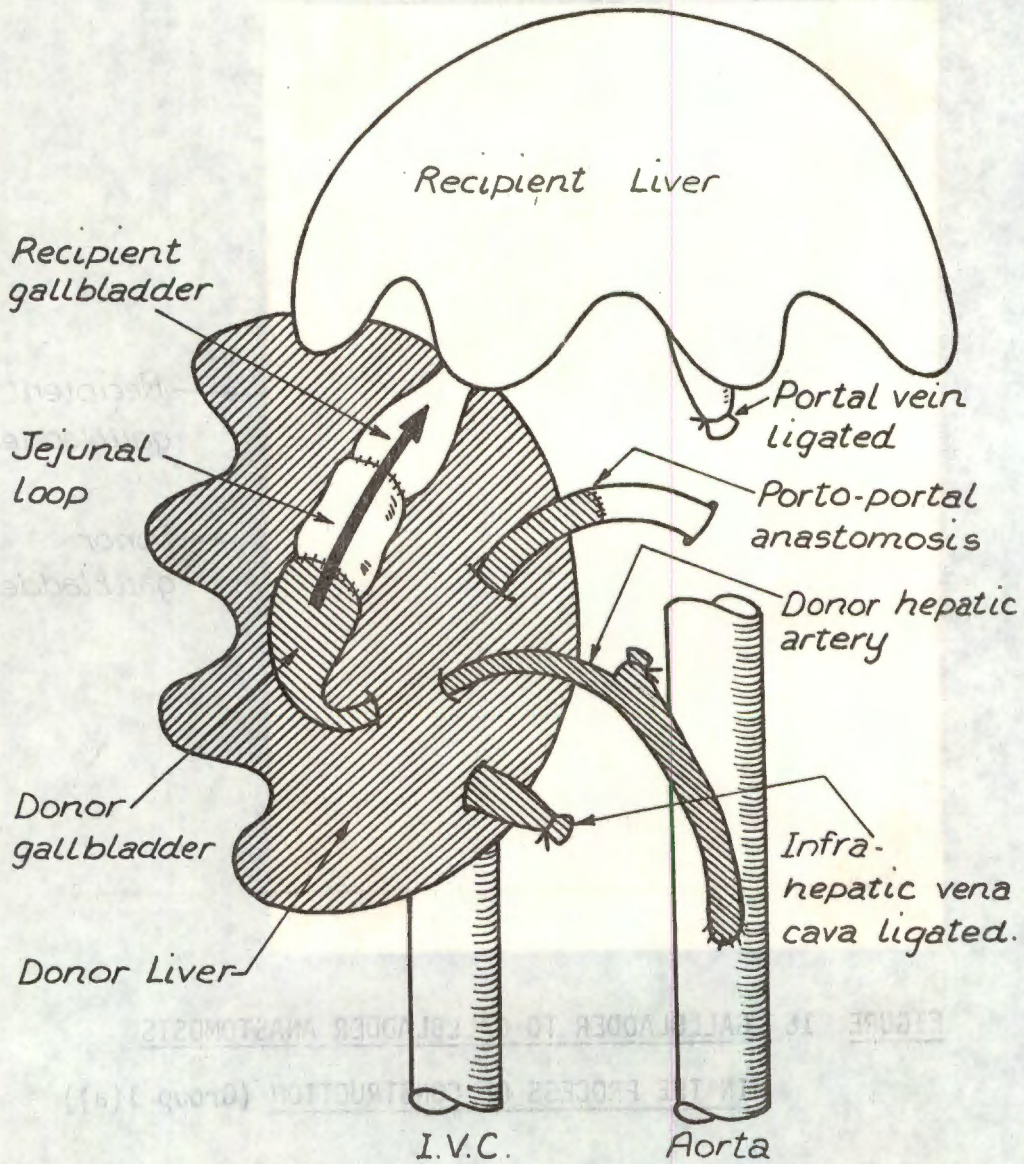


FIGURE 16 GALLBLADDER TO GALLBLADDER ANASTOMOSIS IN THE PROCESS OF CONSTRUCTION (Group 1(a))



Interposition of jejunal loop.



Vascular & Biliary reconstruction completed.

FIGURE 17 THE TECHNIQUE OF CHOLECYSTOJEJUNOCHOLECYSTOSTOMY -

(Group 1(b))

(b) GROUP 1(b). Interposition of a jejunal conduit (Cholecystojejunocholecystostomy or GB-JEJ-GB technique). This technique is diagrammatically illustrated in Figure 17 on the opposite page.

A loop of jejunum 10-15 cms. from the ligament of Treitz was selected, the vascular arcades inspected, and a loop of jejunum chosen to contain several supplying vessels, as shown in the photograph below.

FIGURE 18 SELECTION OF THE JEJUNAL SEGMENT FOR INTERPOSITION

FIGURE 19 CHOLECYSTOJEJUNOCHOLECYSTOSTOMY IN THE PROCESS OF CONSTRUCTION

(1) The loop has been rotated through 180°, and end C, is anstomosed to the recipient's gallbladder, while the proximal end B, is anastomosed to the donor gallbladder
(2) The vascular pedicle is arrowed
(3) The loop has been tented to the right for photographic purposes.

Soft bowel clamps were applied to the jejunum approximately 1cm. on either side of the chosen segment. The segment (B-C) was transected proximal to each clamp to avoid trauma to the ends of the segment. The mesentery of the segment was incised in the bloodless areas down to the vascular arcade, the lines of the incision being shown by the dotted lines in the photograph on page 85. A bacteriological swab was taken from within the lumen of the segment, and contents were massaged out by gently stroking with two fingers. A small length of jejunum was excised for histological examination.

The donor gallbladder was incised at the fundus, a bacteriological swab taken, and the incision trimmed to create a circular cystotomy, the excised gallbladder tissue being retained for histology. The isolated loop was rotated through 180^{o} (for isoperistaltic flow), and the proximal end (B) was anastomosed to the donor gallbladder fundus in 2 layers using continuous 0000 chromic catgut. A simple over and over technique was used on the inner layer, while the outer layer created a slight inversion of the suture line. The size of the stoma was noted.

The recipient gallbladder was opened, a bacteriological swab taken, the circular opening constructed, and the distal end of the isolated loop (C) anastomosed to the recipient gallbladder in the same manner as the proximal end. The excised segment of gallbladder was retained for histological examination. The size of the stoma was noted. The jejunal loop thus served as a vascularised conduit between the two gallbladders. The photograph in Figure 19 illustrates a cholecystojejunocholecystostomy in the process of construction.

The transected ends of the jejunum (A & D) were then anastomosed end-to-end using 0000 chromic catgut in 2 layers, thus re-establishing jejunal continuity. The defect in the jejunal mesentery was closed, using 0000 chromic catgut. At this stage, the mesentery of the isolated loop was checked for arterial pulsation and venous engorgement. The colour of the loop was noted, and the loop stimulated to provoke peristaltic movement.

The double biliary anastomosis in addition to the small bowel anastomosis required an average time of 34 minutes. Construction of this type of biliary drainage added 23 minutes to the operative time, compared to the gallbladder to gallbladder technique. (Tables 49 - 51 in the Appendix).

(5) Additional Procedures - Gastroduodenostomy

In 6 group 1(a) animals and 1 from group 1(b) a standard two layered gastroduodenostomy was performed to assess the effect of this procedure in preventing oesophagogastric ulcers in this heterotopic model. The average anastomotic time was 14 minutes.

(6) Closure of the abdomen

The proximal limb of the bypass cannula was removed, the hilum securely ligated, and splenectomy performed. All vascular and biliary anastomoses were checked, and donor and recipient liver biopsies taken in a few cases. Peritoneal lavage was performed with warm saline.

In the early cases a single layer closure was performed using continuous 00 nylon through the peritoneum and linea alba.

In subsequent cases, an additional layer of 00 chromic catgut approximated the subcutaneous tissue. The skin was closed with a continuous 0 nylon over and over suture in the earlier cases. In the later cases, skin closure was performed using a continuous subcuticular 000 nylon suture. Little or no difficulty was encountered in closing the abdomen in the transplanted animals, despite the presence of the extra organ, and no respiratory difficulties appeared to result.

The arterial catheter in the neck was removed, and the carotid artery ligated. The jugular vein was ligated after removal of the distal limb of the bypass cannula. The intravenous subclavian cannula was left in situ, and the incision closed with continuous 00 nylon suture. The cannula was firmly anchored to the skin with additional sutures.

(7) Marking of the animals for identification

Each animal was then marked for subsequent identification. The marking was performed in 3 ways for each animal.

(a) The ears were marked according to the laboratory code.

(b) The number allocated to the animal was cauterised onto the lateral abdominal wall.

(c) The number was written onto a neck band made of adhesive tape.

Triplication of the marking was found to be necessary in our laboratory circumstances.

The numbers allocated to the animals in the operative laboratory differ from those used in the script. The laboratory number,

script number and histological number for each pig is tabulated in Table 39 in the Appendix.

Anaesthesia was discontinued, and the animals kept on the operating table until awake and breathing well. The postoperative care is described on page 94.

The average total anaesthetic periods were 171 minutes (GB-GB group) and 184 minutes (GB-JEJ-GB group). Average operating times were 123 and 149 minutes respectively (Tables 49 - 51 in the Appendix). If the donor hepatectomy is performed by a second surgical team, approximately 1 hour of recipient anaesthetic exposure could be prevented.

2. END-TO-SIDE PORTACAVAL SHUNTS: (Group 2(a))

Lumbar puncture and insertion of cervical arterial and venous cannulae was identical to that described for the donor operation on page 70. A midline approach was used similar to that described for the transplanted animals and exploratory laparotomy was carried out. No bypass was used because portal occlusion time was within that tolerated by the pig (20). A wedge liver biopsy was taken into formol saline.

With the stomach and bowel retracted down to the left, and the liver retracted upwards, the filmy peritoneum enveloping the portal vein was incised, and the hepatic end of the vein mobilised by dissecting it free of adventitia and lymph nodes. Care was taken to

preserve the coronary vein, but this was not possible in a few cases. A curved Potts Satinsky partially-occlusive vascular clamp was positioned on the infrahepatic vena cava. A longitudinal venotomy was created and the edges trimmed to provide an oval opening. A bulldog clamp was applied to the portal vein at the level of the duodenum. The portal vein was ligated close to the hilum, and transected on the clamp side of the ligature.

The portal vein was trimmed to the correct length to prevent kinking and tortuosity, and the end cut obliquely to create a widely patent anastomosis. The anastomosis was constructed using two double armed 00000 silk sutures. Two corner sutures were inserted; thereafter the left side of the anastomosis was performed using a simple over and over technique. The suture was tied at the top corner, and the right side completed in the same manner. On completion of the anastomosis, the clamps were released simultaneously, and haemostasis secured. The portal vein was examined for kinking and rotation prior to closure, and the bowel checked for return of colour. Abdominal closure was performed in three layers as described for the transplanted animals on page 88 and the animals were then marked.

The average portal occlusion time was 12 minutes, the average total anaesthetic period approximately 62 minutes (Table 52 in the Appendix).

3. SHAM LAPAROTOMIES - (Group 2(b))

The procedures on these animals were exactly the same as those previously described for the portacaval-shunted animals, with the exception that a portacaval shunt was not created. Following lumbar puncture and cervical cannulation, exploratory laparotomy was performed through a midline incision, and liver biopsy performed.

The portal vein was then dissected free in exactly the same manner as described for the portacaval shunts. A partially-occlusive Potts Satinsky clamp was applied to the suprarenal infrahepatic vena cava for the average time of clamping recorded in the group 2(a) animals, while simultaneously, a bulldog clamp was applied to the portal vein for the average portal occlusion time recorded in the group 2(a) animals. After removal of the clamps, the animals were closed, and marked for identification, in exactly the same manner as the animals from group 2(a).

The average portal occlusion time was 12 minutes, and average anaesthetic time approximately 66 minutes (Table 52 in the Appendix).

INTRA-OPERATIVE CARE

(1) Physiological monitoring

Temperatures were monitored continuously in all animals, using a telethermometer with the probe placed in the oesophagus or rectum.

The mean arterial pressure was continuously recorded by means of the intra-arterial carotid catheter connected to an anaeroid manometer. Mean arterial pressure invariably fell during the

bypass period, and also whenever the bowel was retracted too vigorously. Pulse rate was recorded on a cardiac monitor, when available, or by counting the deflections on the manometer.

Sequential arterial bloods were taken throughout some of the operations to establish the pattern of acid-base changes, but later on in the series only when indicated by the clinical condition of the animals.

(2) Fluid Balance

Ringers Lactate in Invert Sugar 10% was infused throughout the operation. The infusion rate was adjusted according to requirements, and between 500 ml and 1000 ml were used in each animal.

Sodium Bicarbonate 4,2% (m.v.) was infused immediately prior to and during the first few minutes after revascularisation in the transplant animals (53). On an average 300 ml was used. In the control groups, the infusion was commenced immediately prior to the clamps being released from the portal vein.

Approximately 500 ml donor blood was infused into all transplant recipients while the vascular anastomoses were being constructed. The rate of infusion was increased immediately the clamps were released. The sham and portacaval shunted animals received no blood transfusions.

(3) Medication

Early on in the series, the animals all received 1 000 000 units Penicillin and 1 gram of Chloromycetin intravenously as soon as the neck cannulae had been inserted. Because of the high sepsis

rate in the first few animals, the doses were increased to 3 million units Penicillin and 3 grams of Chloromycetin for the final 75% of animals. The latter 50% of animals received 2 grams of Chloromycetin intravenously, and 1 gram intraperitoneally after closure.

Heparin, 1 mg/Kg., was administered prior to exsanguination in the donor, and prior to implantation in the recipient. The heparinisation was not reversed with protamine, except in a few animals where severe post-revascularisation bleeding occurred.

The transplant recipients were given 50 ml 50% Dextrose, plus 1 ampoule of Vitamin K, as soon as full revascularisation had been completed.

(4) Prevention of hypothermia

The animals all tended to develop severe hypothermia, especially in winter. The operating table was heated with fan heaters, and warmed humidified anaesthetic gases were administered in an attempt to minimise heat loss. In addition, when these measures were inadequate, warm saline was placed into the plastic bag containing the loops of bowel. This proved to be an effective heat exchanger.

(5) Preservation of the donor liver

Prior to cold perfusion, the donor animal was maintained in the best possible physiological state, by preventing hypothermia and hypotension and by correcting acid-base imbalances.

Cold perfusion was commenced with the heart still beating and prior to the occlusion of any vessels. Three litres of perfusate, made

up of 90 ml Rheomacrodex in 3 000 ml of Physiosol, at 4°C was infused from a height of 40 cms via the portal vein. The perfusion was continued until the liver was a homogeneous grey colour, and until the liver had been covered completely by perfusate. The liver was kept immersed in the cold perfusate during ligation of the branches of the aorta, and after weighing, the liver was wrapped in a sterile swab to insulate it, and cold perfusate poured over the swab. The hepatic arterial tree was perfused with cold heparinised saline.

The liver was handled gently and the duration of ischaemia was kept as short as possible.

The donor gallbladder was not opened after infusion, but bile was allowed to drain from the cut end of the common bile duct, which was only ligated once the biliary anastomosis had been completed.

POSTOPERATIVE CARE AND ASSESSMENT

As soon as the animals were awake, the blood pressure and respiration stable, they were returned to the postoperative holding cages. The cages were heated by means of fan heaters. Intravenous infusion of Ringers Lactate in 10% Invert Sugar was maintained for 48 hours - 2 litres per 24 hour period. The animals had free access to water after 24 hours, and were returned to the sty after 48 hours, being allowed free access to food and water.

Intravenous Penicillin (1 000 000 units daily) and Chloromycetin (1 gram daily) were given for 8 days. If the intravenous cannulae ceased

to function or were pulled out, the antibiotics were given intramuscularly.

The animals were seen twice daily and notes made of their vigor, general appearance and wound status.

SERIAL HARVESTING OF BIOCHEMICAL, HAEMATOLOGICAL AND HISTOPATHOLOGICAL SPECIMENS

(1) At the time of definitive operation

CSF and arterial and venous blood samples were taken as soon as possible after induction of anaesthesia. Wedge liver biopsies were taken as soon as the abdomen was opened in all animals. In some cases, repeat liver biopsies were taken immediately prior to closure of the abdomen.

(2) In the first week

Venous blood samples were obtained daily over the first 6 post-operative days, or for as long as the venous cannulae functioned.

(3) Weekly

Every 6th day the animals were starved for 24 hours, but allowed free access to water, in preparation for anaesthesia the following day. On each 7th day the animals were induced with Halothane, Nitrous Oxide and Oxygen administered via a nose cone. Under anaesthesia the animals were examined, weighed, lumbar puncture performed, and arterial and venous blood samples taken.

The transplant recipients were subjected to a small subcostal laparotomy incision in the right lateral abdominal wall, the site of which was varied by 1-3 cms. each week. Adhesions were freed by blunt finger dissection, and the donor liver visualised and palpated and a note made of the colour and consistency. A donor liver biopsy was taken under direct vision, using either a biopsy needle, or the wedge technique. Host liver biopsies were performed percutaneously with a biopsy needle inserted immediately below and 1,5 cms to the right of the xiphisternum.

In the sham and portacaval-shunted animals, liver biopsies were obtained by the epigastric percutaneous needle technique using a disposable 20 mm biopsy needle.

(4) At sacrifice

Simultaneous CSF and blood samples were obtained under anaesthesia in exactly the same manner described for the weekly sampling.

The animals were then euthanised with a large bolus of either Sodium Pentothal or Potassium Chloride, and a full autopsy was performed immediately.

AUTOPSY TECHNIQUE

The animals from all groups were autopsied in the same manner. Autopsy was performed immediately following death in sacrificed animals, and as soon as possible after death had occurred in others.

The animals were weighed and inspected closely. An incision was made from the neck to the pubis, and superficial and deep wound sepsis noted. The peritoneal cavity was carefully opened and a note made of the presence of adhesions, sepsis and ascitic fluid. The peritoneal cavity and tissues adjacent to the extrahepatic biliary tract were carefully examined for evidence of bile leakage.

The livers were gently cleared of adherent bowel, omentum and peritoneum by blunt finger dissection. The external appearance, consistency and lie of the livers was noted. The position, appearance and tension in the various components of the extrahepatic biliary apparatus were observed. The interposed jejunal loops in group 1(b) animals were digitally stimulated to provoke peristaltic movement. Small wedge liver biopsies were taken from each lobe at this time. The livers were further dissected free, and all vessels palpated to detect ante-mortem thrombi. The livers were then removed with vessels and anastomoses intact. All hepatic vessels were opened along their entire length to inspect the anastomoses, and to search for thrombi. The vessels were opened as far as possible into the substance of the livers.

In all sacrificed animals from groups 1(a), 1(b) and 2(b), swabs for bacteriological analysis of the biliary tract were taken through small cystotomies. The whole extrahepatic biliary tract was laid open and a minute inspection carried out. The anastomotic lines in the two transplant groups were carefully examined and palpated. Wall thickness and mucosal appearance were noted, and a search made for bile stasis, stones and sepsis. In most cases, the entire extrahepatic biliary apparatus was excised in continuity with adjacent liver tissue, and preserved in 10% formol saline.

In transplanted animals, the two livers were then separated by blunt dissection. Each liver was weighed approximately 15 minutes after removal. After weighing, multiple slices were made through the livers to assess texture and to look for thrombi and sepsis. Specimens were selected randomly throughout the sliced livers, and submitted for histology. In addition, liver biopsies were taken from any unusual-looking areas. The same procedures were carried out on the control livers.

A search was made for signs of a gastric ulcer, and the stomach removed, opened, washed and inspected. Ulcers were excised in toto, and preserved in formol saline. The duodenum, jejunum, ileum and large bowel were opened and inspected, and biopsies taken. Both kidneys were removed, sliced in half, examined and biopsies taken from each kidney. Bladders were inspected, and in a few animals, biopsies taken. In the control animals, splenic biopsies were taken.

The thoracic cavity was then opened. The lungs were inspected for adhesions, colour and consistency, mobilised and two biopsies taken from each lung. The pericardium and heart were opened and biopsies taken from the right ventricle and right atrium. A large retrosternal node, invariably present, was biopsied, and the thymus, when seen. In a few animals, the thyroid was biopsied.

All histological material was preserved in formol saline.

The author performed every procedure described in this Chapter.

PROCESSING OF SPECIMENS

(1) Haematology specimens were collected into sequestrine tubes and the analyses performed through the routine hospital service, as discussed on page 339.

(2) Blood specimens for biochemical analysis were collected into heparinised tubes, spun, decanted and the plasma stored in a refrigerator. Blood for ammonia determinations was collected into tubes containing 15% trichloro-acetic acid, spun, decanted and the supernatant fluid stored in a refrigerator. CSF was collected into clean test tubes, spun, decanted and stored in a refrigerator. All biochemical determinations were carried out in the Biochemistry laboratory of the Department of Surgery, as described on pages 334-338. Some duplicate blood and CSF samples were processed in the Department of Chemical Pathology.

(3) All histological material was processed in the Histology laboratory of the Department of Surgery.

(4) Bacteriological studies were undertaken by the Division of Bacteriology of the Department of Pathology.

After initial determinations, all the residual serum and CSF samples were deep-frozen awaiting any additional tests.

SUMMARY OF DATA COLLECTION

The following data were obtained, or recorded at the time of initial operation, weekly and at sacrifice.

(1)	Clinical	Appearance, weight, vigor sepsis.
(2)	Haematological	Haemoglobin; white cell, platelet and differential white cell counts.
(3)	Biochemical	CSF glutamine, venous ammonia, (arterial blood ammonia where possible), alkaline phosphatase, SGOT, cholesterol, total protein, albumin, globulin, acid base balance, and in a few animals, urea and creatinine.
(4)	Pathological	Liver biopsies.

In addition, blood was taken for haematology and biochemistry several times during the first week. Bacteriology samples were obtained at the time of the initial operation and at the time of sacrifice.

Comprehensive liver, biliary tract and organ specimens were taken at autopsy.

The preparation and interpretation of the histological material will be discussed on pages 161, 175 and 183.

The analytical methods used for biochemical determinations are outlined on pages 334-338.

The statistical methods used in the analysis of the techniques and results are outlined on pages 340-342.

PART II

CHAPTER 3

RESULTS OF THE LIVE PORCINE STUDY

The results of the live porcine study will be presented in four sections. A detailed index will be found at the beginning of each section.

SECTION I

The clinical course, cause of death, complications and general pathological findings are presented and briefly discussed in pages 102-120.

SECTION II

The macroscopic, microscopic and bacteriological analyses of the gallbladders and interposed loops are detailed and discussed in pages 121-142.

SECTION III

The macroscopic and microscopic changes seen in the livers and blood vessels are presented in detail, analysed, compared and discussed in pages 143-194.

SECTION IV

The study of the serial changes in CSF glutamine, blood biochemistry and haematology is detailed, analysed and discussed in pages 195-225.

The abbreviated case histories of individual animals will be found in the Appendix, pages 269-315.

INDEX TO PART II, CHAPTER 3

SECTION I - GENERAL CLINICAL AND PATHOLOGICAL FINDINGS

PART II

CHAPTER 3

SECTION I - GENERAL CLINICAL AND PATHOLOGICAL FINDINGS

SUMMARY

The quality of life, survival patterns, cause of death, complications and general organ pathology in the four groups of animals is presented and briefly discussed.

It is shown that there were no outstanding differences clinically nor pathologically between the transplant groups 1(a) and 1(b); and that no deaths could be attributed directly to defects or complications of biliary drainage.

The outstanding complications in groups 1(a), 1(b) and 2(a) are shown to be gastric ulceration, pulmonary infection and wound sepsis.

OVERALL SURVIVAL

Heterotopic liver transplantation in the pig proved to be a formidable procedure, as can be seen from the overall survival figures in Table 8.

	TRANSPLANTS		PORTA-CAVAL SHUNTS		SHAMS	
	No.	%	No.	%	No.	%
Operations attempted	69	100	19	100	5	100
Table deaths	19	28	2	10	0	0
Survival less than 24 hours	17	25	0	0	0	0
Survival between 24 hours & 6 Days	4	6	3	16	0	0
Survival 7 Days or more	29	42	14	74	5	100

TABLE 8 OVERALL RESULTS OF THE SERIES

The overall survival pattern in this series was basically similar to the patterns previously described in pigs (19, 36, 95, 111, 131, 198), dogs (24, 76, 80, 170, 216) and in the early experience of liver transplantation in man (4).

Survival increased considerably as technical, anaesthetic, organisational and postoperative management skills developed. Only 10% of the first 20 recipients survived 7 days or longer, while 65% of the final 20 animals survived to more than 7 days.

The main causes of table deaths were technical mishaps, anaesthetic failure, hypothermia, malignant hyperpyrexia and the presence of pre-existing pulmonary or cardiac infections in the donors or recipients. Over transfusion and pre-existing infections were implicated in some deaths within the first 24 hours, but in several animals the cause of death could not be determined. No deaths occurring before 7 days could be attributed directly to biliary tract complications, and in only one animal could biliary leakage be detected.

The 40 recipients that survived less than 7 days will be excluded from further discussion and documentation, together with one recipient that survived longer than this, but in whom gas gangrene had developed in both donor and recipient livers by the time autopsy was performed. The report will be confined to 28 transplanted animals that survived in excess of 7 days.

Survival to more than 7 days was 74% in the portacaval-shunted group. The table deaths in this group were due to gross pre-existing cardio-pulmonary disease in one animal, and a technical mishap in a second. Three animals died within the first week, one from small bowel obstruction, while the cause of death in two could not be determined. The report will be confined to the 14 animals surviving 7 days or longer.

All the animals subjected to sham laparotomy survived in excess of 7 days, and the 5 animals are all included in this series.

GROUP	WEIGHT CHANGE					
	Gained		Static		Lost	
	No.	%	No.	%	No.	%
1(a)	3	25	0	0	9	75
1(b)	4	29	1	7	9	64
2(a)	2	20	5	50	3	30
2(b)	5	100	0	0	0	0

TABLE 9 BODY WEIGHT CHANGES IN THE FOUR GROUPS OF ANIMALS *

* Pre-operative weight compared to weight at autopsy

Group 1(a) - Heterotopic allograft plus cholecystocholecystostomy
Group 1(b) - Heterotopic allograft plus cholecystojejunocholecystostomy
Group 2(a) - End-to-side portacaval shunt
Group 2(b) - Sham laparotomy

FIGURE 21 THREE PIGS, 26, 27 and 28 DAYS AFTER TRANSPLANT

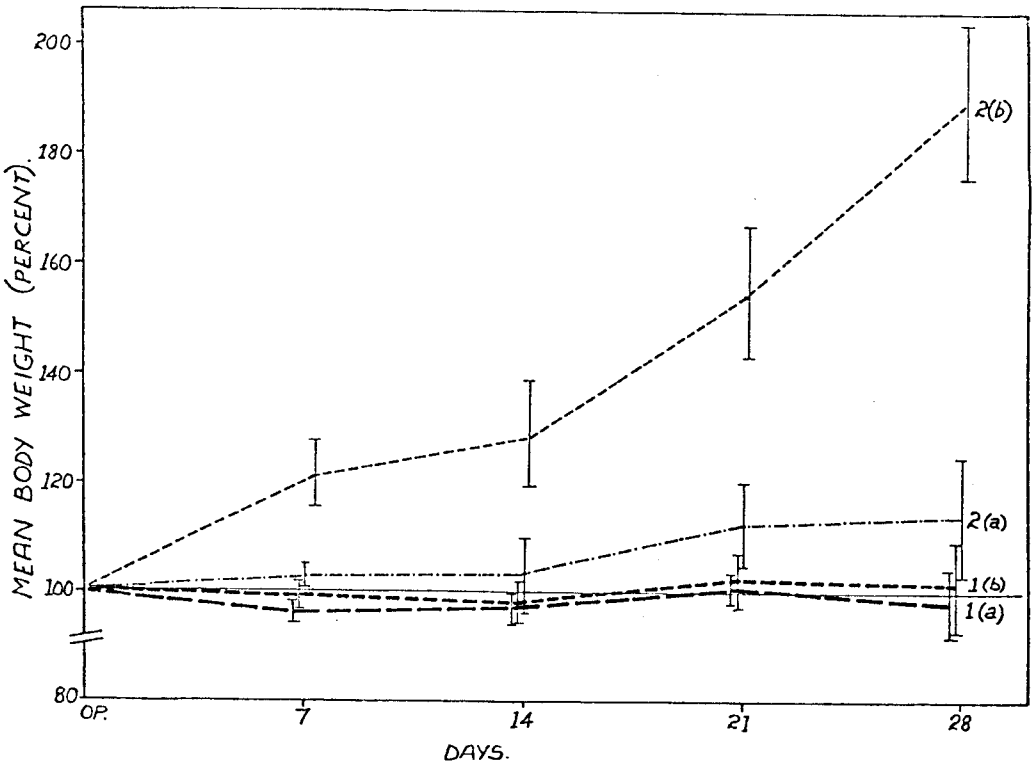
CLINICAL FEATURES OF THE FOUR GROUPS OF ANIMALS

This presentation is limited to the animals that survived 7 days or longer. Fourteen animals were studied in each of groups 1(a), 1(b) and 2(a), and five in group 2(b). The case histories of the animals are summarised in the Appendix on pages 269-315.

1. QUALITY OF LIFE

The five animals in group 2(b) (sham laparotomy) exhibited the best quality of life and will be discussed first. Following the initial few postoperative days, all the animals became vigorous and alert. They strenuously resisted any physical manipulation and required lengthy anaesthetic induction for minor procedures. On visiting the sty, the animals always appeared hungry, and ate voraciously. Body growth was rapid, the general condition excellent, and there was no loss of hair along the spine. Individually, and as a group, the animals showed significant body weight gain, as demonstrated in Table 9 on the opposite page and Figure 20 on page 107F. Wounds healed rapidly, and the rate and degree of sepsis appeared less severe than in the other three groups.

The fourteen animals in group 2(a) (portacaval shunts) had a strikingly different postoperative course when compared with group 2(b) animals. Most of the animals rapidly lost condition, became listless, and, although resentful of handling, did not have the physical vigor of the group 2(b) animals. Some of the animals became ataxic and one developed severe hindquarter weakness. On visiting the sty, all appeared less hungry and to eat less than their simultaneously observed counterparts from group 2(b). The



Graph 20a MEAN PERCENTAGE BODY WEIGHT CHANGE IN THE FOUR GROUPS (Mean ± 1 SEM)

Group	DAY															
	7				14				21				28			
	Mean	SD	SEM	No.	Mean	SD	SEM	No.	Mean	SD	SEM	No.	Mean	SD	SEM	No.
1(a)	96,30	6,66	2,22	10	97,71	8,03	3,27	7	100,22	8,07	2,85	9	98,00	17,22	6,51	8
1(b)	99,00	10,70	2,96	14	98,16	12,54	3,78	12	101,87	14,86	5,61	8	101,00	17,66	7,89	6
2(a)	102,40	6,32	2,10	10	103,57	16,60	6,77	7	112,66	16,29	7,28	6	114,20	23,05	11,52	5
2(b)	121,80	12,25	6,12	5	128,80	19,85	9,92	5	154,80	24,53	12,26	5	189,75	25,40	14,66	4

Table 20b MEAN PERCENTAGE BODY WEIGHT IN THE FOUR GROUPS COMPARED TO PRE-OPERATIVE WEIGHT

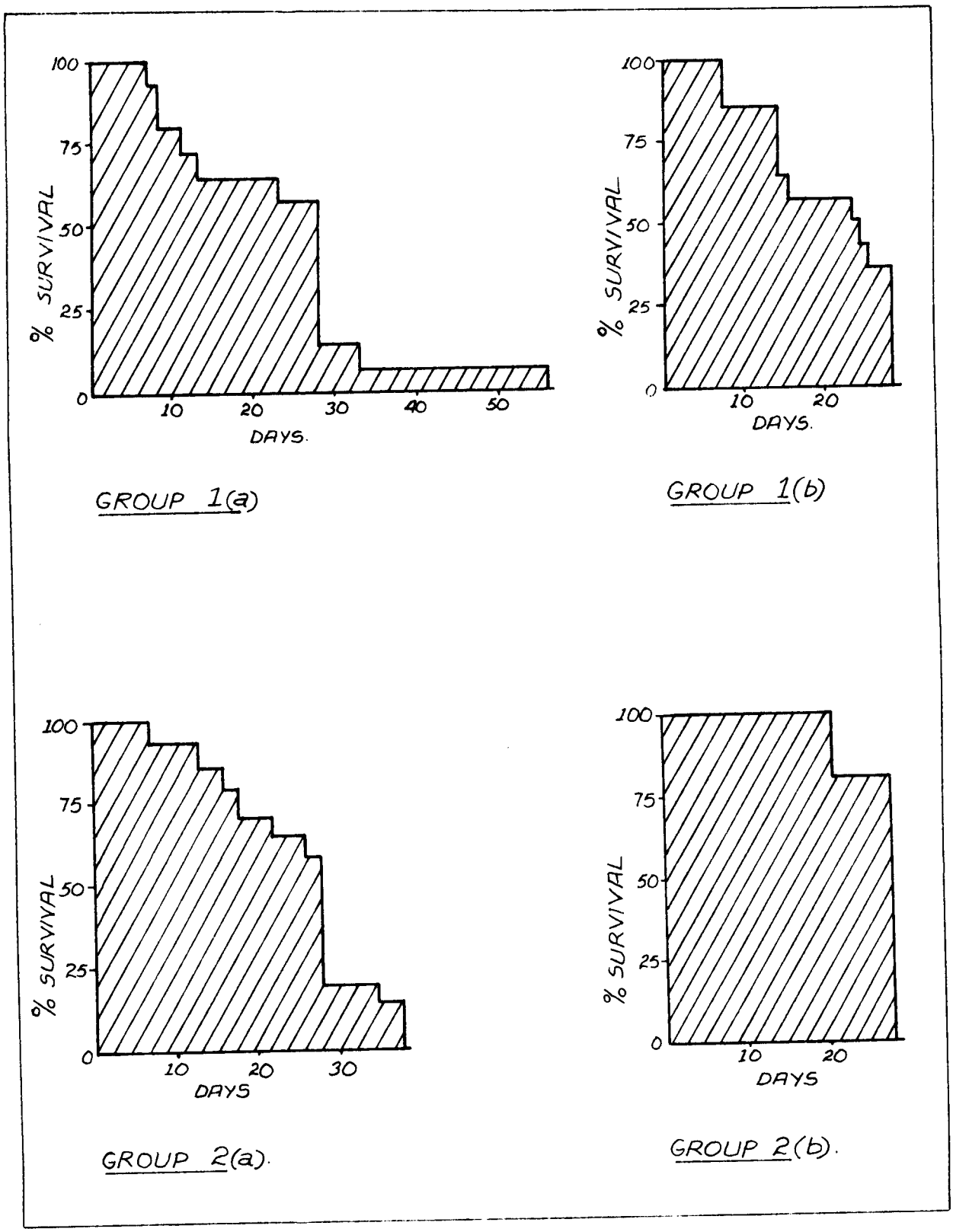
Group 1(a) - Heterotopic allograft plus cholecystocholecystostomy
Group 1(b) - Heterotopic allograft plus cholecystojejunocholecystostomy
Group 2(a) - End-to-side portacaval shunt
Group 2(b) - Sham laparotomy

FIGURE 20 MEAN PERCENTAGE BODY WEIGHT CHANGE IN THE FOUR GROUPS

ribs and spine became prominent, and there was hair loss parallel to the spine. The animals required minimal anaesthetic induction for minor procedures and were slow in waking up. The ten smaller animals, who were of comparable weight range to the animals in group 2(b), showed an insignificant weight gain, as illustrated in Figure 20 on page 107F. Eight (80%) of the 10 smaller animals lost weight or remained at their preoperative weight as shown in Table 9 on page 106F. The preoperative weights of the four larger animals were not recorded. Serial postoperative weights of these 4 animals showed no weight gain in 1 animal, while 3 showed some weight gain, but not of the same order as the animals from group 2(b). Wound sepsis was more severe and slower to heal than in group 2(b). Similar features have previously been reported in dogs after portacaval shunts (27).

The transplanted animals in groups 1(a) and 1(b) revealed a wide spectrum of clinical behaviour, ranging from that seen in the best group 2(b) animals, to that seen in the worst group 2(a) animals. Approximately half of the animals in each group followed the clinical course seen in the majority of group 2(a) animals. They appeared vigorous after the first few postoperative days, but gradually became listless, apathetic and ill as time progressed. Hair loss along the spine was a prominent feature in the poor quality animals.

Approximately half the animals in each group resembled the group 2(b) animals in that they remained physically vigorous, appeared to eat well and resisted physical manipulation. However, with three exceptions, they did not achieve the prime condition of



Group 1(a) - Heterotopic allograft plus cholecystocholecystostomy
Group 1(b) - Heterotopic allograft plus cholecystojejunocholecystostomy
Group 2(a) - End-to-side portacaval shunt
Group 2(b) - Sham laparotomy

FIGURE 22 SURVIVAL PATTERNS IN THE FOUR GROUPS OF ANIMALS

the group 2(b) animals.

Over 70% of the animals in each group lost, or failed to gain weight, as shown in Table 9 on page 106F, and there was no mean weight gain in either group as demonstrated in Figure 20 on page 107F.

Wound sepsis was common in both groups, wounds healed slowly, and wound breakdown was encountered in a few animals in each group. The abdominal cavity appeared to accommodate the additional organ well, as there was little increase in pendulousness.

There were no outstanding clinical differences between animals from groups 1(a) and 1(b) and the two groups were considered physically comparable.

Three transplanted animals, taken 26, 27 and 28 days postoperatively, are demonstrated in Figure 21 on page 106F.

2. SURVIVAL

The survival patterns are shown in Figure 22 on the opposite page. Some of the initial animals were kept for longer than 28 days, but in general their quality of life was so poor, that it was decided to sacrifice the remaining animals at 28 days irrespective of clinical condition, and thus both alleviate suffering, and establish a uniform period of study. In addition, any animal that appeared grossly ill or suffering, was humanely sacrificed, irrespective of the postoperative period. These policies resulted in the following mean periods of study, in days:

MAIN CAUSE OF DEATH	GROUPS							
	1(a)		1(b)		2(a)		2(b)	
	No.	%	No.	%	No.	%	No.	%
Sacrifice - protocol	6	43	4	28	5	36	4	80
- humane	3	21	2	14	2	14	-	-
Anaesthetic - accidental	1	7	1	7	-	-	-	-
- malignant hyperpyrexia	-	-	1	7	-	-	1	20
Iatrogenic haemothorax	-	-	-	-	2	14	-	-
Gastric ulcer - haemorrhage	-	-	2	14	-	-	-	-
- perforation	-	-	1	7	-	-	-	-
Small bowel obstruction	1	7	-	-	1	7	-	-
Wound dehiscence	1	7	-	-	-	-	-	-
Pulmonary infection	-	-	2	14	1	7	-	-
Pericarditis	1	7	-	-	1	7	-	-
No cause determined	1	7	1	7	2	14	-	-
TOTAL	14		14		14		5	

TABLE 10 CAUSE OF DEATH IN THE FOUR GROUPS OF ANIMALS

Group 1(a) - Heterotopic allograft plus cholecystocholecystostomy.
Group 1(b) - Heterotopic allograft plus cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

Group 1(a)	20,84 ± 9,36 S.D.	(13)*
Group 1(b)	20,21 ± 7,71 S.D.	(14)
Group 2(a)	25,21 ± 8,82 S.D.	(14)
Group 2(b)	26,60 ± 2,80 S.D.	(5)

*Pig No. 3 survived 56 days and was excluded from the calculations.

3. CAUSE OF DEATH

The causes of death in the four groups of animals are summarised in Table 10 on the opposite page, and detailed in the case histories in the Appendix, pages 269-315. Many animals exhibited multiple organ pathology and associated sepsis, and in these cases the condition that appeared most serious was taken as the main cause of death.

No animals died of causes directly attributable to complications of the biliary tract.

Anaesthetic-associated death accounted for four deaths. Animals Nos. 10 and 16 died soon after being induced with Halothane via a nose cone for the weekly procedures. Animal 21 was of interest as both donor and recipient animals had had hyperpyrexia at the time of transplant. The donor's temperature was 41,1°C and the recipient's 39,5°C. Halothane was stopped, and the animals were resuscitated by correcting acidosis and administering 10 ml of Procaine. Postoperatively the recipient did well, but as soon as the weekly Halothane anaesthetic was started on day 7, the animal became warm, stiff and died. Pig 43 had an uneventful sham-laparotomy, but on days 7 and 14 light Halothane anaesthesia produced a rapid increase in temperature, but the animal recovered

rapidly from the anaesthetic. On day 21 the animal woke after a light Halothane anaesthetic, and became stiff and died approximately 10 minutes after the anaesthetic. In both pigs immediate autopsy was performed; the internal organs were strikingly warm, and death was attributed to Halothane-induced malignant hyperpyrexia.

Two animals (Nos. 36 and 42) died from massive haemothoraces following percutaneous venepuncture at the base of the neck. Despite the high incidence of gastric ulcers in groups 1(a), 1(b) and 2(a), only 3 animals died from complications directly attributed to gastric ulceration; exsanguinating haemorrhage in animals 23 and 24, and a perforated gastric ulcer with extensive peritoneal soiling in animal 15.

Small bowel obstruction caused the death of animals 6 and 37. The former was due to a retained swab under the donor liver. Animal 11 died from a massive wound dehiscence with disembowelment.

Despite the high incidence of pulmonary infection in all groups, only 2 deaths were attributed to pneumonia - animals Nos. 25 and 27), and one to extensive pulmonary abscesses (animal No. 32). Severe constrictive pericarditis caused the deaths of animals Nos. 4 and 35.

The exact cause of death could not be determined in animals Nos. 2, 22, 29 and 41.

Seven animals were sacrificed for humane reasons as detailed in the case histories, while 19 were sacrificed according to protocol.

GRADE OF WOUND SEPSIS	GROUP							
	1(a)		1(b)		2(a)		2(b)	
	No	%	No	%	No	%	No	%
0	3	21	1	7	3	21	0	0
+	5	36	5	36	4	29	3	60
++	2	14	5	36	6	43	2	40
+++	4	29	3	21	1	7	0	0

TABLE 11 THE INCIDENCE AND DEGREE OF WOUND SEPSIS IN THE FOUR GROUPS

Group 1(a) - Heterotopic allograft plus cholecystocholecystostomy.
Group 1(b) - Heterotopic allograft plus cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

GENERAL COMPLICATIONS AND AUTOPSY FINDINGS IN THE FOUR GROUPS OF ANIMALS

The main complications and autopsy findings will be discussed in this section. The findings in the biliary tracts, livers and transplanted blood vessels will be discussed separately in the next two sections. The macroscopic assessment was performed by the author, while the microscopic analyses were performed by Dr. J. van den Ende. The author viewed all the histology slides with the pathologist.

1. WOUND SEPSIS

The abdominal, neck and subcostal laparotomy wounds were examined weekly and at autopsy, and the degree of wound sepsis roughly graded as:

0 - No sepsis

\+ - Mild superficial sepsis

++ - Severe deep sepsis

+++ - Gross sepsis with wound breakdown.

The crude incidence and degree of wound sepsis is summarised in Table 11 on the opposite page. Only 7 of the 47 experimental animals remained free of wound sepsis during the postoperative period. Severe wound sepsis occurred in all four groups of animals, but, as can be seen in the case histories, wound sepsis cleared more rapidly in the group 2(b) animals.

The worst sepsis occurred early on in the series, but improved with the combination of modifying the holding facilities, increasing the antibiotic dosage and modifying the technique of closure.

GRADE OF ADHESIONS	GROUP							
	1 (a)		1(b)		2(a)		2(b)	
	No	%	No	%	No	%	No	%
0	0	0	1	7	1	7	0	0
+	5	36	5	36	5	36	2	40
++	3	21	3	21	7	50	1	20
+++	6	43	5	36	1	7	2	40

TABLE 12 THE INCIDENCE AND DEGREE OF INTRAPERITONEAL ADHESIONS

Group 1(a) - Heterotopic allograft plus cholecystocholecystostomy.
Group 1(b) - Heterotopic allograft plus cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

The slight difference in grade of wound sepsis in groups 1(a) and 1(b) is probably fortuitous, and not related to the type of biliary drainage used.

2. INTRAPERITONEAL ADHESIONS

Bearing in mind the philosophy that auxiliary liver transplantation may be temporary, leading to later donor hepatectomy when the host's own liver has recovered, or permanent, with later recipient hepatectomy as discussed in the Main Introduction, a crude assessment was made of the extent and incidence of intraperitoneal adhesions at autopsy. The whole peritoneal cavity was explored, and the degree of adhesions roughly graded as follows:

0 - No adhesions

\+ - Minimal adhesions

++ - Moderate adhesions

+++ - Severe adhesions.

The crude incidence and degree are shown in Table 12 on the opposite page.

Only 2 of the 47 experimental animals had not developed adhesions. Moderate and severe adhesions were seen in all four groups of animals. The adhesions were tenacious and severe over the transplanted livers.

The incidence and degree of adhesions found was more severe than that seen, in the author's experience, in major human surgery, and the propensity to form adhesions may be a property of the pig,

as other research workers have also encountered severe adhesions in the pig (Immelman - personal communication). Donor liver or recipient liver excision 28 days postoperatively would have been extremely difficult in most allografted animals in this series, and would have entailed considerable blood loss from oozing due to separation of the adhesions.

3. INTRAPERITONEAL SEPSIS

The presence of intraperitoneal, extrahepatic abscesses was recorded.

Three abscesses were found in group 1(a):-
a large pelvic abscess was present in pig No. 2, a huge subphrenic abscess in pig No. 4, while in pig No. 7 a small abscess was found between the liver and lateral abdominal wall at the site of the weekly subcostal laparotomy incisions.

Two abscesses were seen in group 1(b) animals. In pig No. 20 a small abscess was related to the subcostal laparotomy scar, while in pig No. 23 a small abscess was found on the lesser curvature of the stomach.

No intraperitoneal abscesses were found in the groups 2(a) and 2(b).

The incidence of intraperitoneal sepsis was surprisingly low, bearing in mind the magnitude of the operations and the incidence of wound sepsis.

	GROUP							
	1(a)		1(b)		2(a)		2(b)	
	No.	%	No.	%	No.	%	No.	%
No demonstrable ulcer	4	31	4	29	3	23	4	80
significant anaemia	2	50	2	50	0	-	2	50
Ulcer pars oesophagea	9	69	8	57	9	69	1	20
significant anaemia	5	56	5	63	5	56	0	-
Other ulcers	0	-	2	14	1	8	0	-
significant anaemia	0	-	2	100	0	-	0	-
Not recorded	1	-	0	-	1	-	0	-

TABLE 13 THE INCIDENCE OF GASTRIC ULCERS AND ANAEMIA IN THE FOUR GROUPS

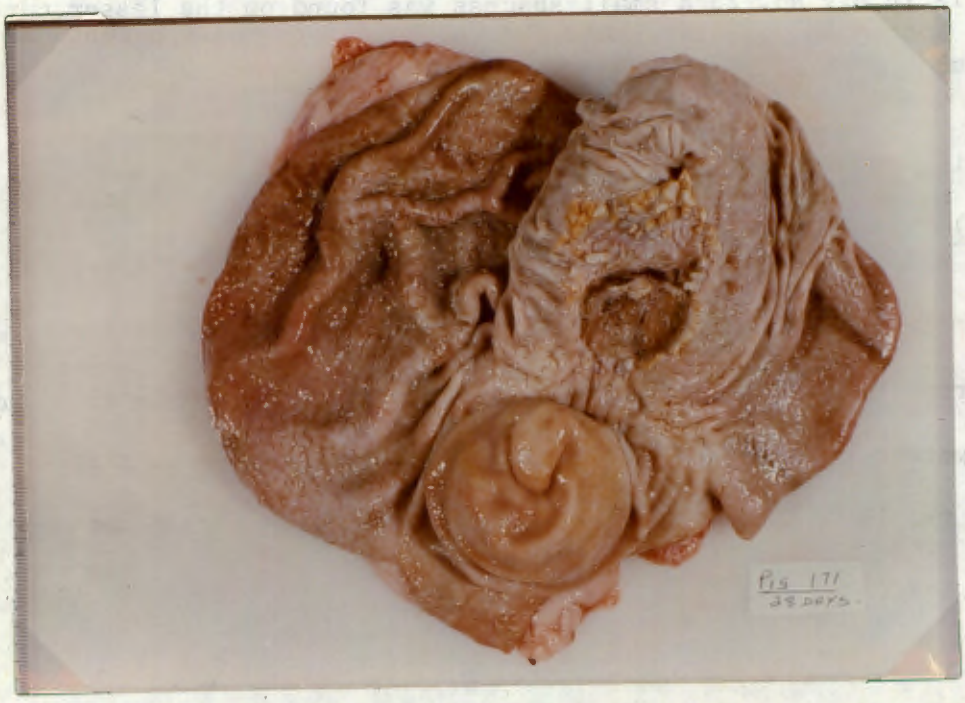


FIGURE 23 GASTRIC ULCER FOLLOWING LIVER TRANSPLANTATION

4. ASCITES

A moderate amount of clear ascites was seen in pigs Nos. 12 and 13 from group 1(a), and Nos. 15, 19 and 28 from group 1(b). Biliary ascites was seen in pig No. 26.

No ascites was seen in groups 2(a) or 2(b).

5. GASTRIC ULCERS

Gastric ulceration occurred in approximately 70% of animals in each of groups 1(a), 1(b) and 2(a), the incidence being summarised in Table 13 on the opposite page. Most of the ulcers occurred in the pars oesophagea (see Figure 23 on the opposite page) as described by Terblanche (198), Dent (54) and Lempinen (111). Two animals, Nos. 25 and 26 in group 1(b), had multiple, shallow ulcers of the greater curvature and antrum, while pig No. 29 had multiple small ulcers throughout the stomach. In 7 transplanted animals, gastroduodenostomies had been performed in an attempt to prevent gastric ulceration - 5 of these developed ulcers of the pars oesophagea, despite the demonstration of widely patent gastroduodenostomies in all 7 at autopsy.

Gastric ulceration was the main cause of death in 3 animals, exsanguinating haemorrhage in animals Nos. 23 and 24, and perforation in animal No. 15. Pig No. 37 died from small bowel obstruction but was found to have had a recent large bleed from a gastric ulcer. Of the 19 transplanted animals with gastric ulcers, 12 (63%) had anaemia of 9g% or less. Four of the seven animals without ulcers (57%) had anaemia of 9g% or less. The gastric ulcers probably contributed to the prevalent anaemia in

	GROUP			
	1(a)	1(b)	2(a)	2(b)
No demonstrable pathology	12	11	11	3
Small bowel obstruction	1	-	1	-
Small bowel perforation	-	1	-	-
Colonic papules	-	-	1	2
Plasma cell infiltrate	1	1	-	-
Peritonitis	-	1	-	-
Not recorded	-	-	1	-

TABLE 14 PATHOLOGY OF THE LARGE AND SMALL BOWEL

	GROUP			
	1(a)	1(b)	2(a)	2(b)
No demonstrable pathology	2	2	1	3
Pneumonia	10	8	6	2
Abscesses	1	3	2	-
Total pulmonary infection	11 (79%)	11 (79%)	8 (62%)	2 (40%)
Atelectasis	-	-	3	-
Hamartomatous tumour	1	-	-	-
Pulmonary embolus	1	-	-	-
Gross pleural adhesions	1	-	-	-
Iatrogenic haemothorax	-	1	2	-
Not recorded	-	-	1	-

TABLE 15 PATHOLOGY OF THE LUNGS AND PLEURA IN THE FOUR GROUPS

the two transplant groups, but other factors were also contributory. In group 2(a), ten animals had ulcers, 5 (50%) of whom had anaemia. The animals without ulcers had no anaemia. In group 2(b) two animals without gastric ulcers developed anaemia. The genesis of the anaemia will be discussed on page 215.

6. INTESTINAL PATHOLOGY

The autopsy findings are summarised in Table 14 on the opposite page. Despite the high incidence of dense adhesions, fatal small bowel obstruction was seen in only 2 animals (Nos. 6 and 37), one of which may have been preventable. One leak was detected - in animal No. 28, with no obvious cause. It was speculated that this may have been due to an inadvertent perforation of the small bowel by the liver biopsy needle, or an inadvertent cautery burn.

Small translucent papules, ± 1 mm in diameter, studded the large bowel in 3 animals. The nature could not be elicited on histology. Mild plasma cell infiltration was noted in the small bowel of 2 animals, while the gross evidence of peritonitis in animal No. 15 was confirmed histologically.

7. PULMONARY PATHOLOGY

Only 8 out of 46 experimental animals were macroscopically and microscopically free of pulmonary pathology, as shown in Table 15 on the opposite page. Severe pneumonia was seen in 11 animals and accounted for the deaths of pigs Nos. 25 and 27. Mild pneumonia was seen microscopically in 15 animals, in most of whom the

	GROUP			
	1(a)	1(b)	2(a)	2(b)
No demonstrable pathology	8	7	7	4
Pericarditis	2	3	3	1
Myocardial fibrosis	2	-	-	-
Excessive pericardial fluid	2	4	3	-
Myocardial parasites	1	1	-	-
Not recorded	-	-	1	-

TABLE 16 PATHOLOGY OF THE HEART AND PERICARDIUM IN THE FOUR GROUPS

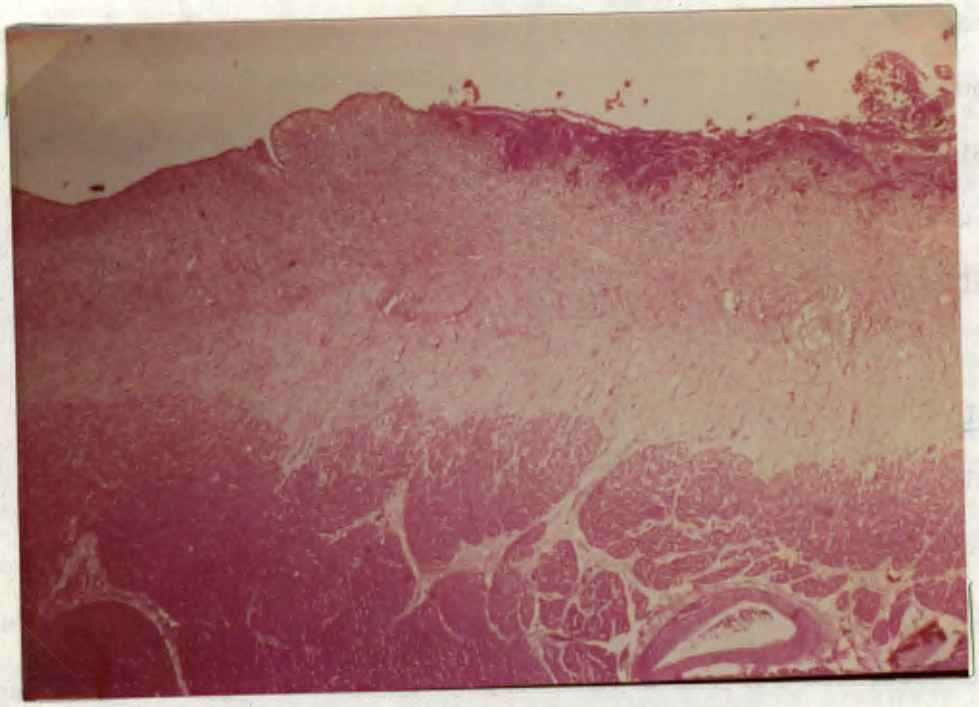


FIGURE 24 THE INTENSITY OF THE PERICARDITIS

lungs appeared macroscopically normal. Pulmonary abscesses were seen in 6 animals, accounting for the death of one - pig No. 32. Pulmonary infection was thus encountered in 70% of all animals. Groups 1(a) and 1(b) had a similar high incidence (79%) while groups 2(a) (62%) and 2(b) (40%) evidenced less, but still significantly high incidences of pulmonary infection.

Iatrogenic haemothoraces due to percutaneous venepuncture at the base of the neck were found in 3 animals, and caused the death of two of these - pigs Nos. 36 and 42. In pig No. 19 a resolving haemothorax was an incidental finding at autopsy. Atelectasis, pulmonary congestion and pulmonary embolism were rarely encountered. A hamartomatous tumour was encountered in the lungs of pig No. 5. Severe bilateral pleural adhesions were encountered in pig No. 9, with a normal underlying lung on microscopy.

Several of the donor animals were found to have pneumonia and/or pleural adhesions, and the high incidence of pulmonary infection may be partly due to inadequate holding facilities, or the delivery to the laboratory of animals with pre-existing disease.

8. CARDIAC COMPLICATIONS

The cardiac pathology encountered in the four groups is summarised in Table 16 on the opposite page. There were no detectable abnormalities of either heart or pericardium in 26 animals. Pericarditis was seen in 9 animals. The pericarditis was gross in 6 animals and mild in 3. A photomicrograph on the opposite page demonstrates the intensity of the disease encountered. In a few animals there was associated pulmonary pathology. Excessive

	GROUP			
	1(a)	1(b)	2(a)	2(b)
No demonstrable pathology	1	3	1	-
Reactive	9	6	9	4
Histiocytic proliferation	1	-	1	-
Extramedullary haemopoeisis	-	1	-	-
Haemorrhagic	-	-	1	-
Acute lymphadenitis	-	-	1	-
Autolysed	-	1	-	-
Not recorded	3	3	1	1

TABLE 17 PATHOLOGY OF THE LYMPH NODES

	GROUP			
	1(a)	1(b)	2(a)	2(b)
No demonstrable pathology	13	11	11	4
Pyelonephritis	-	2	-	-
Hydronephrosis	1	-	1	-
Focal fibrosis	-	-	1	-
Lymphoid infiltration	-	1	-	1
Not recorded	-	-	1	-

TABLE 18 PATHOLOGY OF THE KIDNEYS

pericardial fluid was seen in 9 animals - the histology in most of these was non-contributory and bacteriological cultures failed to reveal any organisms. Parasites were seen in 2 hearts and focal myocardial fibrosis in two.

Pericarditis was seen in a number of donor animals. The pericarditis was a striking feature in the series, and occurred in all groups. The phenomenon may be unrelated to the operative procedures and may be on the basis of pre-existing disease.

9. LYMPH NODE PATHOLOGY

A large, fleshy retrosternal lymph node was present in most animals. The histological findings in these nodes are summarised in Table 17 on the opposite page. Five of the nodes were assessed as normal. Mild to moderate reactive changes were seen in 28 animals. Acute lymphadenitis was seen in pig No. 39. Extramedullary haemopoeisis was seen in one node from pig No. 19. Sinus histiocytic proliferation was prominent in pigs 14 and 37.

There were no gross histological differences between the nodes examined from the four groups of animals.

10. PATHOLOGY OF THE KIDNEYS AND BLADDERS

The kidneys were significantly free of complications as summarised in Table 18 on the opposite page. Normal histology was shown in 39 animals, two of which demonstrated mild unilateral hydronephrosis at autopsy - pigs Nos. 4 and 30. Mild resolving pyelonephritis was seen in pigs Nos. 18 and 20. Focal lymphoid infiltration was seen in pigs Nos. 21 and 45, and focal fibrosis

	GROUP			
	1(a)	1(b)	2(a)	2(b)
No demonstrable pathology	11	10	13	14
Pancreatitis	1	-	-	-
Duct obstruction	-	1	-	-
Autolytic	2	2	1	-
Not recorded	-	1	-	1

TABLE 19 PATHOLOGY OF THE PANCREAS IN THE FOUR GROUPS OF ANIMALS

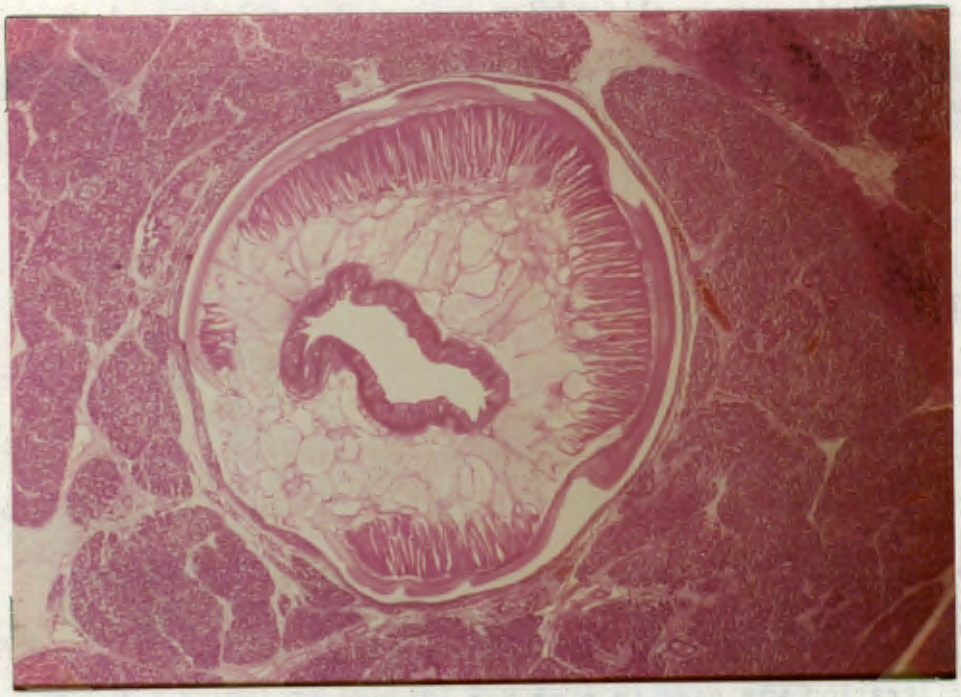


FIGURE 25 ASCARIS OBSTRUCTING THE PANCREATIC DUCT

in pig No. 31. There was no observed difference between the kidneys from the four groups of animals.

The bladders of 44 animals were assessed as normal. In pig No. 5 the bladder was thick and haemorrhagic. The animal had had haematuria during the postoperative period. Histology revealed a haemorrhagic bladder wall with no recognisable mucosa, while the kidneys were normal. The bladder from pig No. 12 revealed microscopic granulomata towards the peritoneal surface, but the remainder of the wall and the mucosa were normal.

11. PANCREATIC PATHOLOGY

The pancreatic pathology is listed in Table 19 on the opposite page. Most pancreases were macroscopically and microscopically normal. Mild pancreatitis with fat necrosis was seen in one animal - No. 4. An adult roundworm (Ascaris sp.) was found in the duct of animal No. 25, and the histology revealed evidence of duct obstruction. The worm is shown in the photomicrograph in Figure 25 on the opposite page.

There was no observed difference in pancreatic histology between the groups.

12. SPLENIC PATHOLOGY

Spleens from the portacaval and sham groups revealed normal histology or very mild reactive changes. In pig No. 33 extramedullary haemopoeisis was seen.

DISCUSSION IN CONTEXT

The two transplant groups, 1(a) and 1(b), have been shown to be roughly comparable with regard to pre-operative weights, operative times, quality of life, postoperative weight changes, survival and mode of death. The incidences of wound sepsis, intraperitoneal adhesions, intraperitoneal sepsis, ascites, gastric ulcers, pulmonary infection and pericarditis were essentially the same in the two groups. Intestinal, pancreatic, renal and bladder complications were infrequent and comparable, while the changes seen in the lymph nodes were similar.

There were thus no outstanding operative, clinical or general pathological differences between groups 1(a) and 1(b), and the two transplant groups can be regarded as comparable.

The incidence of pulmonary infection was significant in all four experimental groups, with groups 1(a) and 1(b) revealing an incidence of almost 80%. The extra organ in the abdominal cavity could possibly be implicated in the high incidence of pulmonary infection, although atelectasis was not seen in the transplanted animals. The high incidence of pulmonary infection in the transplant groups may reflect the combination of pre-existing infection, massive abdominal surgery and inadequate postoperative holding facilities.

Significant pulmonary infection has previously been reported in pigs (55, 137) and dogs (80, 148, 190, 195, 204) following both orthotopic and heterotopic transplantation, while lung infections have frequently been encountered in human liver transplantation (135, 179).

Gastric ulceration was extremely frequent in groups 1(a), 1(b) and 2(a), and less frequent in group 2(b). The incidence in the transplanted animals was similar to that reported by Dent in his orthotopic series from the same laboratory, and the subject has been discussed by him (54).

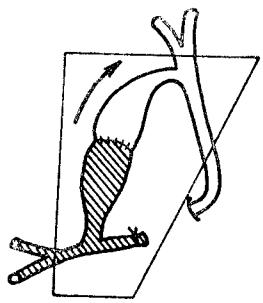
The high incidence of wound sepsis in all four groups was not unexpected, as the abdominal wall was not shaved and prepared pre-operatively, and the operations were performed under clean, but not sterile conditions. In addition, the conditions in the crowded temporary pig sty, together with the pig's habit of lying on its abdomen in the midst of faeces and urine, may also have contributed to the high incidence of wound sepsis.

The remaining complications seen in the four groups of animals were essentially those that could be encountered after any major surgical procedure, or explicable on the basis of pre-existing disease, and do not differ materially from the complications previously reported from other liver transplant series (36, 42, 55, 111, 131, 198).

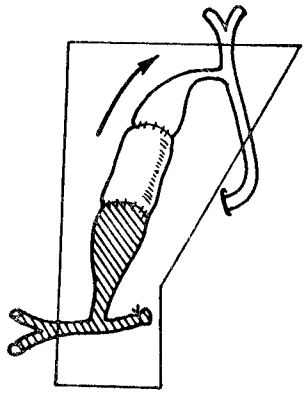
INDEX TO PART II, CHAPTER 3

SECTION II - LOCAL EFFECTS OF THE PROCEDURES ON THE EXTRAHEPATIC BILIARY APPARATUS

Group 1(a)



Group 1(b)



Groups 2(a)+2(b)

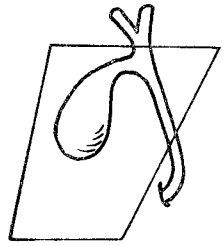


FIGURE 26 DIAGRAMS EXPLAINING THE TERM "EXTRAHEPATIC BILIARY APPARATUS"

PART II

CHAPTER 3

SECTION II - LOCAL EFFECTS OF THE PROCEDURES ON THE EXTRAHEPATIC BILIARY APPARATUS

SUMMARY

The macro- and microscopic findings in the extrahepatic biliary apparatus at initial operation and at autopsy are presented and briefly discussed. The technique of interposing a jejunal loop is shown to be feasible, with negligible changes being produced in the loops.

It is shown that the two types of biliary drainage used in the transplant groups, produced a comparable number of satisfactory results, and that the complications both macro- and microscopically were comparable and few. Negligible changes were observed in the extrahepatic biliary apparatus of the two control groups.

The results of the bacteriological studies of the extrahepatic biliary tracts in groups 1(a), 1 (b) and 2(b) are presented and discussed.

	TRANSPLANT GROUPS					CONTROL GROUPS	
	1(a)		1(b)			2(a)	2(b)
	DGB	RGB	DGB	RGB	IJL	GB*	GB
Normal	10	14	11	14	12	13	5
Thickened	2	-	1	-	-	-	-
Thickened & Bile encrusted	1	-	1	-	-	-	-
Dilated	-	-	-	-	1	-	-
Necrotic	1	-	1	-	1	-	-
Leaks	2		2			-	-
Strictures	-		-			-	-
Ulcers	-		-			-	-
Concretions	2		3			-	-
Sepsis	-		1			-	-
Bile stasis:							
Gross	1		-			-	-
Minimal	3		3			-	-
Soft plugs	2		-			-	-

DGB = Donor gallbladder
RGB = Recipient gallbladder
IJL = Interposed jejunal loop
GB = Gallbladder
* = Results not available in 1 animal

TABLE 20 MACROSCOPIC ASSESSMENT OF THE EXTRAHEPATIC BILIARY APPARATUS AT AUTOPSY

Group 1(a) - Heterotopic allograft plus cholecystocholecystostomy.
Group 1(b) - Heterotopic allograft plus cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

MACROSCOPIC PATHOLOGY OF THE EXTRAHEPATIC BILIARY APPARATUS

Details of the conduct of the experiments have been described in Part II, Chapter 2, as well as the methods used to assess the extrahepatic biliary tract pre-operatively and at autopsy. The macroscopic assessments were all performed by the author.

The term "extrahepatic biliary apparatus" for each group is explained diagrammatically in Figure 26, page 122F. The results are summarised in Table 20 on the opposite page, and detailed in the case histories, pages 269-315 in the Appendix.

1. Pre-Operative Assessment

The pre-operative gallbladders of all animals appeared normal, but mild oedema had developed in a few donor gallbladders by the time the biliary anastomoses were commenced. The jejunum and interposed jejunal loops appeared normal, and peristalsis could be provoked in all loops on completion of the anastomoses, while circulation appeared adequate as judged by colour and arterial pulsation.

2. Autopsy Assessment

Lysis of adhesions made the assessment of the serosal surface difficult in some cases in all four groups of animals, but usually some areas free of adhesions could be found to allow assessment.

(a) Control groups: 2(a) and 2(b)

In both portacaval-shunted and sham groups the gallbladders were macroscopically normal on both serosal and mucosal surfaces with no evidence of leaks, ulceration, stones, sepsis

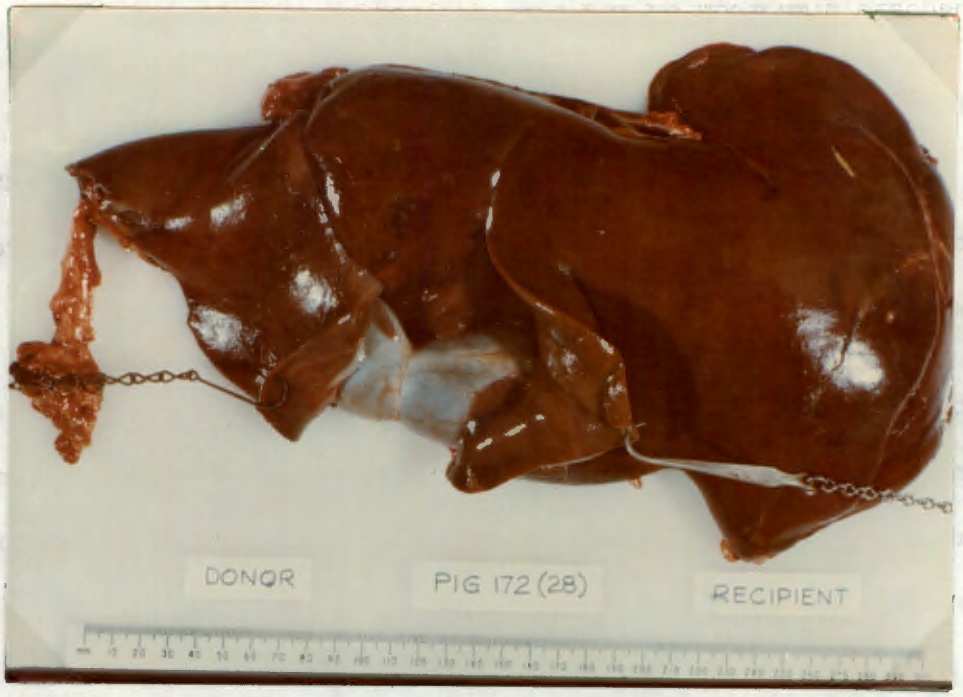


FIGURE 27 ANTERIOR VIEW CHOLECYSTOCHOLECYSTOSTOMY
(28 Days after transplant).

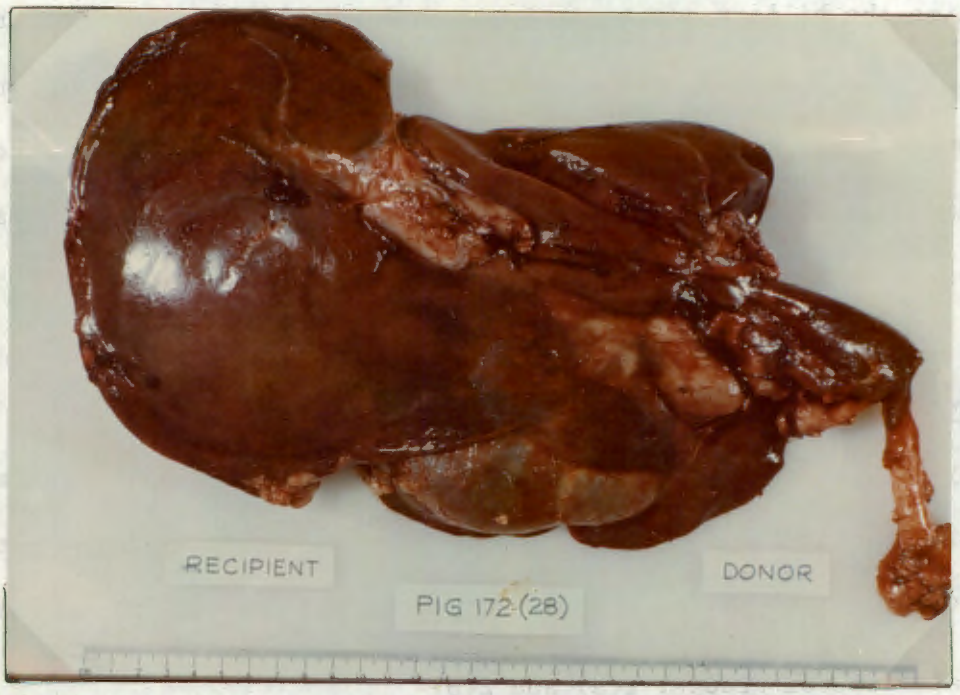


FIGURE 28 POSTERIOR VIEW CHOLECYSTOCHOLECYSTOSTOMY
(Note that the donor and recipient livers and gallbladders are almost identical in colour and appearance).

nor stasis, and colour of the bile was normal. The common bile ducts were free of obstruction. The findings in the gallbladder of one portacaval-shunted animal, No. 41 was not recorded as the animal had been dead too long for accurate assessment.

(b) Cholecystocholecystostomy - Group 1(a)

On external appearance, the conjoined gallbladders had a normal appearance in 10 animals. The gallbladders were comfortably apposed with no evidence of tension, kinking or twisting. The anterior and posterior appearances of a cholecystocholecystostomy are shown in Figures 27 and 28 on the opposite page. All the recipient gallbladders appeared normal on serosal and mucosal surfaces, while 10 donor gallbladders were assessed as normal. The mucosal surfaces of two conjoined gallbladders assessed as normal are shown in Figure 29.

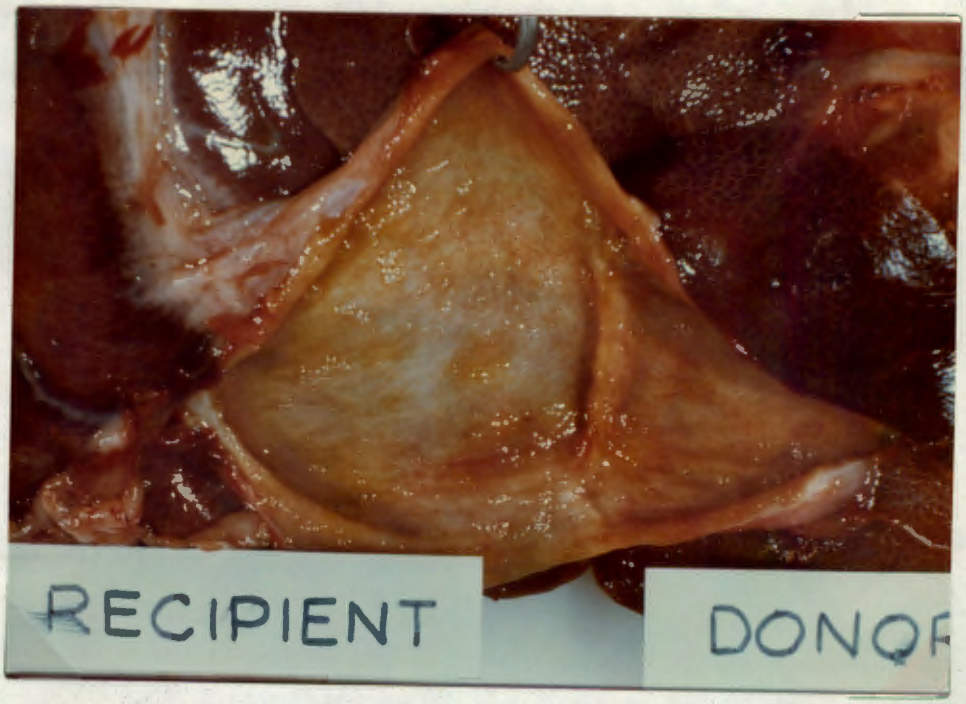


FIGURE 29 DONOR AND RECIPIENT GALLBLADDER MUCOSAL SURFACES AT 28 DAYS

In three animals (Nos. 4, 12 and 13) the donor gallbladders had shortened and retracted almost completely into the substance of the donor livers, as shown in Figure 30 and on

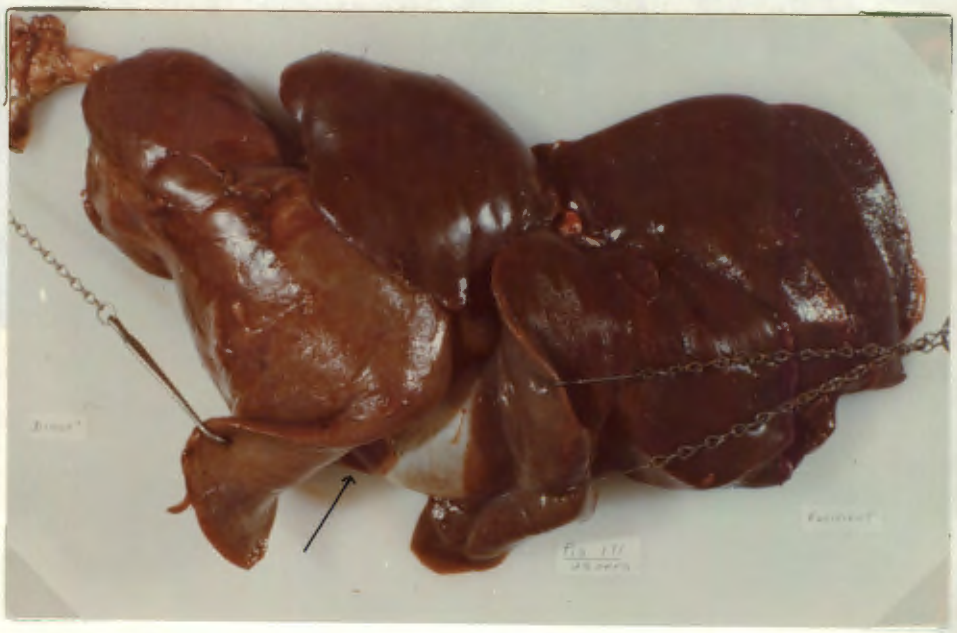


FIGURE 30 SHRUNKEN DONOR GALLBLADDER (arrowed)

opening, the walls of the donor gallbladders were found to be thickened and discoloured as shown in Figure 31. The recipient gallbladders, however, appeared normal.

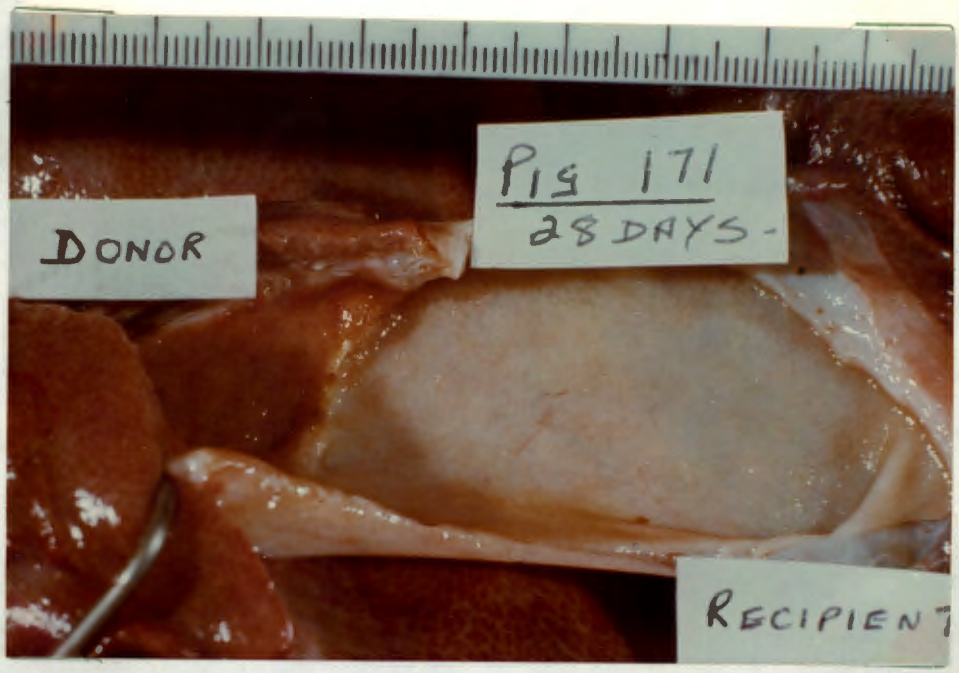


FIGURE 31 DONOR AND RECIPIENT GALLBLADDER MUCOSAL SURFACES

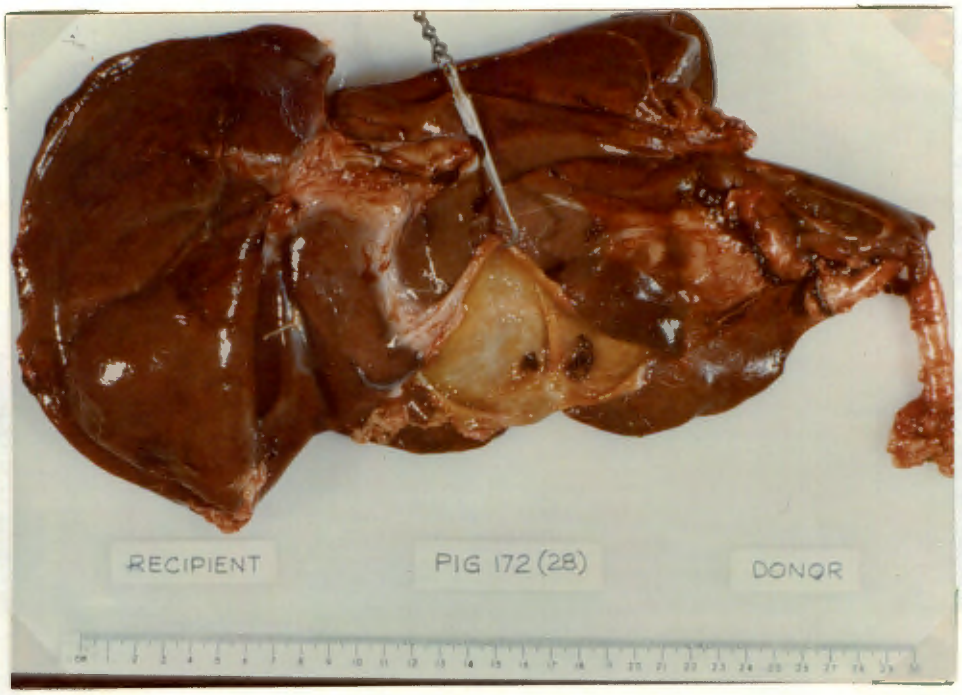


FIGURE 32 ENCRUSTATIONS AT SUTURE LINE 28 DAYS AFTER TRANSPLANT (Group 1(a))

In one animal (No. 3 at 56 days) the liver had sloughed and the donor gallbladder could not be identified; however, the recipient gallbladder was normal on opening and the anastomotic site had closed spontaneously.

The complications seen this group are summarised in Table 20 on page 123F. Two leaks were detected. In animal No. 1, dense adhesions surrounded the biliary apparatus, and a small inspissated bile deposit was present adjacent to the suture line. The peritoneal cavity was not bile stained, and it appeared that the leak had occurred early on, and had been sealed off. Pig No. 12 demonstrated mild, nonbiliary ascites, and a small, fresh sealed-off leak was found. This leak appeared to be associated with the inadvertent perforation of the biliary apparatus when doing a needle biopsy on day 21.

The anastomoses were widely patent in eleven animals with no evidence of strictures. In two animals done early on in the series (Nos. 5 and 6), the anastomotic stomata were small 0,5 cms in diameter, but patent. No anastomotic ulcers or abscesses were detected. In several animals, the anastomotic surface was excessively inverted; this was probably technical in origin.

In two animals (Nos. 7 and 9) the anastomoses were gritty to the touch on the mucosal surface, and subsequent microscopy confirmed the presence of small concretions on the suture lines.

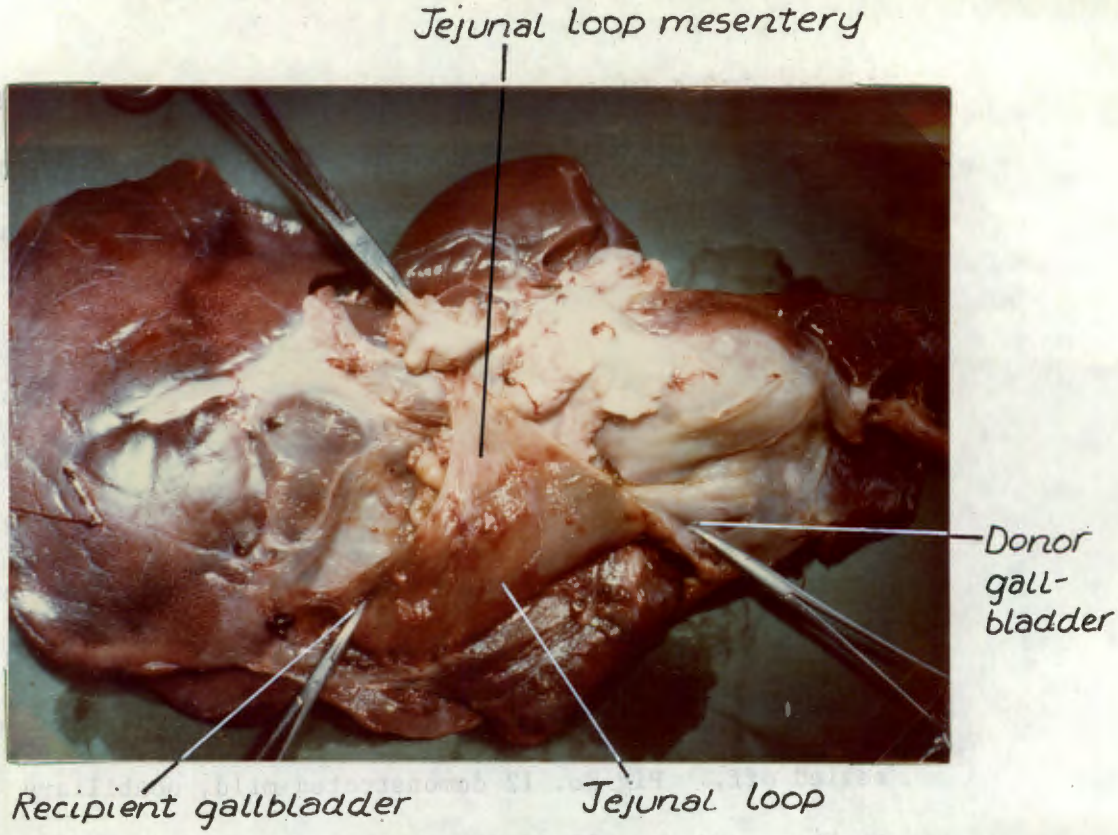


FIGURE 33 ANASTOMOSIS USING THE INTERPOSED LOOP - 28 DAYS AFTER TRANSPLANT

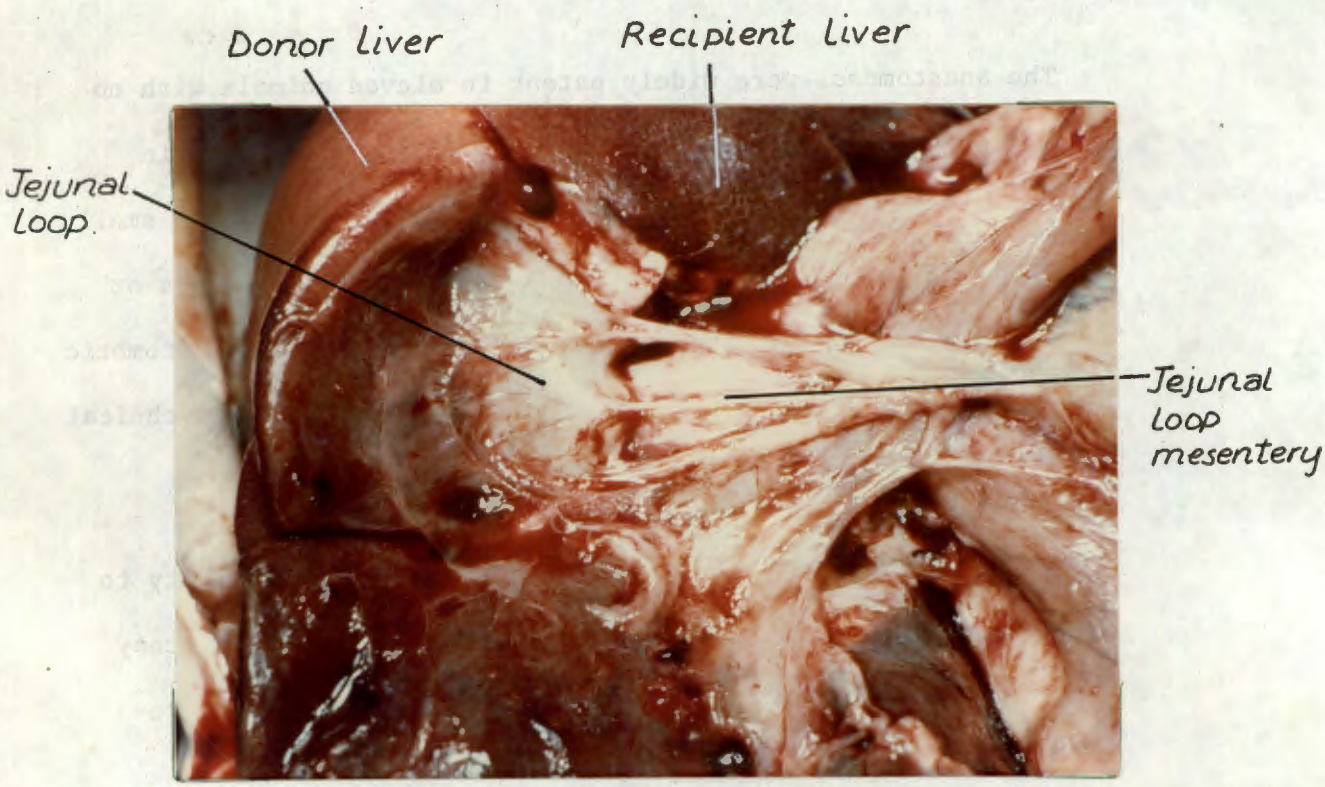


FIGURE 34 INTERPOSED LOOP WITH VASCULAR MESENTERY

In seven animals, the contents of the gallbladders appeared normal. Fine green debris (1-2 mm in size) was seen in the conjoined lumen in two animals (Nos. 5 and 7) and in pig No. 6 the bile was thickened and contained a fine brown granular sediment. These three animals were classified as having minimal bile stasis. Soft brown casts, ± 0,5 cms x 0,3 cms in size, were seen in two animals (Nos. 12 and 14). These casts crumbled on being touched and were featureless on histology. It is thought that these might be digested thrombi from bleeding due to inadequate haemostasis when constructing the anastomosis. Gross bile stasis was present in animal No. 4, with bile encrustation of the entire mucosal surface of the donor gallbladder. Small encrustations were found on the suture line of pig No. 14 (see Figure 32 on page 126F). No common bile duct obstruction was detected in the recipient animals, and no leakage was seen from the ligated donor common bile duct stumps.

(c) Cholecystojejunocholecystostomy - Group 1(b)

The findings are summarised on Table 20, page 123F.

On external appearance, 11 of the cholecystojejunocholecystostomy biliary conduits had a normal appearance. The interposed loops were comfortably accommodated between the two gallbladders, without evidence of tension, kinking or twisting. The position of such an anastomosis is shown on the opposite page in Figure 33.

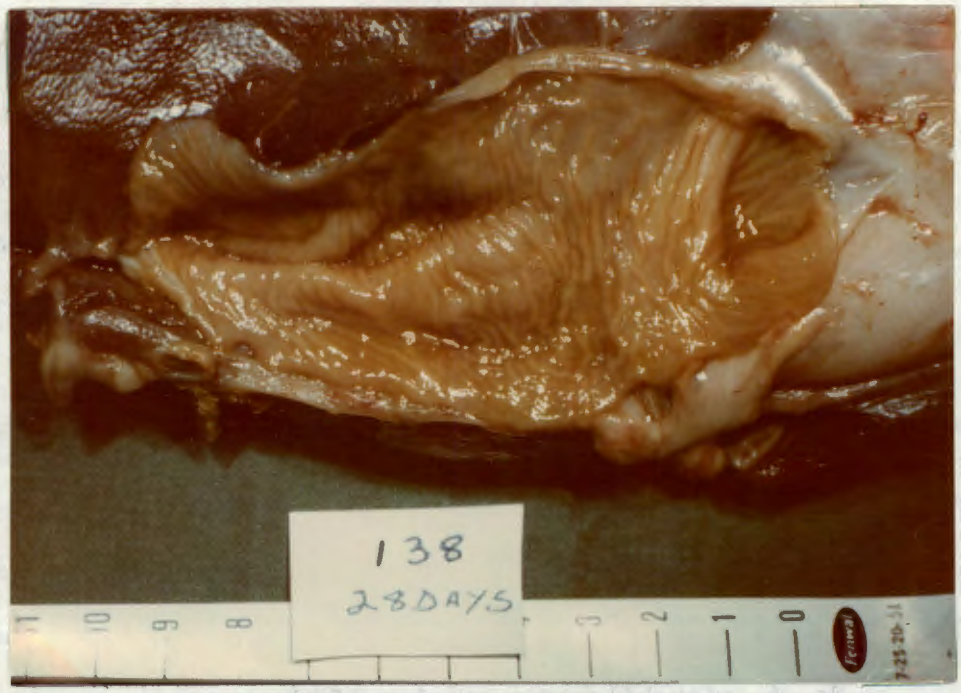


FIGURE 35 MUCOSAL SURFACE INTERPOSED JEJUNAL LOOP AT 28 DAYS

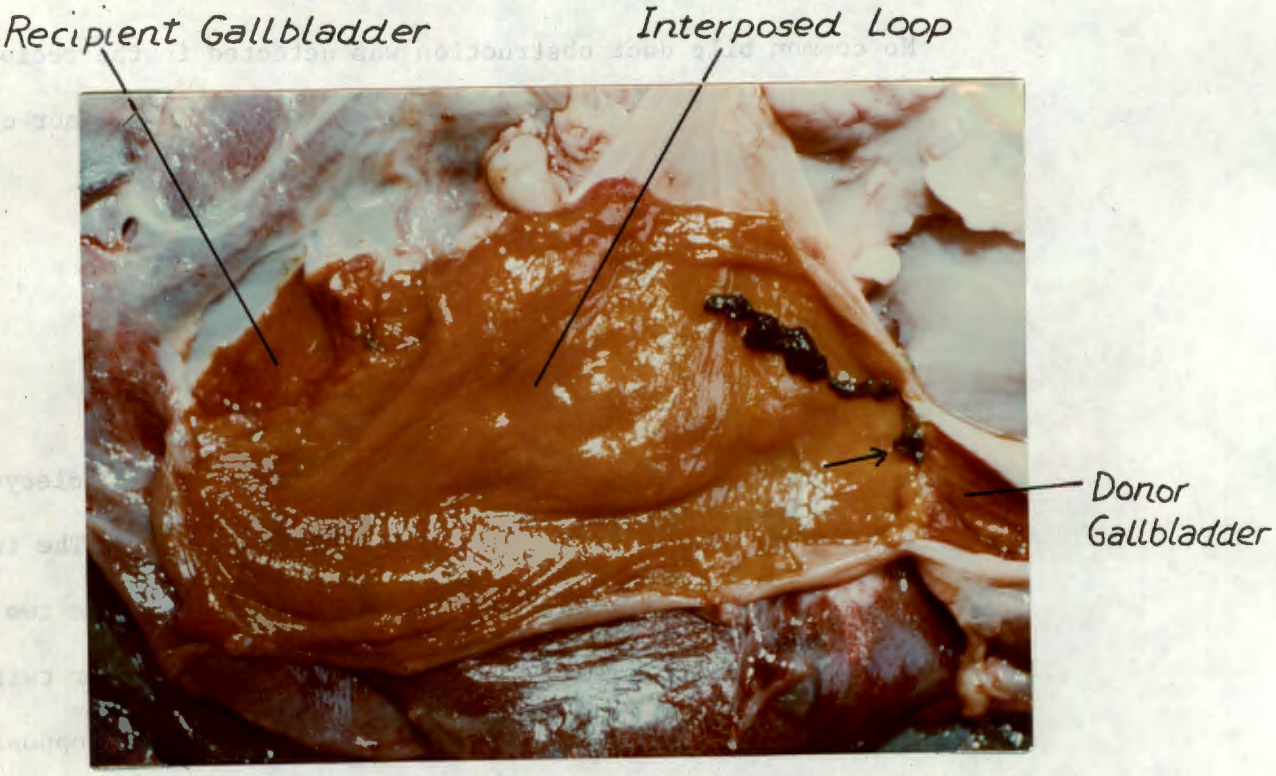


FIGURE 36 MUCOSAL SURFACE INTERPOSED JEJUNAL LOOP AT 28 DAYS

Note: (1) Mucosal surface less rugose.
(2) Encrustation at suture line (arrowed).

Twelve of the interposed jejunal loops appeared macroscopically normal, and appeared to form tension-free, non-obstructive conduits between the donor and recipient gallbladders. The jejunal mesenteries appeared tension-free and well vascularised (See figure 34 on the previous page) and in a few animals in which laparotomy preceded euthanasia, arterial pulsation could clearly be felt. Peristaltic movement could be induced in a few loops. The mucosal surfaces of the loops appeared normal in most animals, as shown in Figure 35 on the opposite page.

In a few animals, however, the rugose pattern was moderately flattened - as shown in Figure 36 on the opposite page. The whole loop was necrotic in animal 18, while in animal 26, the loop was heavily bile stained and dilated. The gross appearance of most of the interposed loops did not differ from the appearance of the jejunum in the same animals.

All the recipient gallbladders in this group appeared normal on both mucosal and serosal surfaces. Eleven donor gallbladders appeared normal, while two were contracted and thickened in the same way as seen in some donor gallbladders in the cholecystocholecystostomy group. The donor gallbladder in animal 18 was completely necrotic and the liver infarcted.

The complications seen in the external biliary tract in this group are summarised in Table 20 on page 123F. Leaks were detected in two animals. In pig 26, the peritoneal cavity was grossly bile stained with gross adhesions. The jejunal

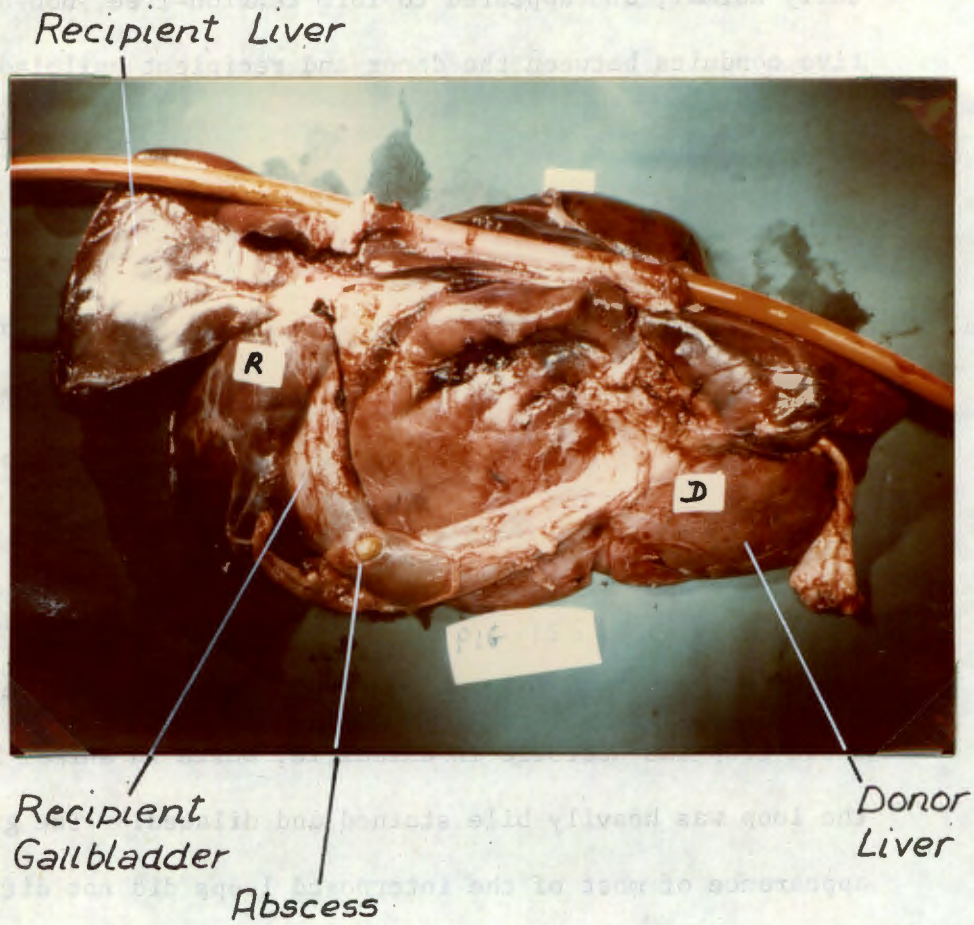


FIGURE 37 ANASTOMOTIC ABSCESS AT DISTAL CHOLECYSTO-JEJUNOSTOMY

loop was dilated and bile stained, and the recipient common bile duct found to be kinked and partially obstructed. The leak is thought to be associated with the inadvertent perforation of the dilated biliary system on day 21 when doing a needle biopsy, as the open biopsy on this occasion revealed no bile peritonitis. A small, sealed-off leak was present at the proximal cholecystojejunal anastomosis in animal 27.

All the anastomoses were widely patent (both proximal and distal) with no evidence of stricture formation or anastomotic ulcers. A large stitch abscess was seen in one animal (No. 28) - see Figure 37 on page 129F. Some of the anastomoses were excessively inverted, and in three, the anastomotic lines were gritty to feel (animals Nos. 19, 25 and 28). Subsequent microscopy confirmed the presence of small concretions on the suture lines in these animals.

The content of the biliary apparatus was completely normal-looking in 9 animals. Marked bile stasis with inspissated bile encrusting the donor gallbladders was seen in two animals (Nos. 16 and 18). Three animals (Nos. 20, 21 and 26) demonstrated fine brown granular sediment plus an aggregation of small green plaques 1-2 mm in size. This was classified as mild stasis. A large green encrustation was seen on the suture line in pig No. 17 - see Figure 36 on page 128F. No soft casts were seen in this group, nor was there evidence of excessive mucus.

With the exception of one animal (No. 26) already discussed above, the recipient common bile ducts were clear, and no leakage was detected from the ligated donor common bile duct stumps.

The additional surgical procedures performed in this group produced no significant complications. The jejunojejunal anastomoses healed well, with no evidence of leakage. The defect created in the jejunal mesentery at the time of doing the operation, had healed in all cases and no internal herniae were seen. In a few cases, small bowel loops were found posterior to the jejunal loop mesentery - a potential source for intestinal obstruction.

3. Additional points

The author was unable to detect any marked differences in macroscopic appearance, texture, or thickness between the recipient gallbladders in the two transplant groups, and the gallbladders in the portacaval and sham control groups. Twenty-one of the donor gallbladders appeared normal.

The patency of the donor and recipient cystic ducts in the two groups was extremely difficult to assess, as the structures were small, covered with adhesions, and difficult to dissect out at autopsy. Lack of radiological facilities in the laboratory precluded the performance of cholangiograms to assess cystic duct size and patency. Most of the donor and recipient cystic ducts appeared patent, but it is felt that the assessment was unreliable, and that no conclusions could be made about changes in the cystic ducts.

MICROSCOPIC PATHOLOGY OF THE EXTRAHEPATIC BILIARY APPARATUS

A number of pre-operative, as well as postoperative specimens taken from the extrahepatic biliary tract were inadvertently discarded in the laboratory. All the remaining material was analysed.

The analyses were performed independently by Professor C.J. Uys and Dr. J. van den Ende. The author viewed all the slides with the two pathologists.

1. Pre-operative specimens

The pre-operative micro-anatomy of the gallbladders in the transplant groups was essentially normal with the gross finding of oedema in a few donor gallbladders being confirmed. Mucosal autolysis was seen in one donor gallbladder. A section from a pre-operative gallbladder is shown in Figure 38. The control jejunal loop biopsies were normal.

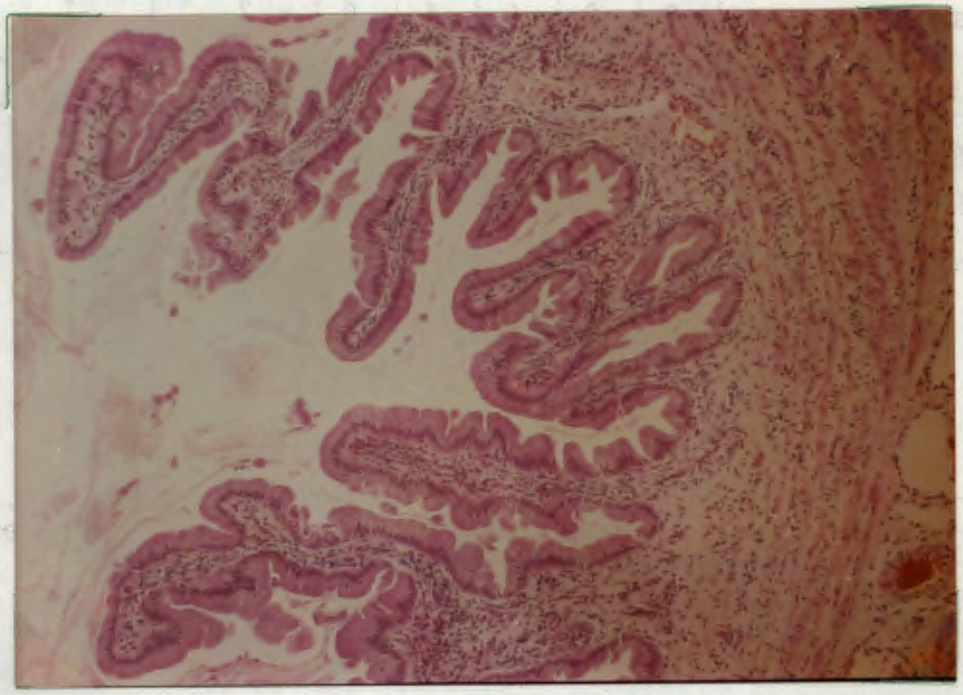


FIGURE 38 MICROSCOPIC APPEARANCE OF A PRE-OPERATIVE GALLBLADDER

	TRANSPLANT GROUPS					CONTROL GROUPS	
	1(a)		1(b)			2(a)	2(b)
	DGB	RGB	DGB	RGB	IJL	GB	GB
No. Examined	11	11	8	7	11	5	4
Normal	0	8	0	5	8	3	3
Inflammation - Acute	1	1	1	1	0	0	0
- Chronic	0	1	0	0	2	0	0
Rejection - Mild	8	0	5	0	0	0	0
- Moderate	0	0	1	0	0	0	0
- Severe	3	0	2	0	0	0	0
Mucosal Ulceration	0	1	0	0	0	0	0
Autolysis	0	0	0	1	0	2	1
Necrotic	0	0	0	0	1	0	0

DGB = Donor gallbladder. IJL = Interposed jejunal loop.
RGB = Recipient gallbladder. GB = Gallbladder.

TABLE 21 MICROANATOMY OF THE EXTRAHEPATIC BILIARY APPARATUS AT AUTOPSY

Group 1(a) - Heterotopic allograft plus cholecystocholecystostomy.
Group 1(b) - Heterotopic allograft plus cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

2. Autopsy specimens

The results are summarised in Table 21 opposite.

(a) Control groups - 2(a) and 2(b)

Gallbladder sections were viewed from five portacaval-shunted animals. Two exhibited severe postmortem autolysis, and three were normal. In the sham-operated group, three were normal, and one showed evidence of postmortem autolysis, despite the autopsy being performed immediately after death. There was no evidence of cellular infiltration in the control gallbladders.

(b) Cholecystocholecystostomy - Group 1(a)

Eight of the eleven recipient gallbladders examined histologically were classified as normal. In animal 14, a mild acute inflammatory cell infiltrate was seen. In pig 10 a mild round cell infiltrate was present, while in pig 9 small mucosal ulcers were present accompanied by a mild acute cellular infiltrate.

Varying degrees of cellular rejection were seen in the eleven donor gallbladders examined. Eight showed mild rejection with maintenance of muscle and mucosa. In pigs Nos. 9, 12 and 13 severe changes were seen, with almost complete necrosis of muscle and mucosa. It was felt that these changes were due to rejection, but the possibility of associated vascular insufficiency due to inadvertent ligation or thrombosis of the cystic arteries could not be excluded, because, in the absence of radiological facilities in our

Recipient Donor

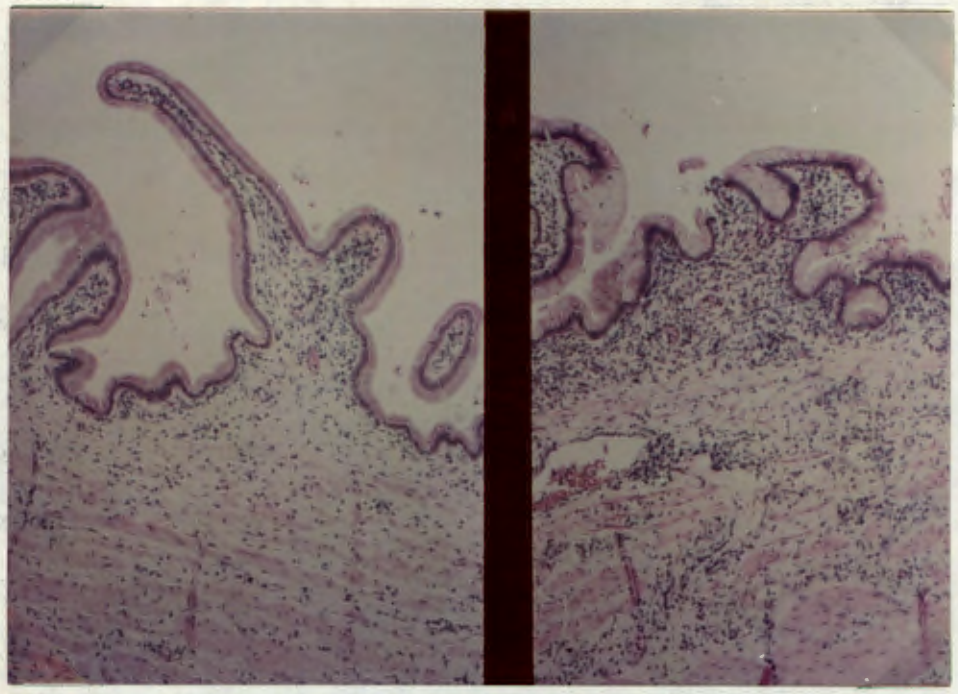


FIGURE 39 MICROSCOPIC APPEARANCES OF THE RECIPIENT AND DONOR GALLBLADDERS AT 7 DAYS

Recipient Donor

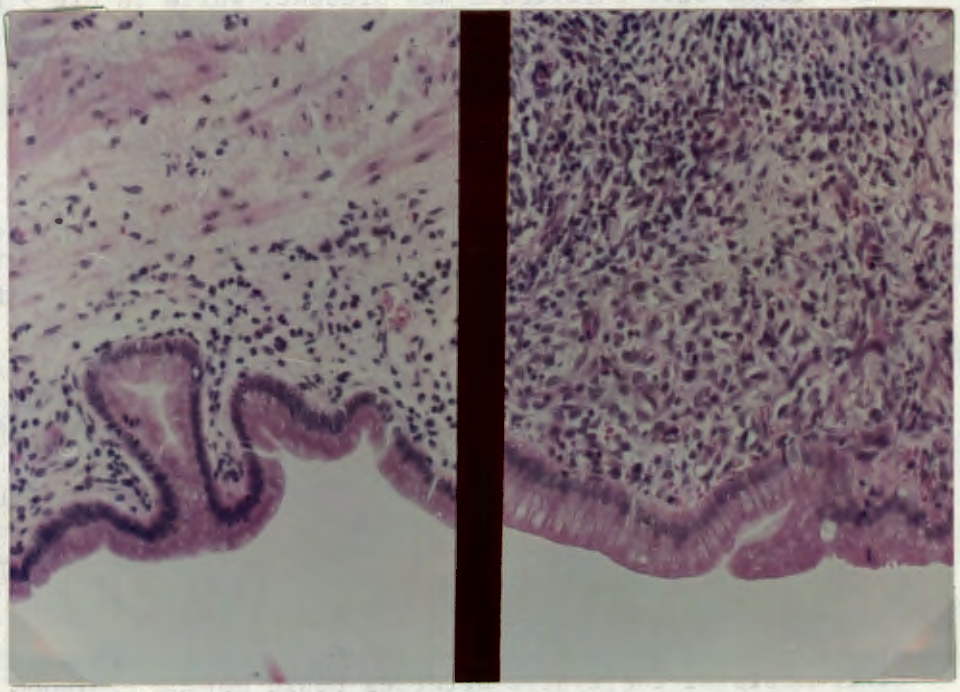


FIGURE 40 MICROSCOPIC APPEARANCES OF THE RECIPIENT AND DONOR GALLBLADDERS AT 7 DAYS

laboratory, arteriography could not be performed. In pig 4 there was evidence of mild cholecystitis. The histological differences between a donor and recipient gallbladder are shown in Figures 39 and 40, and can be compared with the pre-operative specimen shown on page 131.

Seven suture lines were examined and 3 of these showed healing with no abnormalities. The presence of small "concretions" was confirmed in pig 11 (see Figure 43, page 135F), in pig 10 excess granulation tissue was present at the site of anastomosis and a small anastomotic ulcer was seen in pig 8. In pig 7 a mild inflammatory response was seen at the site of anastomosis.

(c) Cholecystojejunocholecystostomy - Group 1(b)

Of the seven recipient gallbladders analysed, five were classed as normal. One, pig 15, showed too much postmortem autolytic change for reasonable comment. Mild cholecystitis was seen in pig 26.

Of the eleven jejunal loops analysed, six were considered completely normal. In Figure 42 the histological appearance of a loop at 28 days is shown for comparison with a pre-operative control jejunal biopsy (Figure 41). Two loops showed minimal changes; in pig 26 the loop showed mild atrophic changes, but was essentially normal, and in pig 16, the loop showed thickening of the wall with thinning of the mucosa. A mild mononuclear infiltrate was seen in pigs 19 and 23, while in pig 18, the loop was necrotic. Thus 10

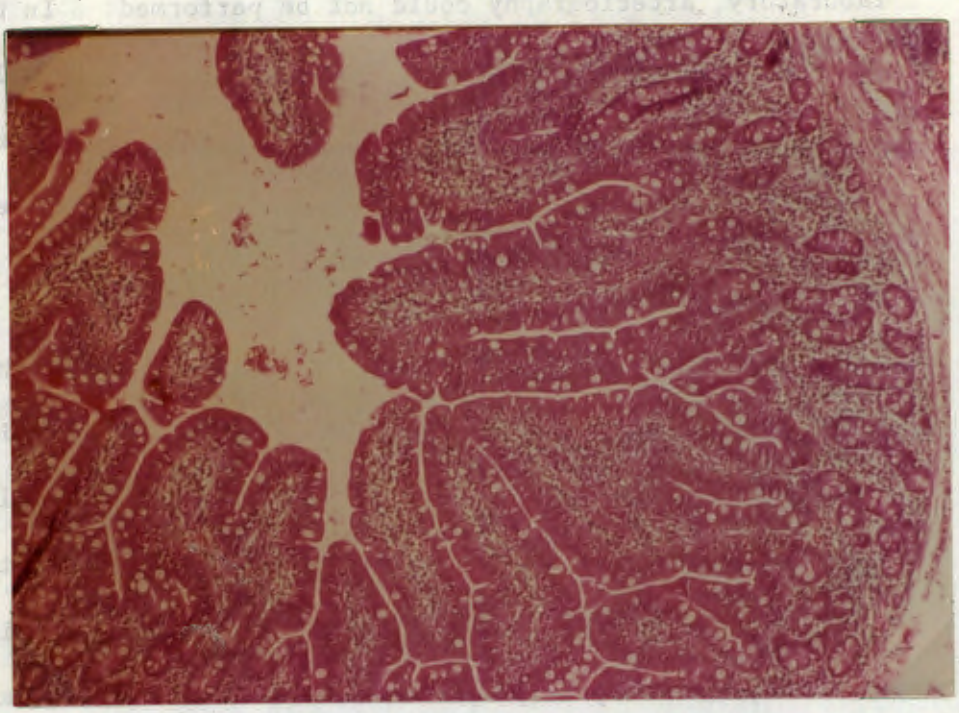


FIGURE 41 PHOTOMICROGRAPH OF PRE-OPERATIVE JEJUNAL LOOP

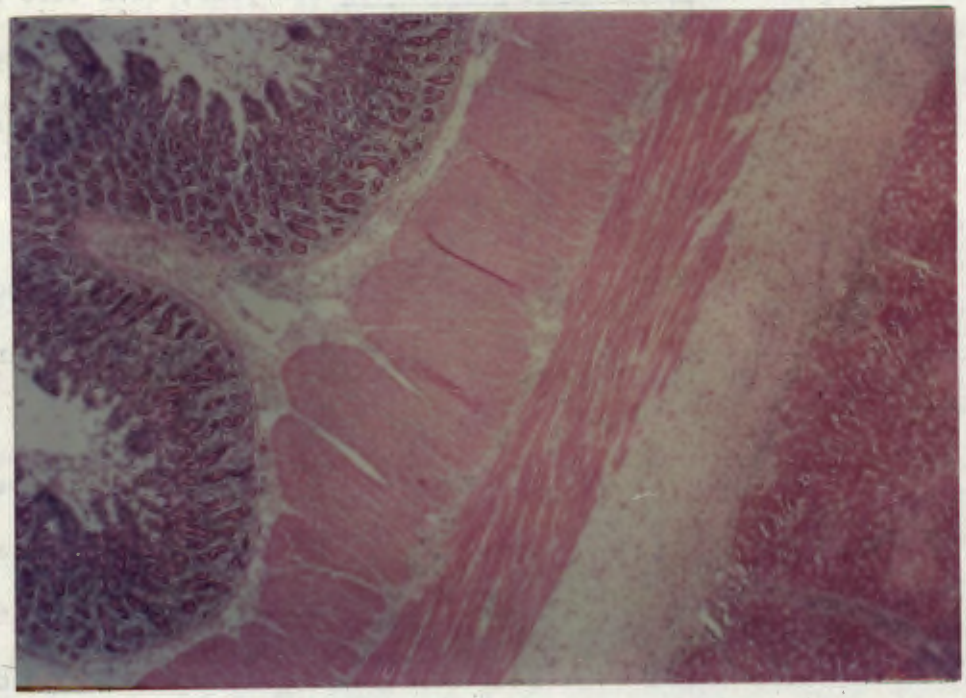


FIGURE 42 PHOTOMICROGRAPH OF INTERPOSED JEJUNAL LOOP AT 28 DAYS

of the 11 loops examined appeared viable and showed minimal changes. The histological appearances of the interposed loops did not differ materially from the simultaneous jejunal biopsies.

Eight donor gallbladder sections were analysed. Mild rejection was seen in five. In pigs 17 and 27 the rejection was severe. In pig 26, there was moderate rejection with evidence of cholecystitis and ulceration.

In pig 26 microscopic ulceration was present at the site of the anastomosis. Large anastomotic concretions were seen in pigs 26 and 28 - an example of which is shown in Figure 44 on page 135F.

No striking histological differences were observed between recipient gallbladders in the two transplant groups, but the degree of donor gallbladder rejection in the cholecystojejunocholecystostomy group appeared less severe than that seen in the gallbladder to gallbladder group.

3. Summary of the effects seen

In summary, the micro-anatomy of 18 recipient gallbladders was studied. Thirteen were considered normal, two showed evidence of mild acute inflammation, mild chronic inflammation was seen in one, and one showed evidence of microscopic ulceration of the mucosa. One specimen was too autolytic for reasonable comment. No striking histological differences could be detected between the recipient gallbladders in the two transplant groups.

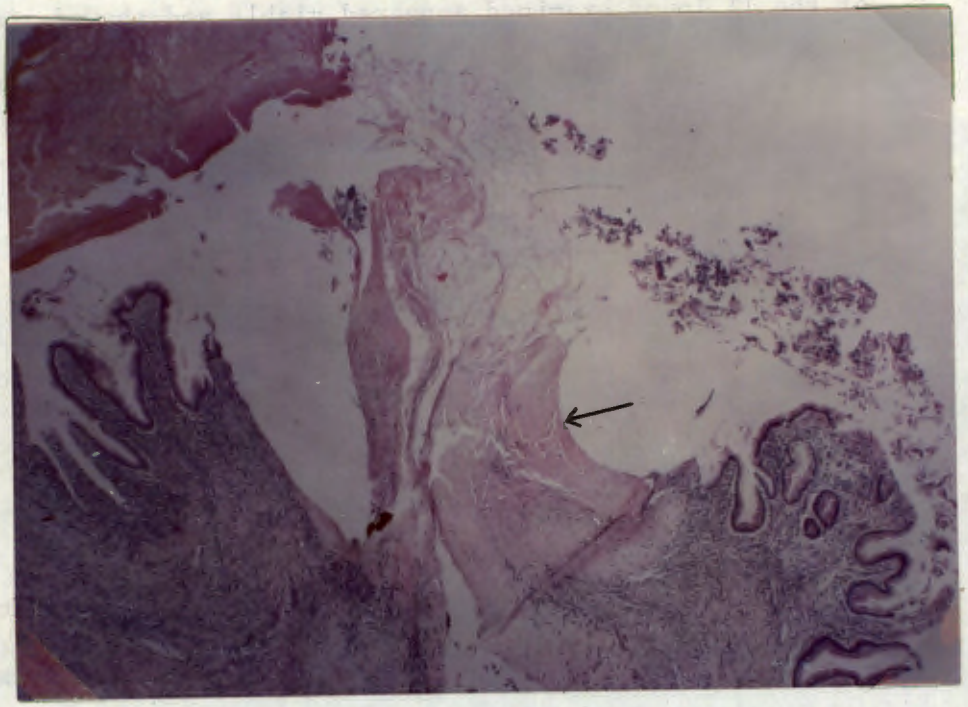


FIGURE 43 ANASTOMOTIC CONCRETION 8 DAYS AFTER CHOLECYSTO-CHOLECYSTOSTOMY (arrowed)

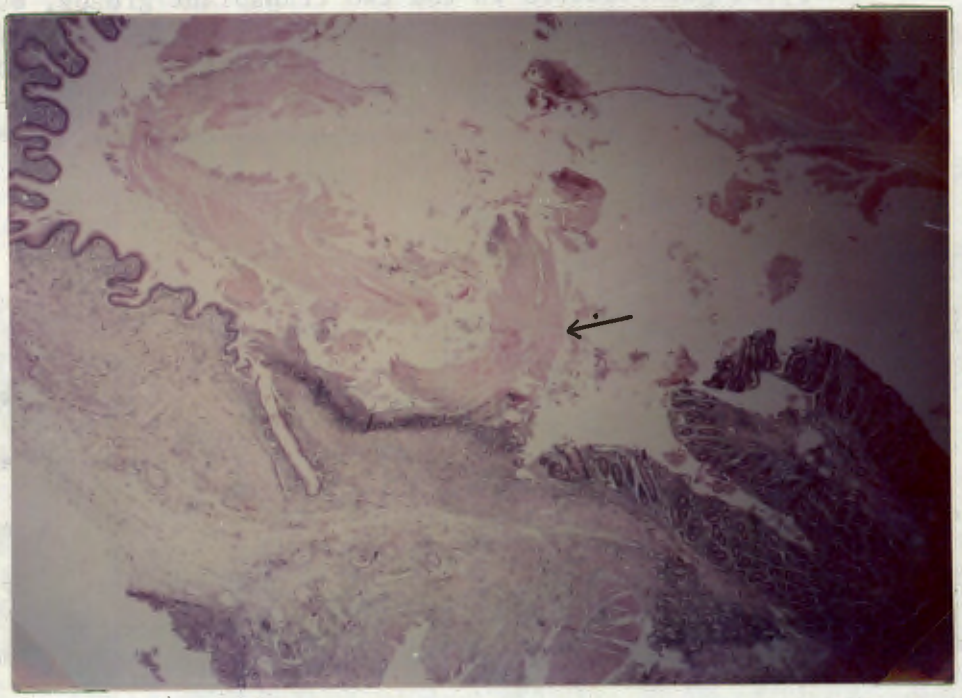


FIGURE 44 ANASTOMOTIC CONCRETION 28 DAYS AFTER CHOLECYSTO-JEJUNOCHOLECYSTOSTOMY (arrowed)

Nineteen donor gallbladders were analysed, all of which showed evidence of rejection, through a wide spectrum. In five animals the rejection was severe, with almost complete necrosis of gallbladder wall; the remaining 14 showing less severe rejection. Acute inflammatory changes were seen in two, and mucosal ulceration in one. The degree of rejection in the cholecystocholecystostomy group, in general, appeared more severe than in the group where the interposed loop was used. As shall be shown later, the degree of rejection seen in the livers in this group was also more severe.

Very mild changes were seen in two of the interposed jejunal loops, the remainder being classed as normal, with no evidence of acute inflammation. One showed macro- and microscopic necrosis.

BACTERIOLOGY OF THE EXTRAHEPATIC BILIARY APPARATUS

Pre-operative donor and recipient gallbladder swabs were taken from most of the animals in the two transplant groups 1(a) and 1(b). In group 1(b), swabs were also taken from the jejunal loop prior to its interposition between the two gallbladders.

At autopsy, swabs were taken only from sacrificed transplant recipients and sham operated animals. A single swab was taken from the extrahepatic biliary apparatus as soon as it had been opened. No swabs were taken from animals that had been dead for longer than 20 minutes. Culture swabs were not taken from group 2(a) animals, nor from a few of the earlier transplant animals. The results are summarised on Table 22 on the following page.

Group	PRE-OPERATIVE				AUTOPSY
	Pig No.	Gall Bladder Donor	Gall Bladder Recipient	Jejunum	Extrahepatic Biliary Apparatus
1(a)	7	N	N	-	E. coli
	8	N	N	-	N
	9	N	N	-	Coliforms Enterococci
	10	N	N	-	-
	11	N	N	-	N
	12	N	-	-	N
	13	N	N	-	Paracolon
	14	N	N	-	Coliforms Achromobacter
1(b)	18	N	Pseudomonas	Staph. aureus	-
	19	N	N	Coliforms Paracolon	Coliforms (2 Strains)
	20	Coliforms	N	Coliforms Paracolon Enterococci	-
	21	Coliforms	N	Coliforms	Coliforms Staph. albus
	22	N	N	Coliforms Paracolon	-
	23	N	N	N	Enterobacter
	24	N	N	N	Coliforms
	25	N	N	N	-
	26	N	N	Coliforms	-
	27	N	N	Pseudomonas Coliforms	-
	28	N	N	Pseudomonas Flavobacter sp. Prot. rettgeri	Pseudomonas
2(b)	43	-		-	N
	44	-		-	Enterococci Staph. aureus
	45	-		-	N
	46	-		-	Enterococci Coliforms
	47	-		-	Coliforms

N = Negative Culture. - = No specimen submitted.

TABLE 22 BACTERIOLOGY OF THE BILIARY APPARATUS

1. Pre-operative specimens

(a) Gallbladders

Thirty-seven swabs were processed. No organisms were cultured from thirty-four of the gallbladders. Coliforms were cultured from two animals, and Pseudomonas from one animal.

(b) Interposed jejunal loops

Of the eleven swabs processed, only three proved to be negative on culture. Eight revealed the presence of organisms, a mixed growth being obtained in 5, and a single growth in 4. Thus over 70% of the loops were shown to harbour organisms at the time of initial operation.

2. Autopsy specimens

In group 1(a), seven swabs were studied. No organisms were cultured from the extrahepatic biliary tract in 3 animals, while 4 were positive for bowel organisms.

Five specimens were studied in group 1(b), and all produced positive cultures. In three of the positive cultures, the same organism was cultured both pre- and postoperatively. In the remaining two, the pre-operative cultures were negative.

Five specimens from group 2(b) were analysed. Bowel organisms were cultured from three, while two produced negative cultures.

3. Additional points

All animals in the series were covered with Penicillin and Chloromycetin intra- and postoperatively. The resistance patterns of

the organisms cultured from the biliary tracts became apparent midway through the series. Approximately 75% of the organisms were resistant to the antibiotics used. The most efficacious combination appeared to be Keflin and Gentamycin. No change was made in the antibiotic therapy for two reasons. Firstly, half the series had been completed using the standard antibiotics, and it appeared undesirable to introduce any variant in treatment, which might affect the comparative nature of the study. The second practical reason was that the liberal use of these two antibiotics would add significantly to the cost of the project.

DISCUSSION ON THE FINDINGS IN THE EXTRAHEPATIC BILIARY APPARATUS

The analysis of the local effects of the surgical procedures in group 1(b) has shown that the interposition of a vascularised jejunal loop as a conduit between donor and recipient gallbladders, is a technically satisfactory procedure. Thirteen of the fourteen loops were viable and showed minimal macroscopic and microscopic changes, compared to the pre-operative control specimens, and the simultaneous autopsy specimens taken from the jejunum. This suggests that the blood supply had been adequate and that the constant passage of concentrated bile had had no morphologically harmful effect on the interposed loops for the time period studied.

The additional surgical procedures required to perform the loop interposition, did not produce any complications in this series, but the potential hazards of small bowel anastomotic leaks, and internal herniae, should not be overlooked.

In both transplant groups, the conjoined biliary drainage apparatuses appeared technically successful, with the components lying comfortably, and without undue tension, kinking or torsion. The viability and contents were comparable.

The recipient gallbladders in the two transplant groups showed comparable, minimal changes both macroscopically and microscopically, from the pre-operative control specimens, and from postoperative specimens from control animals in groups 2(a) and 2(b), even in the presence of confirmed bile infection. This suggests that the two types of drainage had a comparable and negligible effect

on the recipient gallbladders, suggesting good drainage of the conjoined system into the host pylorus.

The macroscopic and microscopic changes in the donor gallbladders were roughly comparable in the two groups, and the spectrum of changes seen was similar to the donor gallbladder changes previously reported with orthotopic transplantation in pigs and dogs (42, 198), auxiliary canine transplantation (191, 201) and in human grafting (128, 193). Donor gallbladder changes in the present series appeared to be due primarily to rejection, and the degree of rejection roughly paralleled the degree of rejection seen in the corresponding livers. Other factors such as cystic artery insufficiency, autolysis of the donor gallbladder mucosa at the time of transplant, ischaemic damage at time of transplant and postoperative biliary infection may also have contributed to some of the changes seen.

The extent to which the donor cystic ducts were involved in these changes could not be assessed, but the Denver group have reported obstruction of the donor cystic ducts in a number of patients with fatal results (128, 184). These cases have led the Denver group to consider abandoning the use of the donor gallbladder in creating biliary drainage (128), but the high incidence of leaks with anastomoses involving the common bile duct in both England (224) and Denver (187), has led the Denver team to persist with cholecystenterostomies. Long term survival has been reported with cholecystenterostomy by both teams (224, 187). The short term morphological changes seen in the donor gallbladders in the present series are unsatisfactory, but this appears to be a problem common to all transplanted gallbladders, and the basic problem appears to lie in the prevention or

adequate treatment of rejection per se, and not in the drainage technique used.

The findings in the biliary anastomotic lines in both groups are of interest. Leaks were infrequent and comparable in the two transplant groups. Small anastomotic concretions were seen with both techniques, and may be due to the encrustation of necrotic tissue in the suture lines with bile salts. These concretions may be the nidus for gallstone formation in the long term survivors. Anastomotic concretions have previously been reported in experimental transplantation (42), and were thought to be due to the use of unsuitable sutures.

In both experimental and clinical liver transplantation, the biliary anastomosis is constructed towards the end of a time-consuming, arduous operation, and it is the author's contention that some of the anastomotic complications reported with both clinical and experimental transplantation might be the result of inadequate care and diminished skill on the part of the surgeon who has been operating usually in excess of four hours. It might be prudent at this stage to introduce a fresh surgeon who would be able to perform the anastomoses with the utmost care.

Although the numbers in this porcine series are small, the bacteriological analysis of the biliary tract swabs has revealed that the interposition of an isolated jejunal loop between the donor and recipient gallbladders presents the danger of introducing gram negative organisms into the biliary tract. However, in gallbladder to gallbladder anastomoses, and in the sham animals in whom the integrity of the biliary tract was not disrupted, a significant percentage of the animals exhibited the same spectrum of organisms postoperatively as seen in the group with cholecystojejunocholecystostomy.

The bacteriological problems specific to the liver in clinical transplantation have been discussed by the Denver group (71, 182) and have been the subject of an extensive investigation in dogs and pigs (31, 120). They reported a low incidence of organisms in the swabs taken from the bile of normal pigs (5 out of 34 positive) and a high incidence of positive swabs from normal pig duodenum (25 out of 34). The percentage positive cultures were almost identical to those found in the present porcine series, as shown in Table 22 on page 136F. Postoperatively, they cultured 18 organisms from the bile of 5 pigs who had been subjected to orthotopic transplantation with biliary drainage by means of cholecystoduodenostomy, and showed convincingly that the incidence of positive bile cultures was significantly higher following cholecystoduodenostomy, than when the common bile duct was left intact, and that the incidence and severity of cholangitis followed a similar pattern (120). In addition, they reported that the administration of appropriate antibiotics significantly decreased the incidence of complications due to these organisms (31).

In the present series, the histology of the interposed loops, and of donor and recipient gallbladders, did not show much evidence of infection despite the positive bile cultures, and, as shall be shown later, the incidence of cholangitis in the donor and recipient livers was low and of mild degree. Similar observations have been reported in human liver transplantation (135).

The potential hazards of any infection in immunosuppressed patients is well known, and it is obligatory to try to avoid any potential source of infection. Starzl (187) has recently reviewed the philosophy of biliary drainage in liver transplantation, and proposes that the biliary

drainage of the transplanted liver should be removed as far as possible away from the mainstream of the gastro-intestinal tract because of the potential hazards of biliary infection from bowel organisms.

The technique of cholecystocholecystostomy used in the present series achieves this aim, while, in principle, the isolated, interposed jejunal loop is also cut off from the mainstream. It appears reasonable to propose that pre-operative bowel sterilization, intra-operative rinsing of the loop with appropriate antibiotic solutions, and the use of appropriate postoperative parenteral antibiotics might decrease the incidence of positive cultures, and thus the hazards of infection.

It is thus evident that drainage via a cholecystocholecystostomy would be safer than that via cholecystojejunocholecystostomy from the point of view of bowel-based infection, and that the interposition of a jejunal loop should be reserved for those cases in which the gallbladders cannot be easily apposed.

Comparative bile flow and biliary tract motility studies could not be performed in the absence of radiological facilities in our animal laboratories.

Overall, the present study has shown that most of the <u>local effects</u> of the two techniques of biliary drainage were comparable. The additional surgical procedures involved in jejunal interposition, plus the hazard of bowel-based infection, would favour cholecystocholecystostomy, unless the two gallbladders cannot be apposed.

The <u>regional effects</u> of the procedures on the livers and blood vessels will be discussed in the next section.

INDEX TO PART II, CHAPTER 3

SECTION III - REGIONAL EFFECTS OF THE PROCEDURES ON THE LIVERS AND BLOOD VESSELS

PART II

CHAPTER 3

SECTION III - REGIONAL EFFECTS OF THE PROCEDURES ON THE LIVERS AND BLOOD VESSELS

SUMMARY

The macroscopic findings in the livers and blood vessels of the four groups of animals are presented, analysed, compared and discussed in the first half of this section.

In the second half of the section, detailed histological analyses of all the serial liver biopsies taken from the animals in groups 1(a), 1(b), 2(a) and 2(b) are presented, together with the results of simultaneous analyses of the liver biopsy material from two previous series (Dent and Immelman). The analyses are presented and discussed in three parts:

(1) Assessment of the incidence and degree of cholestasis and cholangitis

(2) Assessment of the incidence and degree of hepatocyte damage

(3) Assessment of the incidence and degree of rejection.

Finally, towards the end of Section III, the histology of the blood vessels is briefly presented, and the histological findings in the livers and blood vessels discussed.

MACROSCOPIC PATHOLOGY OF THE LIVERS AND BLOOD VESSELS

Details of the conduct of the experiments, and the methods used to assess the livers and blood vessels at initial operation, and at autopsy, have been described in Part II, Chapter 2. The general appearance and physical characteristics, the type and nature of vascular complications, and the site and type of sepsis seen, was recorded for each liver at initial operation and at autopsy. Donor livers were weighed at initial operation. All livers were weighed at autopsy. Weighing was performed 15 minutes after removal from the animal.

The results of this part of the study are presented in detail in the case histories on pages 269-315.

1. MACROSCOPIC ASSESSMENT AT INITIAL OPERATION

(1) Appearance and Physical Characteristics

The livers of the animals used in all four experimental groups 1(a), 1(b), 2(a) and 2(b), including the donor livers used in groups 1(a) and 1(b), appeared essentially normal on capsular and cut surfaces at initial operation. Small circumscribed whitish areas were present on the capsular surface of many livers, and were usually associated with worms in the gastrointestinal tract. This phenomenon was regarded as a normal variant for the pigs used in this study. Bile staining was not detected in any of the pre-operative livers. Adhesions between liver and bowel or peritoneum were extremely infrequent.

The livers were all of fairly uniform soft consistency, and cut easily, with no grittiness, when taking the control wedge biopsies.

(2) Vascular complications

There was no evidence of vascular complications in any of the livers examined. Subcapsular infarcts were not seen, while the portal veins and hepatic arteries appeared patent in all animals. In addition, the hepatic veins of the donor livers were all inspected and found free of thrombi.

(3) Septic complications

No intrahepatic or perihepatic abscesses were detected in the pre-operative animals.

(4) Liver weights

The donor livers were weighed, and the donor liver weight for 33 animals was found to be 3,625% of the total body weight. This compared favourably with Hickman's report (89) in which the liver weight for pigs was found to be 3,64% of total body weight in 50 animals. Hickman's formula was used to assess the pre-operative liver weights for the control animals (groups 2(a) and 2(b)) and for the recipient livers in groups 1(a) and 1(b).

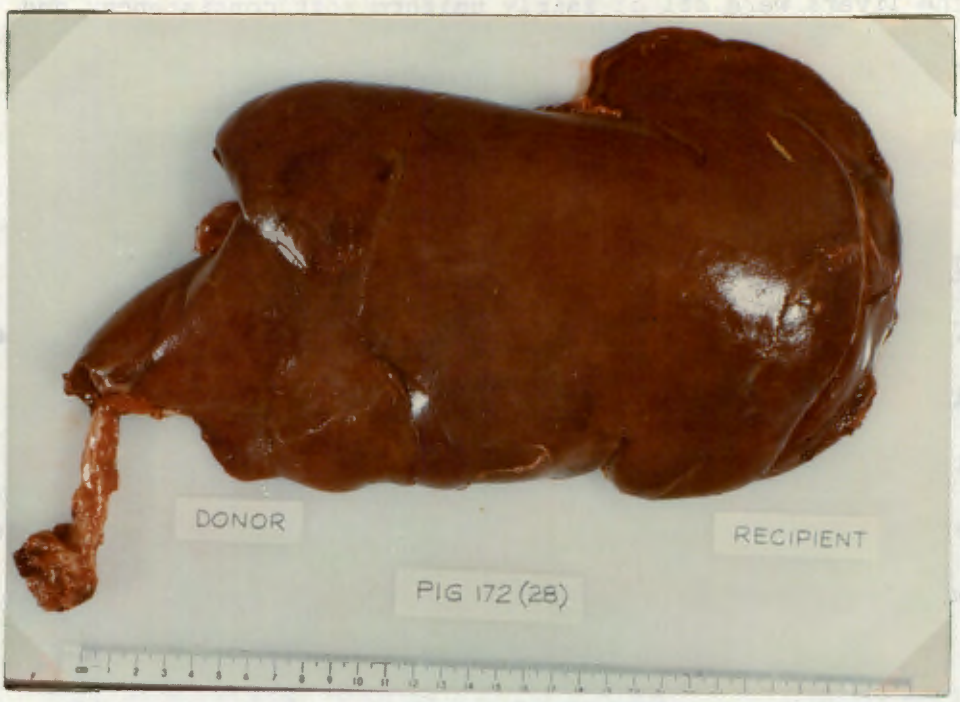


FIGURE 45 CLOSELY APPOSED DONOR AND RECIPIENT LIVERS - GROUP 1(a) (28 days after transplant)

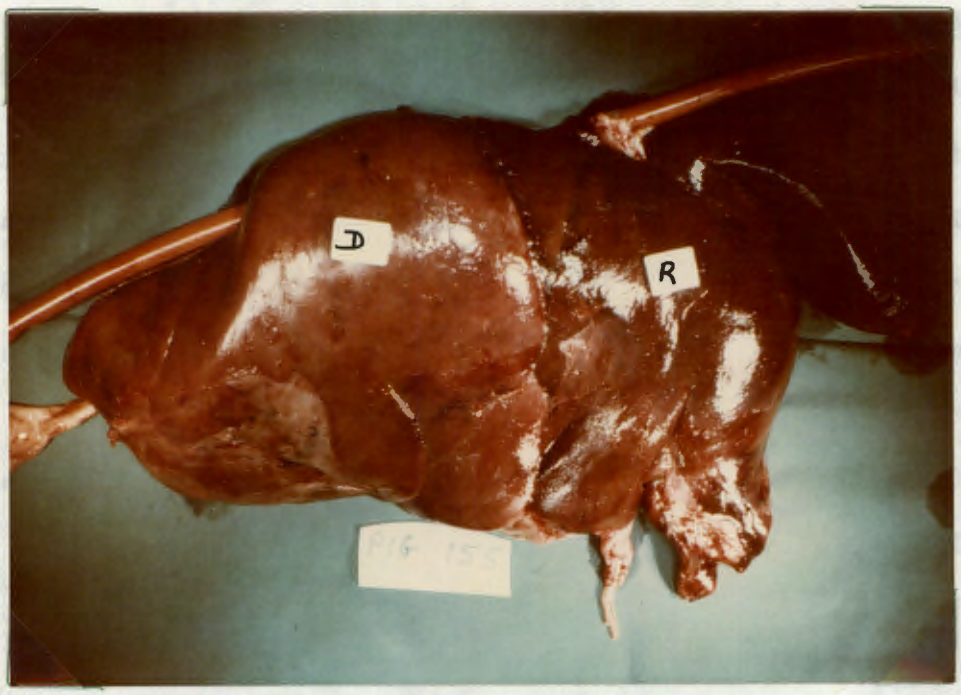


FIGURE 46 CLOSELY APPOSED DONOR AND RECIPIENT LIVERS - GROUP 1(b) (28 days after transplant)

2. MACROSCOPIC ASSESSMENT AT AUTOPSY

(1) Appearance and physical characteristics

At autopsy, loops of bowel, omentum and/or parietal peritoneum were found to be adherent, in varying degrees, to the capsular surface of the livers in most animals from all four experimental groups. The adhesions were usually more widespread and tenacious over the transplanted livers. Lysis of the adhesions tended to interfere with the interpretation of the overall appearance of capsular surface, but in all cases, some areas free of adhesions could be found to allow an assessment to be made.

(a) Groups 1(a) and 1(b) - Donor livers

Throughout the series in both groups 1(a) and 1(b), the donor and recipient livers were found to be closely apposed and firmly adherent to one another at autopsy. On superficial inspection it was often difficult to distinguish between the donor and recipient livers in an individual animal. The appearances of a pair of closely apposed livers from each group are demonstrated in Figures 45 and 46 on the opposite page.

On close inspection, however, even the best quality donor livers from both groups had subtly different appearances from their respective recipient livers. The donor livers were of slightly lighter colour on both capsular and cut surfaces, and minute inspection of the capsular surfaces revealed a fine honeycombed appearance totally different

	DONOR				RECIPIENT				CONTROL			
	1(a)		1(b)		1(a)		1(b)		2(a)		2(b)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Normal	-	-	-	-	14	100	12	86	13	100	5	100
"Normal"	8	62	10	71	-	-	2	14	-	-	-	-
Firm	-	-	2	14	-	-	-	-	-	-	-	-
Congested	1	8	1	7	-	-	-	-	-	-	-	-
Patchy infarcts	3	23	-	-	-	-	-	-	-	-	-	-
Segmental infarcts	-	-	-	-	-	-	-	-	-	-	-	-
Total infarction	1	8	1	7	-	-	-	-	-	-	-	-
Not assessed (gas)	1		-		-		-		1		-	

TABLE 23 PHYSICAL CHARACTERISTICS OF THE LIVERS AT AUTOPSY

Group 1(a) - Heterotopic allograft plus cholecystocholecystostomy.
Group 1(b) - Heterotopic allograft plus cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

from that of the recipient livers. In addition, the donor livers were tougher to slice, and on cutting into them, there was grittiness similar to that encountered when cutting into a pear. These subtle differences from normal might have been missed had the respective recipient livers not simultaneously been available for comparison.

Not a single donor liver from either group 1(a) or group 1(b) could therefore be classed as completely normal. As shown in Table 23 opposite, 8 livers from group 1(a) and 10 from group 1(b) appeared superficially normal on cut and capsular surface, were of soft consistency, but had the subtly different characteristics already outlined, and were classed as "normal". Two livers in group 1(b), (pigs Nos. 16 and 21) were found to be firmer than normal, and firmer than their respective recipient livers, and were classed as "firm". One liver in each of groups 1(a) and 1(b) (pigs Nos. 8 and 23) exhibited either patchy or generalised congestion. Solitary or multiple infarcts of varying severity were seen on capsular or cut surface of 3 livers (pigs Nos. 5, 6 and 9) in group 1(a). Total infarction of the whole donor liver and external biliary tract was seen in one animal from each group (pigs Nos. 3 and 18). The donor liver from animal No. 2 had developed gas gangrene by the time autopsy was performed, and the characteristics could not be accurately assessed. Bile staining was not detected in any of the donor livers.

(b) Groups 1(a) and 1(b) - Recipient livers

The appearance and physical characteristics of 14 recipient livers in group 1(a) and 12 in group 1(b) were assessed as completely normal. Two livers from group 1(b) deviated slightly from normality, and were classed as "normal". In pig 20, a fine reticular pattern was seen on the capsular surface of the recipient liver, similar to that seen in its donor liver. In pig No. 26, the recipient liver was uniformly brown, but normal in other respects. Bile staining was not detected in any of the recipient livers.

(c) Groups 2(a) and 2(b) - Control livers

The livers from groups 2(a) and 2(b) all had characteristics indistinguishable from the pre-operative assessment, and from the recipient livers, and were all assessed as normal. Bile staining was not detected in any of the control livers. One liver from group 2(a) had developed gas gangrene and could not be assessed.

(2) Vascular complications

(a) Groups 1(a) and 1(b) - Donor livers

The incidence of vascular problems in the donor livers and vessels from both groups 1(a) and 1(b) was distressingly high. The results are tabulated in Table 24 on the following page.

	DONOR				RECIPIENT				CONTROL			
	1(a)		1(b)		1(a)		1(b)		2(a)		2(b)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
ARTERIAL												
- no evidence thrombi	9	64	13	93	14	100	14	100	13	100	5	100
- non occlusive thrombi	2	14	0	0	0	0	0	0	0	0	0	0
- complete occlusion	3	21	1	7	0	0	0	0	0	0	0	0
- anastomotic stenosis	0	0	0	0	-	-	-	-	-	-	-	-
PORTAL VENOUS												
- no evidence thrombi	13	93	9	64	-	-	-	-	13	100	5	100
- non occlusive thrombi	0	0	2	14	-	-	-	-	0	0	0	0
- complete occlusion	1	7	3	21	-	-	-	-	0	0	0	0
- anastomotic stenosis	0	0	0	0	-	-	-	-	0	0	-	-
HEPATIC VENOUS												
- no evidence thrombi	11	79	8	57	14	100	14	100	13	100	5	100
- non occlusive thrombi	2	14	5	36	0	0	0	0	0	0	0	0
- complete occlusion	1	7	1	7	0	0	0	0	0	0	0	0
- anastomotic stenosis	0	0	1	7	-	-	-	-	-	-	-	-
Not assessed	0	0	0	0	0	0	0	0	1	7	0	0

TABLE 24 HEPATIC VASCULAR COMPLICATIONS IN THE FOUR GROUPS OF ANIMALS AT AUTOPSY

In group 1(a) only four donor livers were macroscopically completely free of vascular complications. The donor aortic cuff was completely occluded in pigs Nos. 1 and 3, while in pig No. 7, one distal branch of the hepatic artery was completely occluded. In pigs Nos. 9 and 13 a thrombus was present in the donor aortic cuff immediately distal to the origin of the coeliac axis, but in each case was non-occlusive, and related to tying off the aortic cuff too far distally from the origin of the coeliac axis. Although these thrombi were non-occlusive, they are included as complications as it is felt that they may have the potential of propagating. All the portal veins were patent and clear of thrombi except in pig No. 3, where the liver had sloughed. The hepatic veins were patent and free of thrombi in 11 animals. In pig No. 9 a large antemortem thrombus was present in the right hepatic vein, with accompanying congestion and thrombi being seen on cut section of part of the liver. A small non-occlusive thrombus was found in the right hepatic vein of pig No. 10. Infarcts were seen in several livers. Multiple small infarcts were present in pigs Nos. 5, 6 and 9. In pig No. 8 small patchy areas of antemortem thrombi were seen in the left anterior lobe.

In group 1(b), 4 of the donor livers revealed no evidence of vascular complications. The hepatic arteries were patent and free of thrombi in 13 livers, while in pig

No. 18 the aortic segment and hepatic artery had thrombosed, and the liver was completely infarcted. The portal veins and anastomoses were normal and patent in 9 livers. A small non-occlusive thrombus was present in the portal vein in pigs Nos. 23 and 27, while in pigs Nos. 17, 18 and 19 the portal veins were completely occluded with antemortem thrombus. The cavo-caval anastomosis was satisfactory and the hepatic veins patent and clear of thrombi in 8 livers. Small, non-occlusive thrombi were seen in 4 animals (Nos. 19, 23, 25 and 26). In pig No. 20 the right hepatic vein was completely occluded with a clearly demarcated area of congestion and thrombi on cut surface. The hepatic vein was occluded and epithelialised in pig No. 18, the donor liver of which was completely infarcted. Mild cavo-caval anastomotic stenosis was found in pig No. 16, but there was no evidence of thrombotic occlusion.

(b) Groups 1(a) and 1(b) - Recipient livers

All recipient livers from groups 1(a) and 1(b) were free of vascular complications.

(c) Groups 2(a) and 2(b) - Control livers

Hepatic vascular complications were not detected in groups 2(a) and 2(b). The portacaval shunts in groups 2(a) were all patent at autopsy with no evidence of partial occlusion or anastomotic strictures.

	DONOR				RECIPIENT				CONTROL			
	1(a)		1(b)		1(a)		1(b)		2(a)		2(b)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Nil	10	77	13	93	14	100	14	100	12	92	5	100
Intrahepatic	3	23	1	7	0	0	0	0	1	8	0	0
Perihepatic	(1)	(8)	0	0	0	0	0	0	0	0	0	0
Not assessed	1		0		0		0		1		0	

TABLE 25 SEPTIC LIVER COMPLICATIONS IN THE FOUR GROUPS AT AUTOPSY

Group 1(a) - Heterotopic allograft plus cholecystocholecystostomy.
Group 1(b) - Heterotopic allograft plus cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

(3) Septic complications

(a) Groups 1(a) and 1(b) - Donor livers

The macroscopic evidence of sepsis was roughly the same in the donor livers from the two groups, see Table 25 opposite.

In group 1(a) ten of the donor livers showed no evidence of intrahepatic sepsis. Intrahepatic abscesses were found in the donor livers of pigs Nos. 1 and 2. In pig No. 7, an abscess was found extending from the site of the weekly subcostal biopsy incision into the substance of the liver. A large subphrenic abscess was present over the donor liver of pig No. 4, and appeared to involve part of the substance of the liver. The sloughed donor liver of pig No. 3 could not be accurately assessed for sepsis.

In group 1(b), 13 livers were macroscopically free of sepsis. Multiple small abscesses were present throughout the donor liver of Pig No. 15. Twenty-three out of the 27 donor livers that could be assessed were thus free of gross sepsis.

(b) Groups 1(a) and 1(b) - Recipient livers

All recipient livers in both groups 1(a) and 1(b) were macroscopically free of sepsis.

	DONOR				RECIPIENT				CONTROL			
	1(a)		1(b)		1(a)		1(b)		2(a)		2(b)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Increase >5%	2	17	5	36	0	-	0	-	1	11	5	100
Static	2	17	0	-	0	-	1	7	1	11	0	-
Decrease >5%	8	66	9	64	13	100	13	93	7	78	0	-
Not assessed	2		0		1		0		5		0	

TABLE 26 LIVER WEIGHT CHANGES IN THE FOUR GROUPS OF ANIMALS

Group 1(a) - Heterotopic allograft plus cholecystocholecystostomy.
Group 1(b) - Heterotopic allograft plus cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

(c) Groups 2(a) and 2(b) - Control livers

Twelve livers from group 2(a) were free of sepsis, while one liver, from pig No. 31, had multiple small abscesses throughout the liver. In pig No. 41, autolysis had occurred and the liver could not be analysed accurately.

All five livers from group 2(b) remained macroscopically free of sepsis.

(4) Liver weight changes

(a) Groups 1(a) and 1(b) - Donor livers

The liver weight changes that occurred between initial operation and autopsy are detailed in the case histories in the Appendix, pages 269-296, and summarised in Table 26 on the opposite page. For the purposes of this presentation, a weight change of 5% or less has arbitrarily been regarded as insignificant, and the livers regarded as being static in weight.

In group 1(a), 8 of the donor livers lost weight (range 19% - 54%), while 2 livers showed insignificant weight change. Weight gain was seen in 2 livers, 5,2% in pig No. 13, and over 200% in pig No. 9. However, the liver from pig No. 9 was abnormal and shown to have intrahepatic thrombi and congestion. The livers from pigs Nos. 2 and 3 were not weighed on account of gas gangrene and gross infarction respectively.

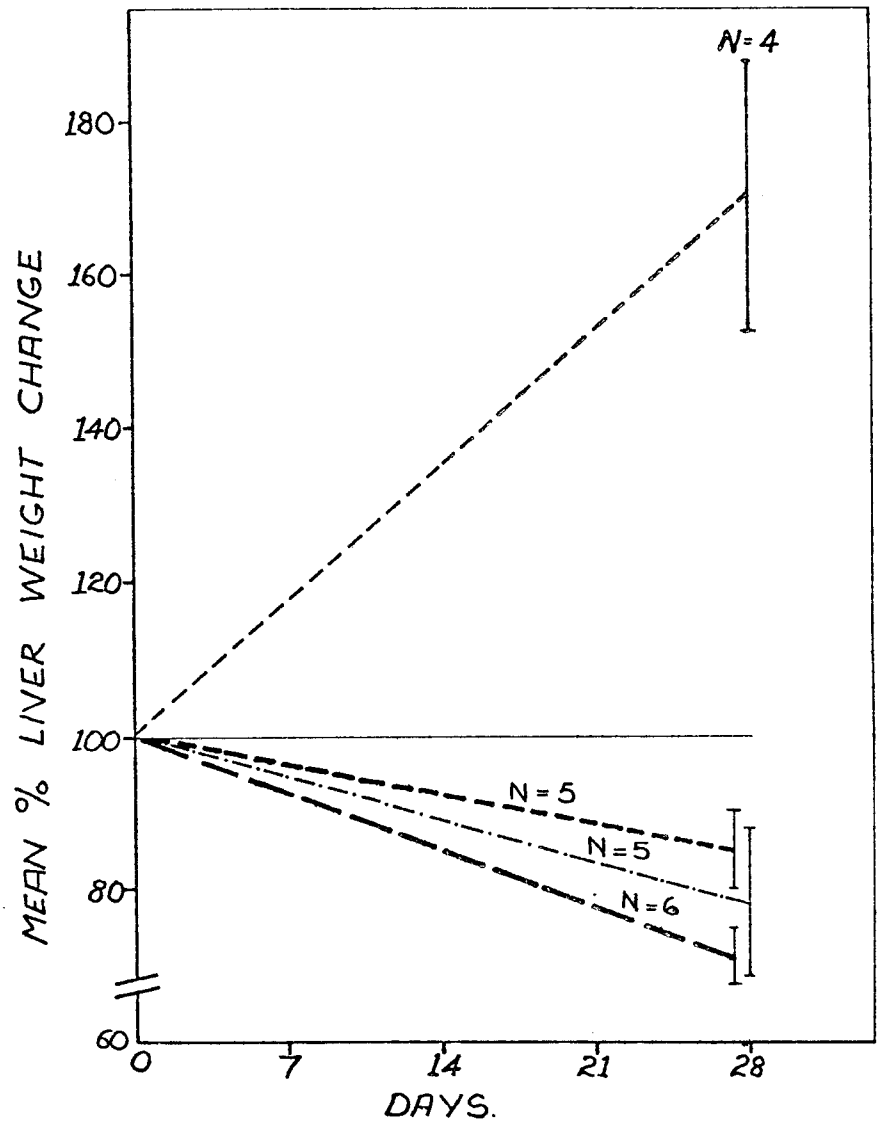
In group 1(b), 9 of the donor livers showed weight loss in excess of 5% (range 13% - 54%), while no livers remained static. Five donor livers had gained weight. In pig No. 18, the liver had shown a 33% increase in weight but the whole liver was infarcted and congested. The remaining 4 livers, from pigs Nos. 20, 22, 24 and 28 revealed a weight increase ranging from 23% - 172%, and were judged macroscopically to be of good quality.

Nineteen of the transplanted livers thus lost weight, or failed to gain weight. Two showed weight gain, but were macroscopically abnormal. One liver in group 1(a) showed slight weight gain, while four in group 1(b) showed marked weight gain.

(b) Groups 1(a) and 1(b) - Recipient livers

The 13 recipient livers from group 1(a) showed weight loss in excess of 5% compared to the pre-operative weights (range 5,8% - 52%). One liver was not weighed - animal No. 2. When compared to the estimated weight at autopsy, 9 of the recipient livers weighed less than the expected weight (range 14% - 46%) while in two the actual and expected weights were similar.

In group 1(b), 13 recipient livers lost weight in excess of 5% (range 5,7% - 34%). One liver remained static. In 11 animals, the actual liver weight was less than the expected liver weight at autopsy (range 8% - 42%) while



= Group 1(a) - Recipient livers
= Group 1(b) - Recipient livers
= Group 2(a)
= Group 2(b)

All the livers used to compile this graph came from animals that had survived to 28 days. The livers were weighed 15 minutes after removal from the animal. Pre-operative weights were calculated as being 3,64% of total body weight.

FIGURE 47 MEAN PERCENTAGE LIVER WEIGHT CHANGE

Group 1(a)- Heterotopic allograft + cholecystocholecystostomy.
Group 1(b)- " " + cholecystojejunocholecystostomy.
Group 2(a)- End-to-side portacaval shunt.
Group 2(b)- Sham laparotomy.

in three animals the actual and expected weights were similar.

The weight change patterns of the recipient livers of animals surviving to 28 days are shown in Figure 47 on the opposite page. The pattern of recipient liver weight changes in these animals is similar and comparable in groups 1(a) and 1(b), and roughly similar to the changes seen in the portacaval-shunted animals from group 2(a), but significantly different from that seen in group 2(b) animals.

(c) Groups 2(a) and 2(b) - Control livers

In group 2(a), 7 livers had lost weight in excess of 5% (range 11% - 49%), while one remained static and one gained weight of 6% (pig No. 34). In 10 animals, the actual liver weight was less than the expected weight (range 8% - 64%), while the actual and expected weights were similar in only one animal. A broken scale precluded analysis of 5 livers. The mean liver weight changes for the 5 animals surviving to 28 days are shown in Figure 47 on page 157F. The liver weight loss appeared disproportionate to the loss of body weight in the same animals, as shown in Figure 48 on the following page.

In group 2(b), all the livers showed weight gain in excess of 5% (range 22% - 101%). In three of these animals the actual liver weight was less than the expected weight

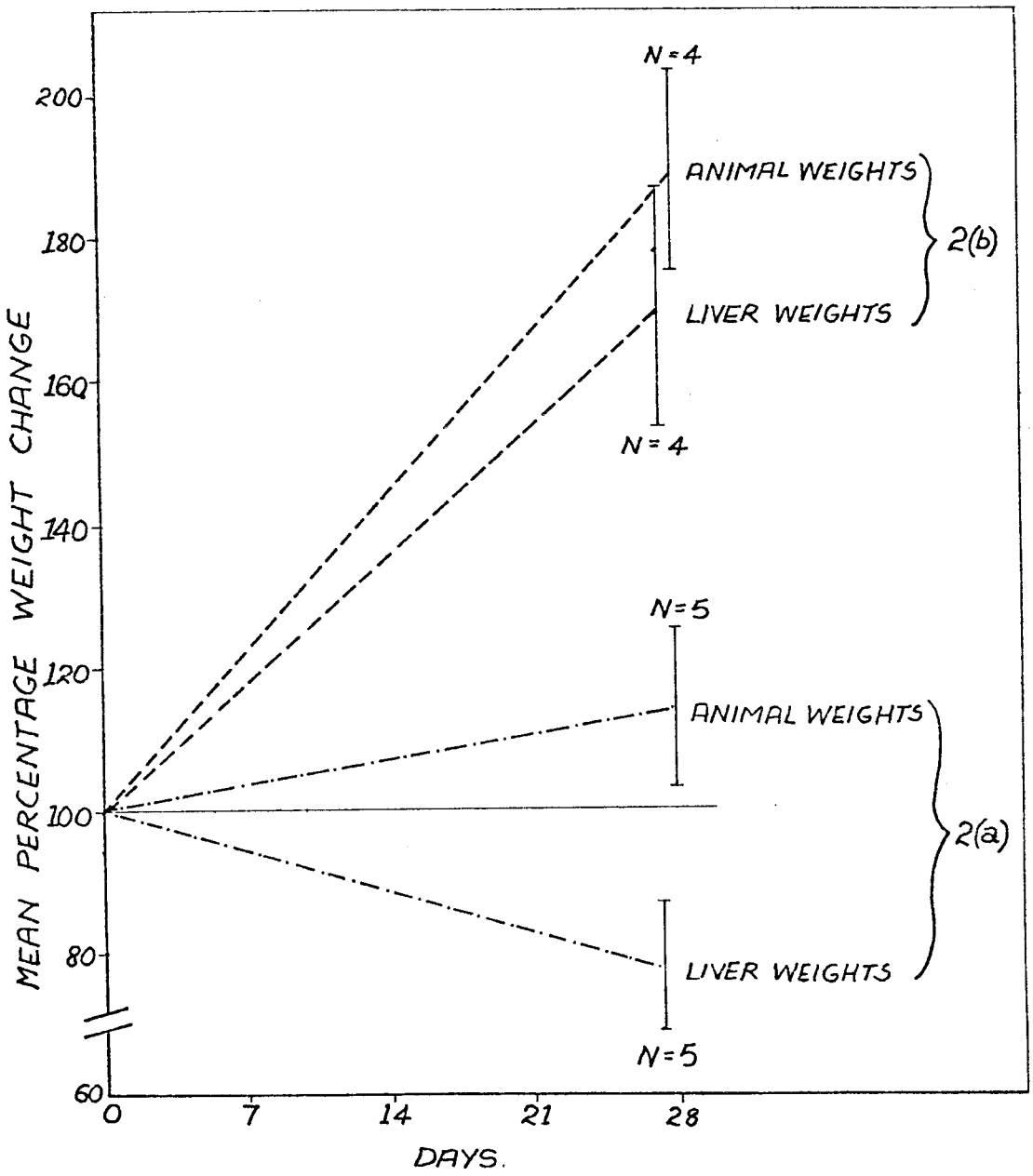


FIGURE 48 COMPARISON OF THE MEAN PERCENTAGE CHANGES IN LIVER AND BODY WEIGHT IN GROUPS 2(a) and 2(b) (Mean ± 1 SEM)

.—.—.—. = Group 2(a) - End-to-side portacaval shunt
------ = Group 2(b) - Sham laparotomy

The criteria and data used to compile this graph are the same as those used for Figure 47

(range 10% - 21%), while in 2 the actual and expected weights were similar. The mean weight changes of the four animals sacrificed on day 28 are shown in Figure 47 on page 157F. The increase in liver weight was roughly commensurate with the increase in body weight as shown in Figure 48 on the opposite page.

DISCUSSION IN CONTEXT

The macroscopic assessment of the livers from all four experimental groups, both pre-operatively and at autopsy, have been presented.

The general appearance and physical characteristics of the donor livers in groups 1(a) and 1(b) were roughly similar at autopsy. None of the livers could be classed as completely normal, but over 60% in each group were of good quality, with only subtle deviations from normal. By contrast, almost all the recipient and control livers were adjudged completely normal.

Vascular complications were common and comparable in the donor livers from groups 1(a) and 1(b), while the recipient and control livers were macroscopically free of any vascular complications. Unfortunately facilities for arteriography did not exist in the laboratory, and neither the time of onset, nor the actual extent of the vascular impairment could be assessed accurately. It had been hoped to use a cuffed indwelling flowmeter in an attempt to detect changes in donor liver blood flow, but technical problems associated with the machine (45) precluded this and the problem had only been solved in vitro (88) by the time the operations on the pigs had been completed.

Septic complications were infrequent and roughly comparable in the donor livers of groups 1(a) and 1(b), and absent in all but one of the livers from the recipient and control groups.

The pattern of weight changes in the donor livers from groups 1(a) and 1(b) were slightly different. Most of the livers evidenced weight loss, while only 5 "normal" livers gained weight.

The recipient livers all lost or failed to gain weight, the mean weight losses for the livers from groups 1(a) and 1(b) being comparable, and similar to the mean weight loss seen in group 2(a). This may be due to the fact that the recipient livers were deprived of primary access to portal blood as discussed in the Main Introduction, pages 25-27. By contrast none of the sham operated animals in group 2(b) showed loss of liver weight. All gained liver weight compared to the estimated pre-operative weight, and the liver weight gain was roughly commensurate with total body weight gain.

Gross bile staining was absent in all the livers analysed, despite, in the case of pig No. 26, partial obstruction to the common bile duct.

CONCLUSIONS IN CONTEXT

Bearing in mind the objectives of this animal study, a number of pertinent conclusions can be derived from the macroscopic analysis of the livers.

The absence of bile staining in the donor livers from both groups 1(a) and 1(b), indicates that neither of the

two methods of biliary drainage that were used, caused gross biliary obstruction. The comparable, low incidence of gross sepsis in the donor livers from the two groups suggests that the two types of biliary drainage did not predispose to septic intrahepatic complications.

The comparable absence of complications in the recipient livers in the two groups, indicates that the two methods of biliary drainage used had no deleterious effect on the animals' own livers. The weight changes seen in the recipient livers were comparable to those seen in the portacaval-shunted livers, and may be due to the fact that the recipient livers were deprived of primary access to portal blood.

None of the donor livers had completely normal physical characteristics at the time of autopsy, and the spectrum of deviation from normality was comparable in the two groups, suggesting that factors other than the biliary drainage were responsible for the changes seen.

TYPE OF OPERATION	GROUP	DONOR BILIARY DRAINAGE		NUMBER OF LIVERS ANALYSED	SERIES
		Type	Method		
AUXILIARY HETEROTOPIC ALLOGRAFT	1(a)	Biliary-Biliary	Cholecystocholecystostomy	14 Recipient 12 Donor	PRESENT
	1(b)	Biliary-Biliary	Cholecystojejunocholecystostomy	14 Recipient 13 Donor	
CONTROL -PORTACAVAL SHUNT	2(a)	Normal	Unmodified	14	
-SHAM LAPAROTOMY	2(b)	Normal	Unmodified	5	
ORTHOTOPIC ALLOGRAFT	3(a)	Biliary-Enteric	Cholecystoduodenostomy	9 Donor	PREVIOUS (Dent)
	3(b)	Biliary-Biliary	Choledochocholedochostomy	8 Donor	
AUXILIARY HETEROTOPIC ALLOGRAFT	4(a)	Biliary-Biliary	Cholecystocholecystostomy	11 Donor	PREVIOUS (Immelman)
	4(b)	Biliary-Enteric	Cholecystojejunostomy-en-y	3 Donor	
	4(c)	External	Cholecystostomy	4 Donor	

TABLE 27 PIG LIVERS SUBJECTED TO THE SAME THREE HISTOLOGICAL ANALYSES

MICROSCOPIC ANALYSIS OF THE LIVERS FROM THE PRESENT AND PREVIOUS SERIES

Three separate histological analyses were carried out on all the liver biopsy material from the four groups of animals in the present series, and, for comparative purposes, on the available histological material from two previous liver transplant series performed in the same laboratory by Dent (55) and Immelman (97, 99). The three analyses were performed on a blind observer basis by the author. The groups of animals from which the liver biopsies were derived, are shown in Table 27 on the opposite page. The detailed results of the analyses are presented in Tables 40-47 in the Appendix, pages 316-326 and, for easy reference in the individual case histories.

The methods used to collect the liver biopsies from the four groups in the present series, have been described in detail in Part II, Chapter 2. Essentially, a single wedge biopsy was taken from each liver at initial operation, needle biopsies at weekly intervals, and multiple, randomly selected cubed sections at autopsy. The biopsies were fixed in formol saline. Following fixation, the tissues were processed by a Shandon-Elliot automatic tissue processor, and embedded in Paraplast embedding medium (melting point 56-57^{o}C). Sections 2-4 μ in thickness were cut using a Jung base sledge microtome.

All the liver sections were stained routinely with Haematoxylin and Eosin, Methyl Green Pyronine (Unna Pappenheim), and Goddard and Sweet's reticulin stain (counter-stained with Von Giesson). When deemed necessary selected sections were also stained for Bilirubin (Hall's bilirubin stain), Iron (Perles' Prussian Blue stain), and Glycogen (Best's Carmine stain and periodic acid Schiffs stain).

The histological material from the two previous series (Dent - groups 3(a) and 3(b), and Immelman - groups 4(a), 4(b) and 4(c)) had been harvested, processed and stained in a similar manner.

The three analyses performed on each histological section were:

(A) An assessment of the incidence and degree of cholangitis and cholestasis, i.e. an assessment of the intrahepatic biliary tract.

(B) An assessment of the incidence and degree of hepatocyte damage, i.e. to establish an index of the functional integrity of each liver.

(C) An assessment of the incidence and degree of rejection.

Each analysis will be presented and discussed individually.

The results of these analyses were used:

(1) As part of the comparative study of the effects of the two techniques of biliary drainage used in the present series

(2) In an attempt to correlate serial biochemical and haematological changes with hepatocellular damage and graft rejection.

(A) ASSESSMENT OF THE INCIDENCE AND DEGREE OF CHOLANGITIS AND CHOLESTASIS

1. Introduction

The frequent development of intrahepatic cholangitis and cholestasis in both clinical and experimental liver transplantation has been discussed in the Main Introduction. The review suggested that the incidence and severity of these these two complications may be associated with the type of biliary drainage used for the donor liver. Dent (55),

Immelman (97,99) and Calne (32) have shown in pig liver transplantation, that cholangitis and cholestasis occur less frequently when donor bile is ultimately drained through the recipient's sphincter of Oddi, than when direct biliary enteric anastomosis is used.

This part of the study was designed and undertaken with four objectives in mind. A detailed blind assessment of the incidence and degree of cholangitis and cholestasis in each serial liver biopsy from all the groups outlined on Table 27 page 161F would allow:

(a) A comparison of the effects of the two types of biliary drainage used in groups 1(a) and 1(b) on the intrahepatic biliary tracts of the respective donor livers.

(b) An assessment of the effects of the two types of donor biliary drainage on the intrahepatic biliary tracts of the recipient livers of groups 1(a) and 1(b).

(c) The exclusion of other factors, common to the four groups, as causes of cholangitis and cholestasis, by using groups 2(a) and 2(b) as controls.

(d) Accurate comparison of the incidence and degree of these two complications in the donor livers of groups 1(a) and 1(b), with those seen in Dent and Immelman's previously published work.

2. Criteria and methods of analysis

Using publications by Herbertson (84), Hunt (94), Porter (143, 144, 145), Sandritter (151) and Uys (205) as a guide, the author established arbitrary criteria for classifying the incidence and severity of cholangitis and cholestasis.

(a) Criteria used to assess the degree of cholangitis

Grade:		Histological findings
0	-	The absence of any acute inflammatory cells in the lumen or wall of any bile duct or ductule in any of the sections from an individual liver on a particular day.
+	-	The presence of relatively few acute inflammatory cells in only a small number of bile duct lumina.
++	-	The presence of acute inflammatory cells in both lumina and walls of many bile ducts and ductules.
+++	-	Plugs of acute inflammatory cells in the bile ducts and ductules, with diffuse infiltration of the bile duct walls and surrounding tissues, and cholangitic abscesses.

(b) Criteria used to assess the degree of cholestasis

Grade:		Histological findings
0	-	The absence of bile throughout every section from an individual liver on a particular day.
+	-	The presence of small amounts of intracytoplasmic or intracanalicular bile
++	-	The presence of sparsely scattered bile plugs or casts in the bile ducts or ductules.
+++	-	The presence of diffusely scattered bile casts in the bile ducts and ductules, intracellular bile, and bile lakes.

	GRADE OF CHOLANGITIS							
	0		+		++		+++	
	No.	%	No.	%	No.	%	No.	%
PRESENT SERIES								
Group 1(a)	8	67	2	17	1	8	1	8
Group 1(b)	7	54	4	30	1	8	1	8
DENT'S SERIES								
Group 3(a)	3	33	3	33	1	11	2	22
Group 3(b)	6	86	1	14	0	0	0	0
IMMELMAN'S SERIES								
Group 4(a)	7	64	4	36	0	0	0	0
Group 4(b)	0	0	1	33	1	33	1	33
Group 4(c)	0	0	0	0	2	50	2	50

TABLE 28 THE INCIDENCE AND DEGREE OF CHOLANGITIS IN THE POSTOPERATIVE DONOR LIVERS

	GRADE OF CHOLESTASIS							
	0		+		++		+++	
	No.	%	No.	%	No.	%	No.	%
PRESENT SERIES								
Group 1(a)	11	92	1	8	0	0	0	0
Group 1(b)	12	92	0	0	1	8	0	0
DENT'S SERIES								
Group 3(a)	4	44	0	0	1	11	4	44
Group 3(b)	3	43	2	29	1	14	1	14
IMMELMAN'S SERIES								
Group 4(a)	9	82	2	18	0	0	0	0
Group 4(b)	0	0	2	66	1	33	0	0
Group 4(c)	0	0	0	0	4	100	0	0

TABLE 29 THE INCIDENCE AND DEGREE OF CHOLESTASIS IN THE POSTOPERATIVE DONOR LIVERS

The analyses were carried out using a Nikon binocular microscope. In every case the whole section was studied under high and low power. Each bile duct or ductule in each section was examined individually under both magnifications. Light was reduced to search for refractile bile.

The degree of cholangitis and cholestasis for each section was established using the criteria outlined above. Where multiple biopsies had been taken from a liver, as in the autopsy material from the present series, the section with the most severe degree of cholangitis and cholestasis was noted as being representative of the biopsy period.

3. Results from the assessment of cholangitis and cholestasis

The incidence and degree of cholangitis and cholestasis are summarised in Tables 28, 29, 30 and 31, and detailed in the Appendix – Tables 40–47 on pages 319–326. On review, Professor C.J. Uys – see page 171 – disagreed with the grading of Grade I cholangitis. The author has, however, felt in duty bound to record his opinion at the time of the analysis.

(a) Pre-operative specimens

There was no evidence of cholangitis or cholestasis in any of the liver biopsies taken at initial operation in groups 1(a), 1(b), 2(a) and 2(b) from the present series nor in the 10 pre-operative specimens from groups 3(a) and 3(b).

(b) Serial postoperative specimens

(i) Groups 1(a) and 1(b) – Donor livers

Tables 40 and 41 in the Appendix, pages 319-320.

Eight out of 12 donor livers in group 1(a) showed no evidence of cholangitis throughout the period of study. Minimal cholangitis (+) was seen in two animals (pigs Nos. 7 and 14), moderate cholangitis (++) in one animal (pig No. 9) and severe cholangitis with cholangitic abscesses (+++) in one animal (pig No. 4). Eleven of the livers remained free of cholestasis while minimal cholestasis (+) was seen in one animal (pig No. 5). Pigs Nos. 2 and 3 could not be analysed accurately, on account of gas gangrene and gross infarction respectively.

In group 1(b), seven out of 13 donor livers were free of cholangitis throughout the period of study. Minimal cholangitis (+) was seen in four animals (pigs Nos. 17, 21, 26 and 28). Moderate cholangitis (++) was seen in one biopsy (pig No. 19) and severe cholangitis (+++) in one animal (pig No. 16). Twelve of the donor livers remained free of cholestasis, while moderate cholestasis (++) was detected in one animal (pig No. 26) in which the common bile duct was found to be obstructed. The donor liver from pig No. 18 was infarcted and could not be analysed accurately.

(ii) Groups 1(a) and 1(b) - Recipient livers

Tables 42 and 43 in the Appendix - pages 321-322.

	GRADE OF CHOLANGITIS							
	0		+		++		+++	
	No.	%	No.	%	No.	%	No.	%
RECIPIENT LIVERS								
Group 1(a)	13	93	1	7	0	0	0	0
Group 1(b)	11	79	3	21	0	0	0	0
CONTROL LIVERS								
Group 2(a)	11	79	3	21	0	0	0	0
Group 2(b)	4	80	1	20	0	0	0	0

TABLE 30 THE INCIDENCE AND DEGREE OF CHOLANGITIS IN THE POSTOPERATIVE "CONTROL" LIVERS

	GRADE OF CHOLESTASIS							
	0		+		++		+++	
	No.	%	No.	%	No.	%	No.	%
RECIPIENT LIVERS								
Group 1(a)	13	93	1	7	0	0	0	0
Group 1(b)	11	79	1	7	1	7	1	7
CONTROL LIVERS								
Group 2(a)	14	100	0	0	0	0	0	0
Group 2(b)	5	100	0	0	0	0	0	0

TABLE 31 THE INCIDENCE AND DEGREE OF CHOLESTASIS IN THE POSTOPERATIVE "CONTROL" LIVERS

Group 1(a) - Heterotopic allograft plus cholecystocholecystostomy.
Group 1(b) - Heterotopic allograft plus cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

Thirteen out of 14 recipient livers from group 1(a) were free of cholangitis throughout the period of study. Mild cholangitis (+) was seen in only one animal (pig No. 1 on day 14). Thirteen of the livers remained free of cholestasis while mild cholestasis (+) was present in one liver (pig No. 6 on day 14).

In group 1(b), eleven out of 14 recipient livers were free of cholangitis throughout the period of study. Mild cholangitis (+) was seen in three animals (pigs Nos. 15, 25 and 26), in one biopsy only. In pig No. 26 the cholangitis was associated with partial obstruction to the common bile duct. Eleven of the livers remained free of cholestasis. Mild cholestasis (+) was seen in pig No. 25. Moderate cholestasis (++) was seen in pig No. 18 on days 7 and 14, and at autopsy the donor liver and gallbladder were found to be infarcted. In pig No. 26, progressively more severe cholestasis was seen and donor cholestasis was present. At autopsy the recipient's common bile duct was found to be acutely angulated with presumed partial obstruction.

(iii) Groups 2(a) and 2(b) - Control Livers

Tables 44 and 45 in the Appendix - pages 323-324.

In group 2(a), eleven of the portacaval-shunted livers were free of cholangitis throughout the

period of study. Mild cholangitis (+) was seen in three animals (pigs Nos. 34, 36 and 38). All 14 livers remained free of cholestasis.

In group 2(b) four out of 5 sham operated livers were free of cholangitis throughout the period of study. Mild cholangitis (+) was seen in one animal (pig No. 46 on day 21). All 5 livers remained free of cholestasis.

(iv) Groups 3(a) and 3(b) - Orthotopic allografts - Dent's series

Tables 28 and 29 on page 165F and Table 46 in the Appendix - page 325.

Three out of 9 donor livers in group 3(a) were free of cholangitis. Mild cholangitis (+) was seen in 3 animals, moderate cholangitis (++) in one animal and severe cholangitis (+++) in two animals. Four animals exhibited no cholestasis, moderate cholestasis (++) was seen in one animal, and severe cholestasis (+++) in 4 animals.

In group 3(b), six out of 7 livers were free of cholangitis, while one liver exhibited mild cholangitis (+). Three animals showed no cholestasis, mild cholestasis (+) was seen in 2 animals, and moderate (++) and severe (+++) cholestasis in on animal each.

Some of Dent's needle biopsies were excluded from analysis, as the sections were felt to be too small for accurate comparison with the other series. Only sections big enough to include a portal tract and a good representative portion of a lobule were included in the analysis.

(v) Groups 4(a), 4(b) and 4(c) - Heterotopic Allografts - Immelman's series

Tables 28 and 29 on page 165F and Table 47 in the Appendix - page 326.

Seven out of 11 donor livers from group 4(a) were free of cholangitis, while four exhibited mild cholangitis (+). Cholestasis was absent in 9 livers, while mild cholestasis (+) was seen in two.

In group 4(b), none of the three livers remained free of cholangitis and cholestasis, as shown in Tables 28 and 29 on page 165F.

The four livers from group 4(c) revealed the most severe incidence and degree of cholangitis and cholestasis.

The numbers in the latter two groups (4(b) and 4(c)) are too small for accurate comparison.

TYPE OF BILIARY DRAINAGE	ANIMAL GROUP	GRADE OF CHOLANGITIS							
		0		+		++		+++	
		No.	%	No.	%	No.	%	No.	%
Biliary-Biliary	1(a)	8	67	2	17	1	8	1	8
	1(b)	7	54	4	30	1	8	1	8
	3(b)	6	86	1	14	0	0	0	0
	4(a)	7	64	4	36	0	0	0	0
Biliary-Enteric	3(a)	3	33	3	33	1	11	2	22

TABLE 32 THE INCIDENCE OF CHOLANGITIS IN THE DONOR LIVERS WITH DIFFERENT METHODS OF BILIARY DRAINAGE

TYPE OF BILIARY DRAINAGE	ANIMAL GROUP	GRADE OF CHOLESTASIS							
		0		+		++		+++	
		No.	%	No.	%	No.	%	No.	%
Biliary-Biliary	1(a)	11	92	1	8	0	0	0	0
	1(b)	12	92	0	0	1	8	0	0
	3(b)	3	43	2	29	1	14	1	14
	4(a)	9	82	2	18	0	0	0	0
Biliary-Enteric	3(a)	4	44	0	0	1	11	4	44

TABLE 33 THE INCIDENCE OF CHOLESTASIS IN THE DONOR LIVERS WITH DIFFERENT METHODS OF BILIARY DRAINAGE

Group 1(a) - Heterotopic allograft plus cholecystocholecystostomy.
Group 1(b) - Heterotopic allograft plus cholecystojejunocholecystostomy.
Group 3(a) - Orthotopic allograft plus cholecystoduodenostomy.
Group 3(b) - Orthotopic allograft plus choledochocholedochostomy.
Group 4(a) - Heterotopic allograft plus cholecystocholecystostomy.

DISCUSSION IN CONTEXT

The incidence and degree of cholangitis and cholestasis in the donor livers analysed, are summarised in Table 32 and 33, on the opposite page.

It can be seen that the incidence and degree of cholangitis and cholestasis in the donor livers from groups 1(a) and 1(b) are similar and comparable, indicating that donor biliary drainage by means of either cholecystocholecystostomy or cholecystojejunocholecystostomy has a similar effect on the donor intrahepatic biliary tract. In Section II it was shown that the effects and rate of complications on the donor extrahepatic biliary apparatus were also similar in groups 1(a) and 1(b).

The incidence and degree of cholangitis and cholestasis in the recipient livers from groups 1(a) and 1(b) were roughly similar to one another, and of low order, suggesting that the two types of biliary drainage used to drain the donor livers had comparable, negligible effects on the recipients' intrahepatic biliary tracts. In Section II, it was shown that the effects on the recipients' extrahepatic biliary apparatus were comparable and minimal in the two groups.

Cholangitis and cholestasis were infrequent and of low order in the control livers from groups 2(a) and 2(b), indicating that an operation, the housing conditions and the rigorous postoperative course had little deleterious effect on the intrahepatic biliary tract, and that any changes that occurred in the donor livers would be due to other factors. The changes in the extrahepatic biliary apparatus of groups 2(a) and 2(b) were also shown to be negligible in Section II.

Comparison of the results obtained in the donor livers of groups 1(a) and 1(b) with the results in groups 3(b) and 4(a), reveal a marked similarity. This indicates that the intrahepatic biliary tract complication rate in the present series was comparable with that seen in Dent's group 3(b) and Immelman's group 4(a). Dent's group 3(a) showed a higher incidence of both cholangitis and cholestasis. This confirmed his published findings that the degree and incidence of cholangitis and cholestasis was less when using a choledochocholedochostomy, than when using direct biliary-enteric anastomosis by means of a cholecystoduodenostomy.

Subsequent to the author completing this analysis of the intrahepatic biliary tract, the sections from this series were re-analysed by Professor C.J. Uys of the Department of Pathology – see page 165. He confirmed the comparable low incidence of cholangitis and cholestasis in the donor livers from groups 1(a) and 1(b). He disagreed with the grading of mild cholangitis (+) as cholangitis, stating that this was not true cholangitis, but simply a normal variant often seen in pigs' livers. Using Professor Uys's criteria, the incidence of cholangitis in groups 1(a) and 1(b) becomes negligible, and the two groups even more comparable.

EXAMPLES OF HISTOLOGICAL GRADING

(Photomicrographs on following pages)

FIGURE 49 Histological section from a normal pre-operative liver - (pig No. 22). The section was graded as:

FLI - N
RI - N
cholestasis - 0
cholangitis - 0

FIGURE 50 Histological section of a recipient liver at 23 days - (pig No. 22). The section was graded as:

FLI - N
RI - N
cholestasis - 0
cholangitis - 0

FIGURE 51 Histological section from a donor liver at 28 days - (pig No. 28). The liver was of good quality and this section was graded as:

FLI - I
RI - II
cholestasis - 0
cholangitis - +

FIGURE 52 Histological section from a donor liver at 21 days - (pig No. 26). The section was graded as:

FLI - II
RI - II
cholestasis - 0
cholangitis - 0

FIGURE 53 Histological section from a donor liver at 14 days - (pig No. 12). The section was graded as:

FLI - III
RI - IV
cholestasis - 0
cholangitis - 0

FIGURE 54 Histological section from a donor liver at 28 days - (pig No. 8). The section was graded as:

FLI - IV
RI - IV
cholestasis - 0
cholangitis - 0

FLI - Functional Liver Index
RI - Rejection Index or Degree of Rejection

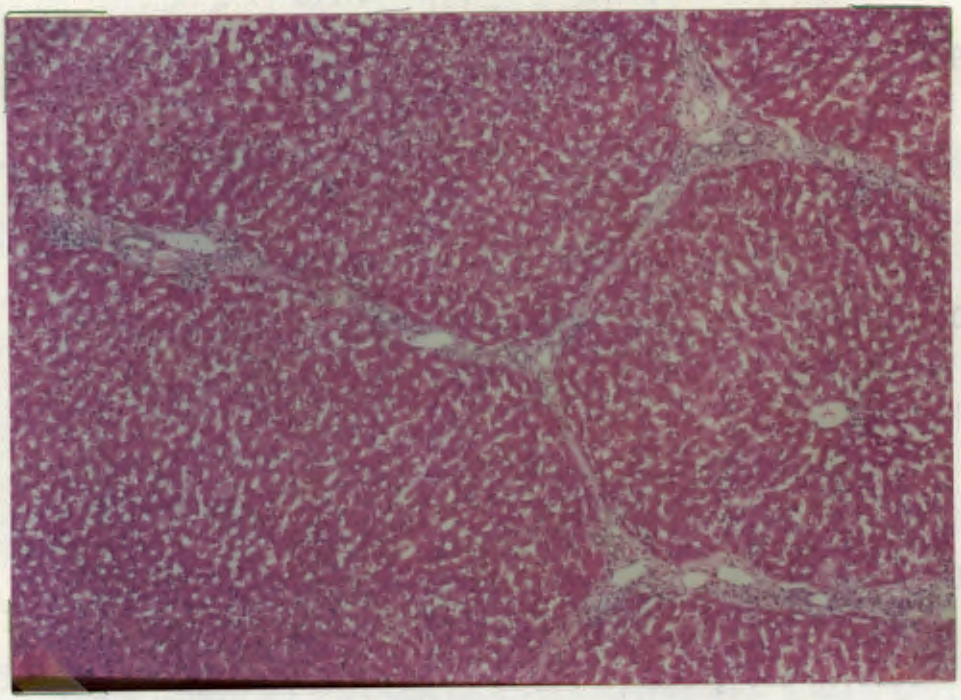


FIGURE 49 (Pig No. 22)

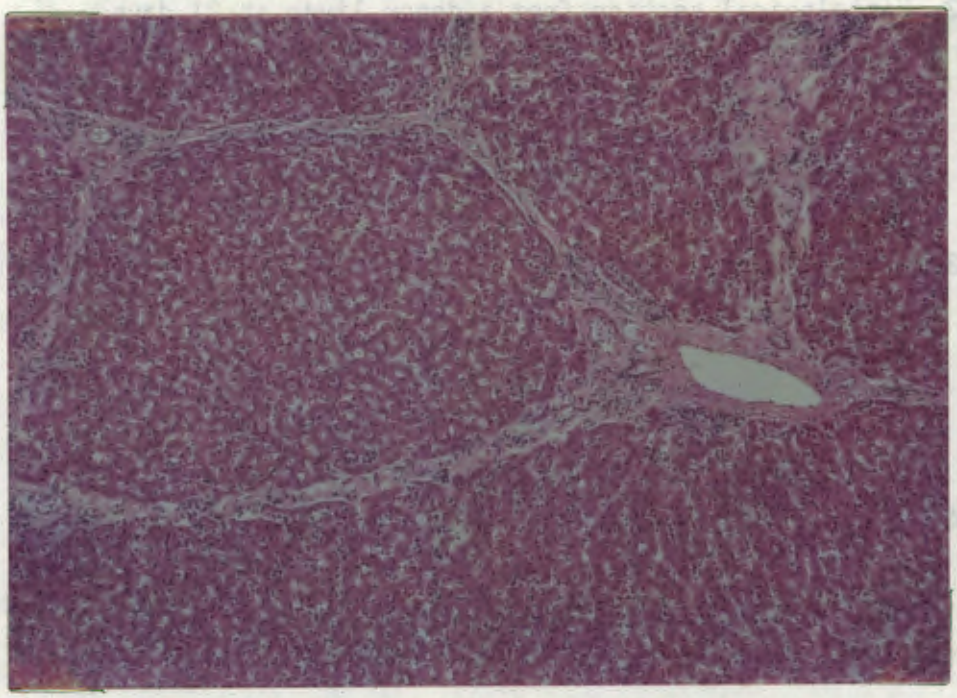


FIGURE 50 (Pig No. 22)

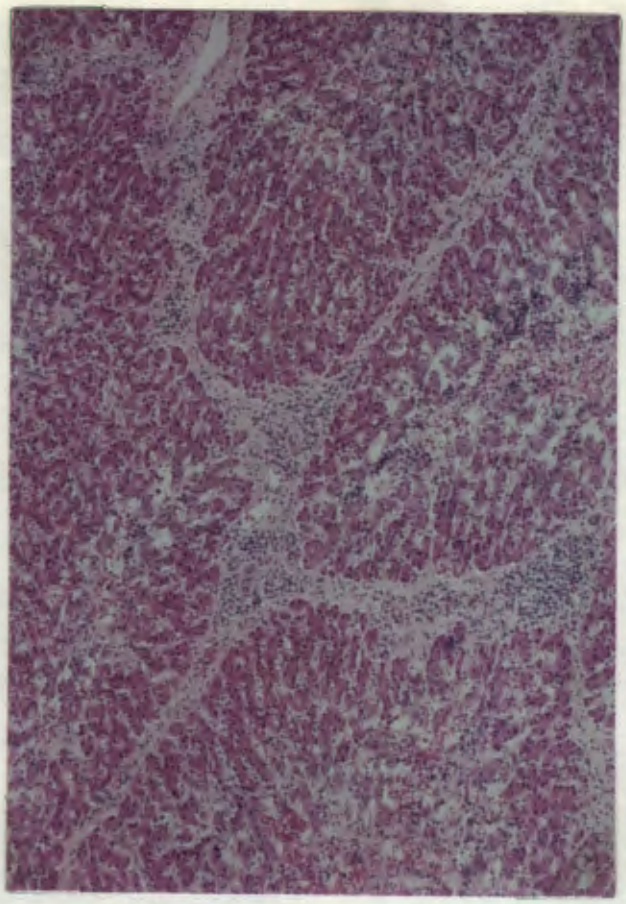


FIGURE 51 (Pig No. 28)

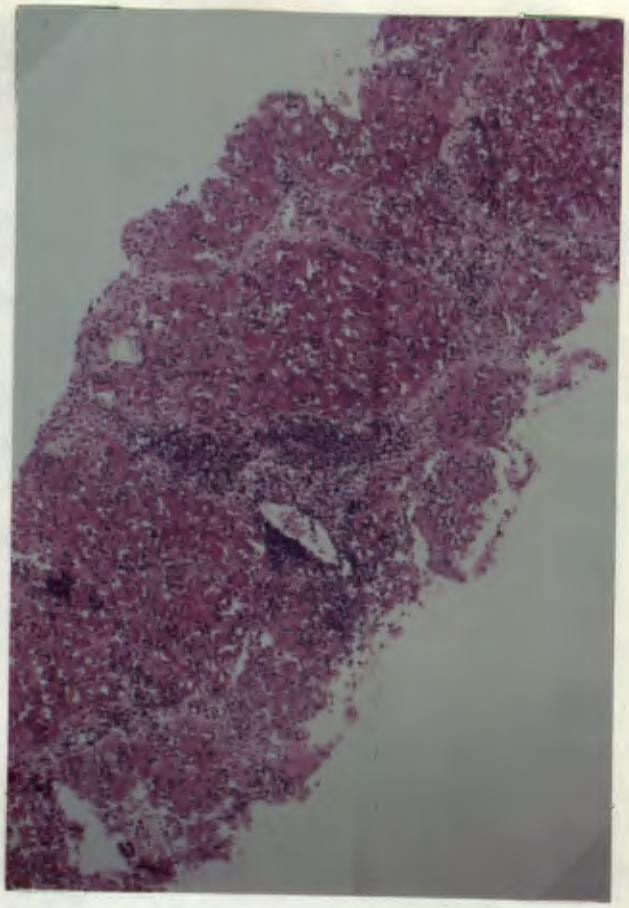


FIGURE 52 (Pig No. 26)

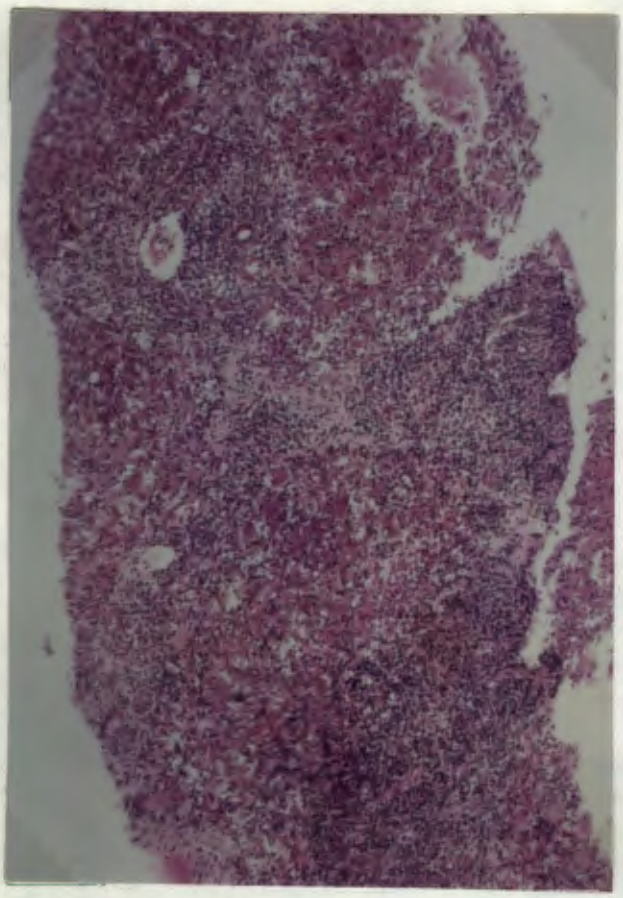


FIGURE 53 (Pig No. 12)

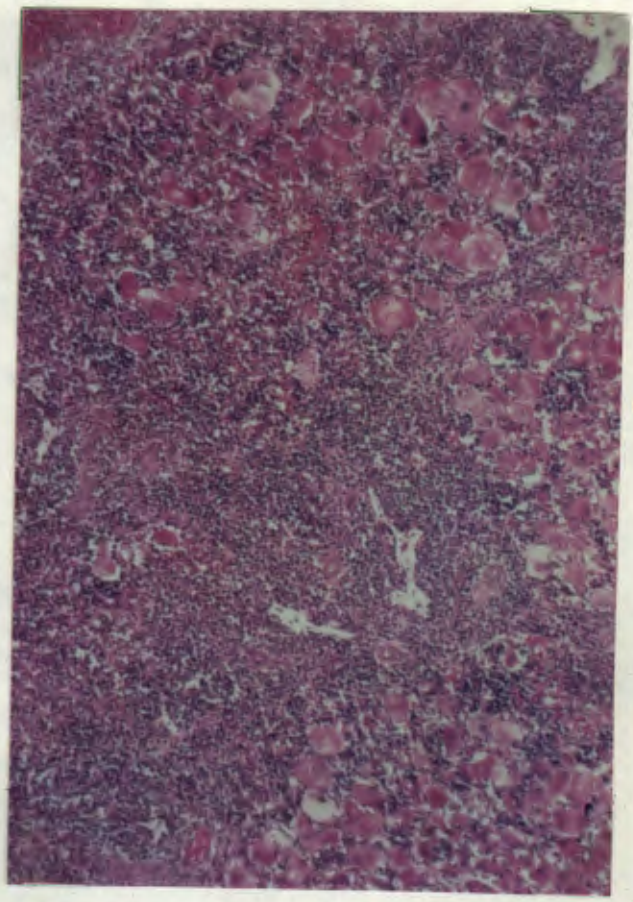


FIGURE 54 (Pig No. 8)

(B) ASSESSMENT OF THE INCIDENCE AND DEGREE OF HEPATOCYTE DAMAGE

(The Functional Liver Index)

1. Introduction

A second blind histological analysis of all the biopsy material outlined in Table 27, page 161F was undertaken, with two main objectives in view.

The first objective was to attempt to detect differences that could be attributable to the type of biliary drainage used, by assessment and comparison of the Functional quality of the donor and recipient livers in groups 1(a) and 1(b), as reflected by the percentage of hepatocytes surviving after the operation. The livers from groups 2(a) and 2(b) would serve as controls, while the donor livers from groups 3(a), 3(b) and 4(a) would allow accurate comparison with work previously performed in our laboratory.

In the Main Introduction the problem of the early diagnosis of rejection was reviewed, and it was stressed that this problem was compounded in the presence of two livers in the heterotopic transplant situation. Immelman (97) had suggested, from a few preliminary observations, that the level of the CSF glutamine appeared to relate to the quality of the donor liver in the present auxiliary pig liver transplantation model. The second objective in undertaking this analysis was to assess the value of Immelman's observation by attempting to correlate changes in the functional quality of the donor livers with changes that occurred in simultaneously harvested CSF glutamine

levels, and in the levels of other commonly used biochemical and haematological factors.

The same index of functional quality, viz, the percentage of hepatocytes surviving, would be used in the pursuance of both objectives.

2. Criteria and methods of analysis

Haematoxylin and Eosin stained sections from all the livers outlined in Table 27 on page 161F were examined under low and high power, on a blind observer basis. Publications by Elias (64, 65), Sandritter (151) and Scheuer (157) were used in the understanding and interpretation of the biopsy material. An estimate was made of the percentage of normal-looking hepatocytes in each section. The large, regenerative hepatocytes were not regarded as normal. Following the analysis, the average percentage of normal-looking hepatocytes was calculated from all the sections taken from each liver at a particular time, and that average percentage regarded as representative of the hepatocyte status of the whole liver at that time.

On this basis, the livers were graded as follows:

N - Hepatocytes normal in number and appearance

I - 75-100% normal hepatocytes present

II - 50-75% of normal hepatocytes present

III - 25-50% of normal hepatocytes present

IV - 0-25% of normal hepatocytes present

EXPERIMENTAL GROUPS	FUNCTIONAL LIVER GRADE									
	N		I		II		III		IV	
	No.	%	No.	%	No.	%	No.	%	No.	%
PRESENT SERIES										
1(a)	0	0	2	15	0	0	7	54	4	31
1(b)	0	0	3	21	3	21	3	21	5	36
DENT'S SERIES										
3(a)	1	11	1	11	5	56	2	22	0	0
3(b)	0	0	4	57	2	29	1	14	0	0
IMMELMAN'S SERIES										
4(a)	1	9	6	55	1	9	1	9	2	18

TABLE 34 FUNCTIONAL GRADES OF THE DONOR LIVERS AT AUTOPSY

Group 1(a) - Heterotopic allograft plus cholecystocholecystostomy.
Group 1(b) - Heterotopic allograft plus cholecystojejunocholecystostomy.
Group 3(a) - Orthotopic allograft plus cholecystoduodenostomy.
Group 3(b) - Orthotopic allograft plus choledochocholedochostomy.
Group 4(a) - Heterotopic allograft plus cholecystocholecystostomy.

This grading system was named the Functional Liver Index (F.L.I.), as it was postulated that some biochemical derangement should be detectable in the blood or CSF of the animals with livers having such gross differences in numbers of hepatocytes.

The photomicrographs in Figures 49 - 54, pages 173F and 173 illustrate the differences in grades of function.

3. Results

The functional quality of the donor livers, as represented by their Functional Liver Indices, are summarised in Table 34 on the opposite page, and the detailed analysis of all the livers is tabulated in Tables 40-47, on pages 319-326.

(a) Pre-operative specimens

All the pre-operative sections from groups 1(a), 1(b), 2(a) and 2(b) as well as the 10 pre-operative specimens from groups 3(a) and 3(b), had hepatocytes normal in number and appearance, and were graded into functional category N (Appendix pages 319-326). Mild fatty infiltration was seen in a few pre-operative sections from each group, but this finding was regarded as a normal variant for the purposes of this study.

(b) Serial postoperative specimens

(i) Groups 1(a) and 1(b) - Donor livers

Tables 40-41 in the Appendix, pages 319-320.

No transplanted liver from group 1(a) could be classed as normal at any stage during the post-operative period. As can be seen in Table 40 on page 319, over 80% of the livers had undergone severe hepatocyte destruction by the time autopsy was performed, and only two livers exhibited mild hepatocyte loss. In some animals the hepatocyte destruction was progressive as seen in serial biopsies, while in others the index of destruction was established by day 7. In one animal, pig No. 13, the quality of the liver improved as time progressed. No donor liver from group 1(b) could be classed as normal at any stage during the postoperative period. A wide spectrum of hepatocyte damage was seen. Over 55% of the donor livers had developed severe hepatocyte destruction (grades III and IV) by the time autopsy was performed (Table 41, page 320). As a group, the degree of hepatocyte damage appeared less severe than that seen in group 1(a). As in group 1(a), the destruction of hepatocytes was progressive in some animals, and established by day 7 in others. In pigs Nos. 20 and 22 the liver quality improved as time progressed.

(ii) Groups 1(a) and 1(b) - Recipient livers

Tables 42 and 43 in the Appendix pages 321-322.

The hepatocytes in the recipient livers from both groups 1(a) and 1(b) remained normal in number and appearance throughout the period of study, with one exception. In pig No. 25 (group 1(b)) mild centrizonal necrosis was present. This might have been due to terminal changes as autopsy was performed several hours after death. This liver was placed in Functional Category I. All the others were adjudged N. The mild fatty infiltration seen in a few serial biopsies from both groups was disregarded as being a normal variant. The interlobular septae in a few animals appeared more prominent than in the pre-operative specimens - a feature also seen in a few of the livers from the portacaval-shunted group.

(iii) Groups 2(a) and 2(b) - Control livers

Tables 44 and 45 in the Appendix pages 323-324.

The liver cell sizes decreased significantly in the postoperative period in some of the portacaval-shunted animals. The method used to compare the liver cell sizes is described in the Appendix, page 327. Despite the decrease in cell size, the cells remained essentially normal in appearance and number, and all group 2(a) livers were placed in Category N. Mild fatty infiltration was seen in a few biopsies, and prominent portal tracts

in others - these features were disregarded for the purposes of this study.

There were no obvious changes in any of the livers from the sham operated group 2(b) throughout the period of study, and all biopsies fell into category N. The mild fatty infiltration seen in a few sections was disregarded.

(iv) Groups 3(a) and 3(b) - Orthotopic Allografts - Dent's series

Table 46 in the Appendix page 325.

The functional quality of the donor livers from group 3(a) at autopsy was significantly better than that seen in either of groups 1(a) and 1(b) (Table 34, page 176F). Only 2 livers (22%) had severe hepatocyte damage (grade III), while the bulk of the livers were placed in grade II. One liver was completely normal at autopsy, while one had only the mildest hepatocyte damage.

Most of the donor livers from group 3(b) were of strikingly good quality at autopsy, 57% falling into grade I. Only 1 liver (14%) had severe hepatocyte damage (grade III). The functional quality of these livers was slightly better than those of group 3(a), and the trend of liver quality strinkingly superior to the trend seen in groups 1(a) and 1(b).

Many of the serial needle biopsies from groups 3(a) and 3(b) were of excellent quality, but most of the sections were too small to make valid comparisons with the present series.

(v) Group 4(a) - Auxiliary Heterotopic Allografts - Immelman's Series

Table 47 in the Appendix, page 326.

The functional quality of the donor livers ranged over the full spectrum. Most of the livers were of good functional quality (55% grade I), while 2 livers (18%) evidenced gross hepatocyte loss (grade IV). The quality trend was similar to that seen in group 3(b) (Table 34, page 176F), and strikingly superior to that seen in groups 1(a) and 1(b). Groups 1(a) and 4(a) reflected markedly different functional qualities in donor livers subjected to the same transplantation technique by two different operators working in the same laboratory, albeit on different groups of pigs.

Groups 4(b) and 4(c) will not be discussed as numbers are too small for valid comparison.

DISCUSSION IN CONTEXT

The analyses of the livers into histological grades of functional quality have been presented in pages 174-180. It has been shown that the functional quality of group 1(b) donor livers was marginally superior to that seen in group 1(a). The bulk of the donor livers in these two groups were, however, of comparably poor functional quality. The poor functional quality does not appear to relate to the type of biliary drainage used, as cholestasis and cholangitis were minimal and of low order in the two groups. The biopsies from Immelman's animals (group 4(a)) showed strikingly good quality grafts using the same technique of drainage as used in group 1(a). Clearly some other factor or factors are implicated.

The functional quality of the recipient livers from both groups 1(a) and 1(b) remained excellent throughout the series. This suggests that the biliary drainage used had no deleterious effects on the recipient hepatocytes, and in addition, that the changes seen in the donor livers were not due to general factors such as an operation, the housing conditions, or the frequent postoperative procedures.

The uniformly excellent functional quality of the control livers from groups 2(a) and 2(b) reinforces the conclusion that factors other than general were responsible for the poor quality of the grafts.

Comparison of the donor livers from this series with those of the two previous series has shown that the functional quality of the donor livers in the present series was strikingly inferior to that achieved in the

previous two series. The poor quality of the donor livers in the present series (groups 1(a) and 1(b)) may reflect poor operative technique, anoxic damage to the graft at operation, vascular complications, or the sequel to severe rejection. Cholangitis and cholestasis have been excluded as contributing causes. The reasons for the poor quality will be discussed further at the end of this section.

The serial functional liver indices of the donor livers in groups 1(a) and 1(b) will be used in Part III, pages 236-258 in a series of correlative studies with the changes that occurred in the levels of CSF glutamine, and other biochemical and haematological factors, which were harvested simultaneously with the liver biopsies.

(C) ASSESSMENT OF THE INCIDENCE AND DEGREE OF REJECTION

1. Introduction

An analysis of the incidence and degree of rejection in all the livers from the groups outlined in Table 27 on page 161F, was undertaken with two objectives in view.

The first objective was to compare the incidence and degree of rejection in the donor livers from groups 1(a) and 1(b). The recipient livers from groups 1(a) and 1(b) and the control livers from groups 2(a) and 2(b) would serve as controls for the present series. In addition, the incidence and degree of rejection in the present series would be compared with that seen in the two previous series (groups 3(a), 3(b) and 4(a)).

The second objective was to grade the donor livers from groups 1(a) and 1(b) into rough categories of rejection, to permit correlation studies with simultaneously harvested levels of CSF glutamine, and other biochemical and haematological factors. In this manner an assessment could be made of the value of CSF glutamine as an index of rejection, and its value could be compared to the information provided from other commonly used biochemical and haematological factors.

The same histological analysis would be used to serve both objectives.

2. Criteria and methods of analysis

The sections used in this analysis had all been stained with Haematoxylin and Eosin, Methyl Green Pyronine and for Reticulin.

Using publications by Ham (82), Herbertson (84), Hunt (94), Porter (143), Starzl (193), and Uys (205) as references, a crude grading system was devised by the author, for establishing the degrees of rejection.

Criteria for grading rejection

Grade		Histological findings
N	-	No evidence of pyroninophilic round cell infiltration in any of the sections examined
I	-	Pyroninophilic round cell infiltration minimal and confined to the portal tracts
II	-	More extensive round cell infiltration extending into lobules and interlobular septae
III	-	Diffuse round cell infiltration throughout the lobule, with widening of the portal tracts, severe cellular destruction, some stromal collapse, and evidence of some hepatocyte and bile duct regeneration
IV	-	More diffuse cellular infiltration of the whole lobule, with destruction of most or all of the hepatocytes, stromal collapse, the presence of bizarre giant cells and evidence of bile duct and hepatocyte regeneration.

In each case, a section stained in 3 ways as mentioned, was examined, and the section graded according to the criteria listed.

Following grading, the average grade was calculated from all the sections taken at a particular biopsy period, and that average grade regarded as representative of the degree of rejection in the whole liver at that time.

EXPERIMENTAL GROUPS	GRADE OF REJECTION									
	N		I		II		III		IV	
	No.	%	No.	%	No.	%	No.	%	No.	%
PRESENT SERIES										
1(a)	0	0	1	8	2	17	5	42	4	33
1(b)	0	0	0	0	5	38	3	23	5	38
DENT'S SERIES										
3(a)	0	0	3	33	4	44	2	22	0	0
3(b)	0	0	5	71	1	14	1	14	0	0
IMMELMAN'S SERIES										
4(a)	1	9	5	45	2	45	2	18	1	9

TABLE 35 THE INCIDENCE AND DEGREE OF REJECTION IN THE DONOR LIVERS AT AUTOPSY

Group 1(a) - Heterotopic allograft plus cholecystocholecystostomy.
Group 1(b) - Heterotopic allograft plus cholecystojejunocholecystostomy.
Group 3(a) - Orthotopic allograft plus cholecystoduodenostomy.
Group 3(b) - Orthotopic allograft plus choledochocholedochostomy.
Group 4(a) - Heterotopic allograft plus cholecystocholecystostomy.

Examples of the grades of rejection accorded to liver sections can be seen in Figures 49-54 on pages 173F and 173.

3. Results

The results of the analysis whereby the livers were graded for rejection, are detailed in the Appendix, Tables 40-47 on pages 319-326.

(a) Pre-operative specimens

No changes suggestive of rejection were seen in any of the pre-operative specimens from groups 1(a), 1(b), 2(a) and 2(b), or in the 10 pre-operative specimens from groups 3(a) and 3(b). In a few animals from each group, isolated areas of mild periportal cellular infiltration were seen, consisting mainly of eosinophils. This type of infiltration was considered a normal variant for the animals used in this study.

(b) Serial postoperative specimens

This analysis proved to be more difficult than the analysis used to grade the Functional Liver Index. Differentiation between grades II and III was particularly difficult.

(i) Groups 1(a) and 1(b) - Donor livers

Tables 40 and 41 in the Appendix, pages 319-320.

Rejection was seen in all the livers from group 1(a) throughout the period of study. At autopsy 75%

of the donor livers exhibited severe rejection (grades III and IV), while only 1 liver exhibited mild rejection (pig No. 13). From the serial biopsies (Appendix-page 319), it can be seen that the rejection process was static in some animals, progressive in some, and that the intensity decreased in 2 animals (Nos. 12 and 13). No donor liver from group 1(b) remained free of rejection in the postoperative period. By the time autopsy was performed 61% of the livers had severe rejection (grades III and IV), while 38% had moderate rejection (grade II). As in group 1(a), the rejection process appeared static in some animals, progressive in some, and of diminishing intensity in others.

The rejection changes seen in group 1(b) appeared marginally less severe than those seen in group 1(a) but the general trend in the two groups was the same and comparable.

(ii) Groups 1(a) and 1(b) - Recipient livers

Tables 42 and 43 in the Appendix, pages 321-322.

No changes suggestive of rejection were seen in any of the recipient liver sections throughout the period of study. A mild cellular infiltration was seen in the portal tracts of several animals, but the cells consisted of eosinophils, neutrophils and

non-pyroninophilic round cells. This infiltrate was fairly similar to that seen in some pre-operative specimens, and in some of the postoperative sections from groups 2(a) and 2(b). All the recipient livers were graded as N rejection.

(iii) Groups 2(a) and 2(b) - Control livers

Tables 44 and 45 in the Appendix, pages 323-324.

No changes suggestive of rejection were seen in any of the livers from these two groups at any stage during the postoperative period. The mild periportal cellular infiltration seen in a few livers from each group was similar to that seen in some of the recipient livers, and in a few of the preoperative specimens.

(iv) Groups 3(a) and 3(b) - Orthotopic Allografts - Dent's Series

Appendix-page 325, and Table 35, page 185F.

In contrast to groups 1(a) and 1(b), 77% of the livers from group 3(a) exhibited only mild to moderate rejection (grades I and II). Gross rejection (grade IV) was not seen in any of the livers from this group. By the time of autopsy, no liver could be graded as N, but several serial biopsies were indistinguishable from normal.

Rejection changes in group 3(b) were minimal, 71% falling into grade I. The degree of rejection was less than that seen in group 3(a).

Overall, far less severe rejection was seen in Dent's two groups, than in group 1(a) and 1(b), and none of the livers from his series showed gross rejection (grade IV).

(v) Group 4(a) - Auxiliary heterotopic allografts - Immelman's Series

Appendix page 326, and Table 35, page 185F.

This group of livers exhibited the full spectrum of changes from grades N to IV. The pattern of rejection was far less severe than that seen in groups 1(a) and 1(b), and roughly similar to that seen in Dent's group 3(b).

DISCUSSION IN CONTEXT

Using the criteria defined on page 184 a comparable and distressingly high incidence of severe and gross rejection has been demonstrated in the donor livers from groups 1(a) and 1(b). The type of biliary drainage did not appear to influence the incidence and degree of rejection in these two groups.

By virtue of the same criteria the donor livers from Dent's groups 3(a) and 3(b), and Immelman's group 4(a), revealed a high incidence of low grade rejection, which confirmed their own published findings.

Rejection changes were not seen in the recipient livers from groups 1(a) and 1(b), nor in the control livers from groups 2(a) and 2(b), suggesting that general factors common to the four groups did not cause the changes seen.

Comparison of groups 1(a) and 4(a), in which the transplant and biliary drainage techniques were identical, has shown that group 4(a) donor livers revealed far less severe rejection, again suggesting that the type of biliary drainage played no part in the changes seen.

A full discussion on the reasons for the high incidence of severe rejection in the present series is beyond the aims and scope of this work, but the phenomenon will be discussed briefly at the end of this Chapter.

The grades of rejection established for each serial donor biopsy from groups 1(a) and 1(b) will be used in Part III pages 236-258, for correlation studies with simultaneously obtained CSF, biochemical and haematological specimens.

(D) ANALYSIS BY A SECOND OBSERVER

Professor C.J. Uys of the Department of Pathology, University of Cape Town, subsequently analysed almost all the donor liver sections from groups 1(a) and 1(b) as well as representative numbers of the pre- and postoperative sections from the recipient livers from groups 1(a) and 1(b), the portacaval shunted (group 2(a)) and the sham laparotomy (group 2(b)) livers.

He confirmed the strikingly low incidence and degree of both cholangitis and cholestasis in the donor livers from groups 1(a) and 1(b). He felt that the criteria used for mild cholangitis (+) were too strict, and that the livers graded (+) cholangitis by the author did not reflect cholangitis but simply a normal variant often seen in pig liver sections.

Professor Uys commented on the extraordinary incidence and degree of rejection in the donor livers from groups 1(a) and 1(b), and stated that the pattern of rejection was more severe than in any of the pig liver transplant series previously analysed by him in Cape Town. He noted that the degree of rejection in group 1(a) was more severe than in group 1(b).

With few exceptions, he confirmed the grading accorded to individual sections by the author, in each of the three analyses performed.

On direct questioning, he stated that the changes seen in the donor livers were due to severe rejection, in many cases seen as early as 7 days, and not due to primary vascular problems, infection or bile stasis.

HISTOLOGICAL ANALYSIS OF THE BLOOD VESSELS

The blood vessels were analysed independently by Professor C.J. Uys and Dr. J. van den Ende of the Department of Pathology. The author viewed all the sections with the two pathologists.

1. Arteries

Sections from 20 transplanted aortic segments were analysed. All the transplanted aortas showed evidence of rejection. Where severe rejection was seen, e.g. pig No. 14, changes were seen through the full thickness of the walls. There was subintimal fibrosis, degeneration of the internal elastic lamina, degeneration of the media, and infiltration with immunocytes. In some pigs, e.g. pig No. 21, mild changes were seen. Marked calcification was seen in the transplanted aorta in pig No. 24. The contrast between the donor and recipient vessels was clearly seen on either side of the anastomoses. The recipient aortas showed no changes from normal.

No arterial changes were observed in the portacaval and sham groups of animals.

2. Veins

Marked rejection changes were seen in a few portal veins and vena caval cuffs examined from donor livers. Similar changes were not seen in the recipient vessels, nor in the veins from the control groups 2(a) and 2(b).

Professor Uys commented on the severity of the rejection changes seen in the vessels, and felt that they were roughly in keeping with the severity of the rejection seen in the livers.

DISCUSSION ON THE FINDINGS IN THE LIVERS AND BLOOD VESSELS

1. The incidence and degree of cholangitis and cholestasis

Cholangitis and cholestasis did not feature prominently in the donor livers of the present series. The allografted livers from groups 1(a) and 1(b) showed a comparable, low incidence and degree of these complications, suggesting that neither of the two techniques of biliary drainage used predispose to the development of cholangitis and cholestasis.

The incidence and degree of cholangitis and cholestasis in the present series was significantly less than that seen with direct biliary-enteric anastomosis in Dent's group 3(a), and with direct biliary-enteric anastomosis or external biliary drainage in Immelman's groups 3(b) and 4(c), and compared favourably with the results from biliary-biliary anastomoses in these series (groups 3(b) and 4(a)).

The low incidence of cholangitis and cholestasis in the recipient livers from the present series, suggests that the biliary drainage techniques used were not deleterious to the host's own liver - an important observation which is relevant to the concept of using an auxiliary liver as a temporary liver assist.

2. Functional Liver Index

Hepatocellular loss was not observed in the recipient livers of the allografted animals, nor in the livers of the control animals from groups 2(a) and 2(b). By contrast, a wide range of hepatocellular loss was seen in the donor livers. The loss of liver cells in the trans-

planted livers could therefore not be due to an operation, the diet, drugs used, or a rigorous postoperative regime. Possible causes for the destruction of the hepatocytes in the donor livers were:

(a) Rejection
(b) Inadequate blood supply or drainage
(c) Anoxic damage at initial operation
(d) Infection
(e) Bile stasis

The macro- and microscopic analyses of the livers, blood vessels and biliary tracts suggest that rejection was the main cause of damage to the donor livers.

3. Incidence and degree of Rejection

Histological features of rejection were seen neither in the recipient livers from the allografted animals, nor in the livers from the control animals - groups 2(a) and 2(b). This suggests that the histological changes seen in the allografted livers were due to factors other than an operation, diet, drugs and a strenuous postoperative regime. The histological changes seen in the donor livers from the present series parallel many of the changes reported in severe acute rejection in dogs (145, 188, 201), and in a few reports on severe rejection in pig livers (22, 72, 94, 131, 145). The surprisingly high incidence of rejection, the highest degree yet seen in Cape Town, is intriguing.

Possible reasons are:

(a) The pigs were grossly mismatched genetically

(b) Some other factors may have enhanced the rejection process.

Sepsis and cholestasis appear not to be responsible factors. Poor graft preservation may be responsible (98), but the ischaemic times compare favourably with those of Dent (53). Dent's average portal ischaemic period was 45,5 minutes, while in this series it was 47 minutes. The period for full revascularisation in Dent's series was 60 minutes, and in this series 64 minutes. No hepatic core temperatures were obtained in this series but the technique used was similar to that used by Dent and Immelman, in that the graft was perfused in the same manner and then wrapped in swabs soaked in cold perfusate, and thus insulated. Flow, pressure and angiographic studies were not performed in this series and no opinion can be passed about the influence of haemodynamic factors in the present series.

Professor Uys felt that the primary cause of the damage to the donor livers was an immunological assault, seen even at 7 days in the severely rejecting livers. The evidence of rejection extended to the aortic cuffs, hepatic arteries and gallbladders.

Intrahepatic cholestasis has been reported as a feature of rejection in both the baboon and man (136). In the present series (groups 1(a) and 1(b)) severe rejection was often encountered, with little or no intrahepatic cholestasis being detected. In a few of Dent's (group 3(a) and 3(b)), and Immelman's (group 4(a)) animals, donor livers with severe rejection also exhibited minimal or no intrahepatic cholestasis. This suggests that cholestasis may not be an inevitable feature of rejection in the pig, and that other factors contribute to the phenomenon of intrahepatic cholestasis when it occurs in this animal.

INDEX TO PART II, CHAPTER 3

SECTION IV - THE EFFECTS OF THE PROCEDURES ON BIOCHEMICAL AND HAEMATOLOGICAL FACTORS

PART II

CHAPTER 3

SECTION IV - THE EFFECTS OF THE PROCEDURES ON BIOCHEMICAL AND HAEMATOLOGICAL FACTORS

SUMMARY

The pattern of biochemical and haematological changes that occurred in the four experimental groups during the period of study are presented, analysed and compared.

The analysis revealed that the changes which occurred in the two transplant groups were essentially similar and comparable, and apart from the CSF glutamine, venous blood ammonia, haemoglobin and cholesterol, similar to the changes seen in the control groups 2(a) and 2(b).

BIOCHEMISTRY	No.	MEAN ± 1 S.D.	RANGE
CSF Glutamine	64	9,96 ± 3,23	4 - 26
Blood ammonia-venous	62	219,61 ± 63,48	126 - 410
-arterial	33	177,54 ± 65,89	78 - 415
Alkaline phosphatase	74	7,32 ± 2,90	2,8 - 18,5
Serum glutamic oxalo-acetic transaminase	75	41,40 ± 24,33	15 - 145
Cholesterol	38	97,84 ± 24,10	49 - 167
Total protein	55	6,27 ± 0,73	4,7 - 8,4
Albumin	54	1,84 ± 0,32	1,20 - 2,5
Globulin	55	4,43 ± 0,63	3,3 - 6,4
pH	51	7,31 ± 0,12	7,00 - 7,53
pCO_2	51	43,89 ± 18,02	17 - 110
Standard Bicarbonate	51	20,69 ± 5,67	12,10 - 43,00
HAEMATOLOGY			
Haemoglobin	49	11,1 ± 1,2	8,2 - 13,9
Leucocyte count	51	17550 ± 5302	7200 - 34400
Platelet count	52	430576 ± 157343	50000 - 825000
Neutrophil %	52	45 ± 13	7 - 83
Lymphocyte %	52	40 ± 13	14 - 78
Eosinophil %	52	2 ± 2	0 - 12
Basophil %	52	0 ± 1	0 - 5
Monocyte %	52	9 ± 5	1 - 26

TABLE 36 MEAN PRE-OPERATIVE VALUES*

* Derived from all the animals used in the series.

INTRODUCTION

Serial biochemical and haematological investigations were undertaken in the four groups of animals with three objectives in mind.

The first objective was to analyse and compare the changes that occurred in the two transplant groups 1(a) and 1(b) in order to detect any differences that could be attributable to the type of biliary drainage used. Groups 2(a) and 2(b) would be used as controls.

The second objective was to assess the value of CSF glutamine as an index of donor liver status, and to compare its value with information provided by other biochemical and haematological factors, as discussed in the Main Introduction.

The third objective was to complement the in-depth study of the animals in the four groups.

The biochemical and haematological factors investigated in this study are tabulated on the opposite page, together with the mean pre-operative values for the animals used in this series. The normal pre-operative values in the present series are comparable to those reported by Hickman (92). The techniques and frequency of sampling have been discussed in Part II, Chapter 2. The methods used to perform the estimations are presented in the Appendix, pages 335-338. The results for individual animals are detailed in the case histories on pages 269-315, in the Appendix. The statistical methods used to compare the results in the different groups, are detailed in the Appendix, pages 340-342. P values of 0,05 or less are regarded as "significant" throughout the text, while those greater than 0,05 are regarded as "not significant".

TEST	DATE OF COMPARISON (DAYS)				
	OP	7	14	21	28
CSF Glutamine	-	-	-	-	-
Venous NH_4	-	-	-	<0,01	<0,001
Alkaline Phos.	-	-	-	-	-
S.G.O.T.*	-	-	-	-	<0,02
Cholesterol	-	-	<0,005	-	-
Total Protein	-	<0,02	-	<0,01	-
Albumin	-	-	-	-	-
Globulin	-	<0,05	-	<0,02	<0,05
Haemoglobin	-	<0,05	<0,02	-	-
Leucocytes	-	-	-	-	-
Platelets	-	-	-	-	-
Neutrophils	-	-	-	-	-
Lymphocytes	-	-	-	-	-
Eosinophils	-	<0,01	-	-	-
Basophils	-	-	-	-	-
Monocytes	-	-	<0,005	-	<0,025
pH	-	-	-	-	-
pCO_2	-	-	-	-	-
Standard Bicarb.	-	-	-	-	-

p < = significance of difference in mean levels
- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation

TABLE 37 THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN GROUPS 1(a) and 1(b).

NB: This comparison was performed using all the available data from all the animals in each group, i.e. 14 animals in each of groups 1(a) and 1(b).

METHODS USED TO COMPARE THE GROUPS

1. Comparison of groups 1(a) and 1(b)

A simple statistical analysis was performed, utilising all the biochemical and haematological data available in the 14 animals from each group. The mean and standard deviation was calculated for each group on days 0, 7, 14, 21, and 28, for each of the factors tabulated in Table 36, page 197F.

The serial mean levels of the factors were compared at weekly intervals by means of a Student's t-test, to detect any significant differences between groups 1(a) and 1(b). The significance of these serial differences are tabulated in Table 37 on the opposite page.

It was shown that there were no significant differences in the serial postoperative levels for CSF glutamine, alkaline phosphatase, albumin, leucocyte count, platelets, neutrophils, lymphocytes, basophils, and acid-base status. For S.G.O.T., cholesterol and eosinophils, the mean levels varied on only one test period, and for practical purposes the mean postoperative changes can be regarded as similar. More sustained differences were seen in the mean levels of venous blood ammonia, total protein, globulin, haemoglobin and monocytes.

2. Comparison of the four groups, 1(a), 1(b), 2(a) and 2(b)

Serial glutamine levels were not available in five animals from group 1(a) and in 1 animal from group 1(b). Failed lumbar punctures in some of the earlier animals, together with technical problems associated with the laboratory estimation of CSF glutamine

in the early stages of the project were responsible for the non-availability. Bearing in mind the importance of CSF glutamine in the project, the six animals in whom serial glutamine levels are not available, will be excluded from further analysis in the presentation.

The biochemical and haematological changes that occurred in the 9 animals from group 1(a), 13 from group 1(b), 14 from group 2(a) and 5 from group 2(b), were compared by means of a second simple statistical analysis. The mean, standard deviation and standard error of the mean for each factor in each group, was calculated on days 0, 7, 14, 21 and 28. In view of the close similarity of the two transplant groups 1(a) and 1(b), the same calculations were performed for all the transplant recipients in whom serial CSF glutamine was available - named the combined transplant group - 1(a+b) - 22 animals. The serial mean values for the four main groups, and 1 sub-group are tabulated in the Appendix - Tables 54-58, on pages 344-348.

Student's t-tests were used to calculate the significance of differences between the mean levels for each factor in the five groups. The significances of differences on days 0, 7, 14, 21 and 28 are tabulated in the Appendix - Tables 64-71 on pages 354-361.

Student's t-tests were used to calculate the significance of serial changes in mean levels in the five groups. The significances of these changes are tabulated in the Appendix - Tables 59-63 on pages 349-353.

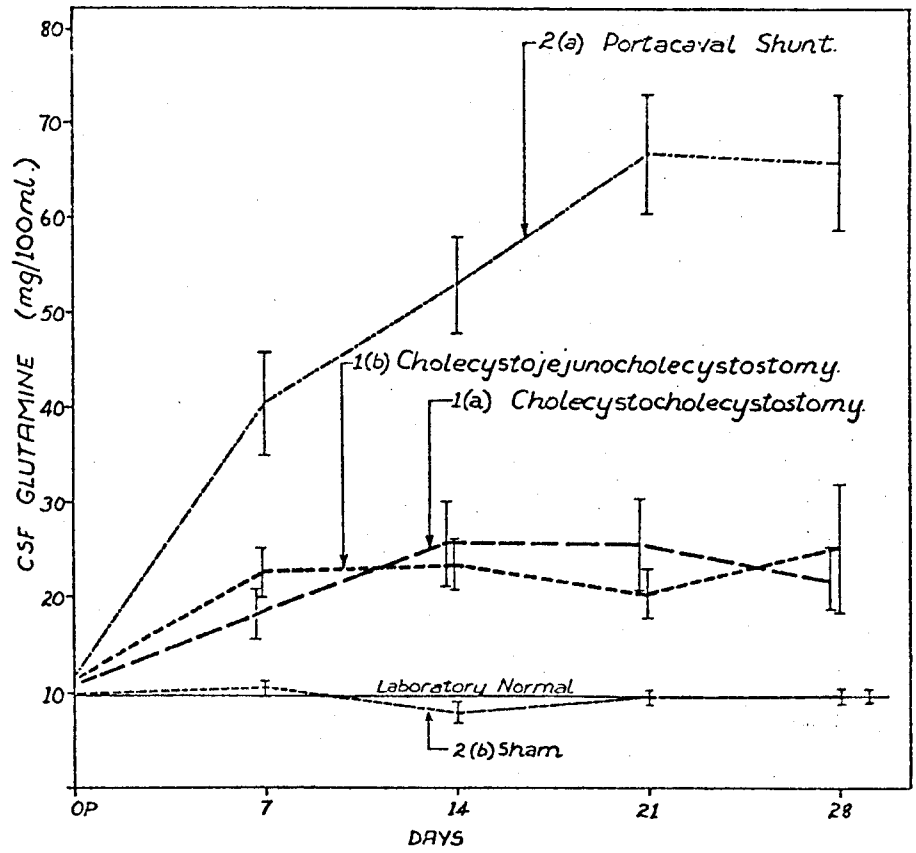
RESULTS

1. Comparison of groups 1(a) and 1(b) using all the available data

The serial mean values for the biochemical and haematological factors in groups 1(a) and 1(b) in the first analysis, in which all the available data was used, were not significantly different from the mean values derived in the second analysis, from the 9 animals in group 1(a) and the 13 in group 1(b). The significances of differences between the two were essentially the same, as can be seen by comparing Table 37 on page 198F (14 animals in each group), with Table 64 on page 354 (9 and 13 animals in each group respectively). All further discussion of groups 1(a) and 1(b) will be limited to the 9 animals from group 1(a) and 13 from group 1(b).

2. Comparative analysis of the changes seen in the individual factors

The comparative analyses of serial changes in the values of the individual biochemical and haematological factors, in each of the experimental groups, are presented on pages 201-225. Roughly the same manner of presentation is used for each factor. The serial mean values for each group of animals are illustrated and tabulated in Graphs "a" and Tables "b" on the left hand pages. The significances of changes in mean levels for each group are tabulated in Tables "c", while the significances of differences in serial mean values between groups are tabulated in the "d" Tables. The results of the statistical analyses are interpreted and briefly discussed for each factor in each group.



GRAPH 55a SERIAL CHANGES IN MEAN CSF GLUTAMINE LEVELS FOR THE GROUPS. (Mean ± 1 SEM)

Group	Day									
	OP	No	7	No	14	No	21	No	28	No
1a	10,60 ± 1,49	8	18,06 ± 7,52	9	25,63 ± 10,57	6	25,40 ± 10,83	6	21,83 ± 7,36	6
1b	11,26 ± 2,91	12	22,57 ± 9,27	13	23,2? ± 8,00	11	20,21 ± 7,04	8	25,26 ± 12,93	5
2a	10,65 ± 3,73	12	40,52 ± 18,47	13	52,95 ± 16,44	12	66,73 ± 18,48	9	66,06 ± 20,12	8
2b	9,88 ± 1,44	5	10,26 ± 1,73	5	7,78 ± 0,93	5	9,42 ± 2,11	5	9,80 ± 1,19	4
1a+1b	11,00 ± 2,47	20	20,73 ± 8,88	22	24,08 ± 9,07	17	22,43 ± 9,23	14	23,39 ± 10,42	11

Mean ± 1 SD No = Number of samples processed

TABLE 55b MEAN LEVELS FOR THE FOUR GROUPS AND ONE SUBGROUP

Group	Time Interval (Days)			
	0-7	0-14	0-21	0-28
1a	<0,025	<0,01	<0,01	<0,01
1b	<0,001	<0,001	<0,005	<0,02
2a	<0,001	<0,001	<0,001	<0,001
2b	-	<0,05	-	-
1a+1b	<0,001	<0,001	<0,001	<0,001

TABLE 55c SIGNIFICANCE OF CHANGES IN MEAN LEVELS

p< = significance
- = no significant difference
0 = no or insufficient data

Groups	Date of Comparison (Days)				
	0-0	7-7	14-14	21-21	28-28
1a-1b	-	-	-	-	-
1a-2a	-	<0,005	<0,005	<0,001	<0,001
1b-2a	-	<0,01	<0,001	<0,001	<0,05
1a-2b	-	<0,05	<0,01	<0,02	<0,02
1b-2b	-	<0,02	<0,005	<0,01	-
2a-2b	-	<0,005	<0,001	<0,001	<0,001
1a+b-2a	-	<0,001	<0,001	<0,001	<0,001
1a+b-2b	-	<0,02	<0,001	<0,01	<0,05

TABLE 55d SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS

FIGURE 55 CSF GLUTAMINE - SERIAL CHANGES IN MEAN LEVELS

(1) CEREBROSPINAL FLUID GLUTAMINE

The serial mean CSF glutamine values for the groups are illustrated and tabulated in Figure 55 on the opposite page. The significances of changes and differences in mean levels are summarised in Tables 55c and 55d respectively.

(a) Groups 1(a) and 1(b)

There were no significant differences between the mean CSF glutamine levels of groups 1(a) and 1(b) at any stage during the postoperative period, as shown in Table 55d on the opposite page.

A significant elevation in mean CSF glutamine levels was seen on day 7 in both group 1(a), ($p < 0,025$) and group 1(b) ($p < 0,001$) see Table 55c.

The elevations in mean levels were sustained throughout the period of study, and did not alter significantly over the ensuing 21 days. As can be seen in the case histories (pages 269-296) individual animals exhibited a varied response, ranging from near normal levels, to levels approaching the levels seen in the portacaval shunted animals from group 2(a).

(b) Group 2(a)

A highly significant increase was seen in the mean CSF glutamine level on day 7 ($p < 0,001$). The increase was progressive from day 7 to day 21 as seen in Table 55b. Individually the animals all exhibited a marked increase

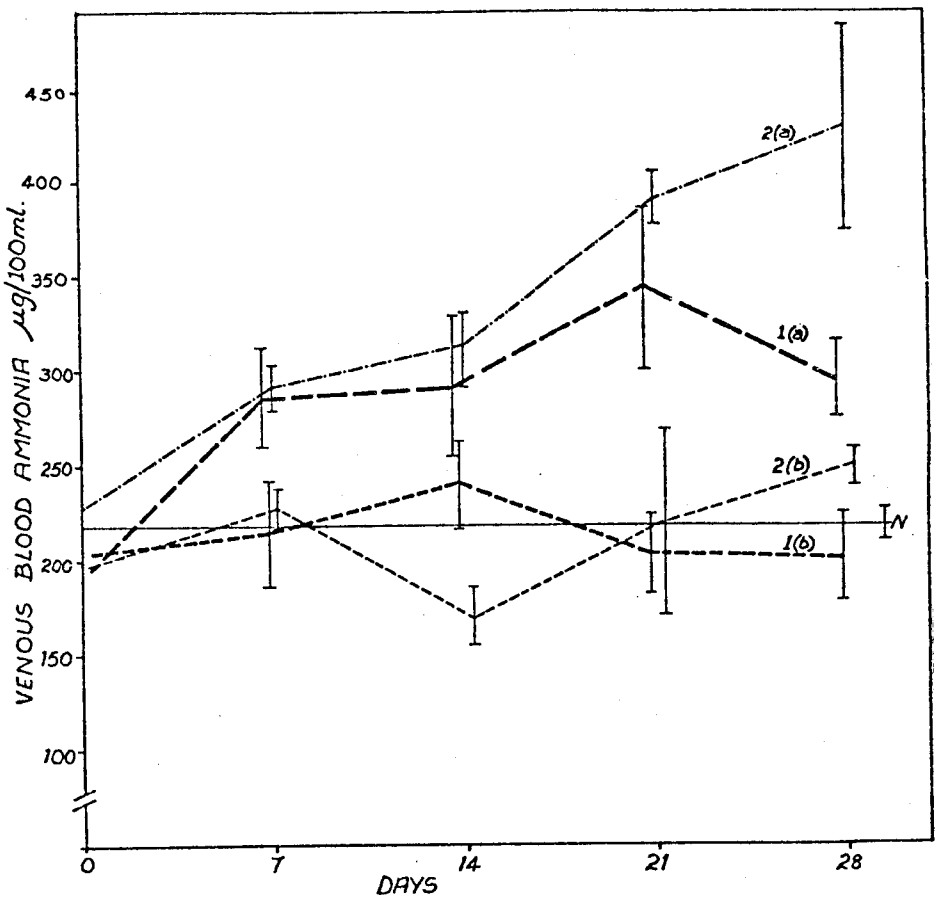
in CSF glutamine levels. Throughout the postoperative period, the mean CSF glutamine levels for group 2(a) were significantly higher than the levels in groups 1(a), 1(b), 2(b) and the levels seen in the combined transplant group 1(a+b), as shown on Tables 55b and 55d.

(c) Group 2(b)

The mean CSF glutamine levels for group 2(b) remained essentially within the normal pre-operative range throughout the period of study. As can be seen in the case histories, pages 311-315 none of the animals revealed elevation of CSF glutamine postoperatively, while a few revealed a slight decrease compared to the pre-operative normal. The mean levels were significantly lower than groups 1(a), 1(b) and 2(a), throughout the postoperative period - see Figure 55 on page 201F.

Interpretation

Three distinct patterns emerged from the postoperative CSF glutamine levels. The group 2(a) animals (subjected to portacaval shunts) all revealed a dramatic elevation, the mean levels being 5 to 6 times normal, while the group 2(b) animals (subjected to sham laparotomy) remained essentially normal. The two transplant groups 1(a) and 1(b) had similar mean levels, elevated significantly above group 2(b) and normal, and significantly lower than group 2(a). The range in the CSF glutamine levels of individual transplant recipients was wide, and it was seen, in general, that the



Group 1(a) - Heterotopic allograft + cholecystocholecystostomy.
Group 1(b) - " " + cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

GRAPH 56a SERIAL CHANGES IN MEAN VENOUS AMMONIA LEVELS FOR THE GROUPS. (Mean ± 1 SEM)

Group	Day									
	OP	No	7	No	14	No	21	No	28	No
1a	193,50 ± 29,97	8	286,85 ± 67.67	7	291,83 ± 76,02	6	344,66 ± 95,88	6	294,50 ± 48,32	6
1b	203,16 ± 44,48	12	212,72 ± 89,09	11	240,10 ± 66,40	10	202,50 ± 51,62	6	201,80 ± 49,30	5
2a	227,22 ± 81,49	9	291,81 ± 39,85	11	312,70 ± 59,53	10	390,10 ± 48,50	10	429,12 ±145,46	8
2b	197,50 ± 29,81	4	227,25 ± 19,53	4	168,00 ± 33,13	5	220,75 ± 92,59	4	249,00 ± 16,09	4
1a+1b	199,30 ± 39,61	20	241,55 ± 89,09	18	259,12 ± 74,63	16	273,58 ±104,79	12	252,36 ± 67,15	11

Mean ± 1 SD No = Number of samples processed

TABLE 56b MEAN LEVELS FOR THE FOUR GROUPS AND ONE SUBGROUP

Group	Time Interval (Days)			
	0-7	0-14	0-21	0-28
1a	<0,01	<0,02	<0,005	<0,001
1b	-	-	-	-
2a	<0,05	<0,02	<0,001	<0,005
2b	-	-	-	-
1a+1b	-	<0,01	<0,02	<0,02

TABLE 56c SIGNIFICANCE OF CHANGES IN MEAN LEVELS

p< = significance
\- = no significant difference
0 = no or insufficient data

Groups	Date of Comparison (Days)				
	0-0	7-7	14-14	21-21	28-28
1a-1b	-	-	-	<0,01	<0,02
1a-2a	-	-	-	-	-
1b-2a	-	<0,02	<0,02	<0,001	<0,01
1a-2b	-	-	<0,01	-	-
1b-2b	-	-	<0,05	-	-
2a-2b	-	<0,01	<0,001	<0,001	<0,05
1a+b-2a	-	-	-	<0,005	<0,01
1a+b-2b	-	-	<0,02	-	-

TABLE 56d SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS

FIGURE 56 VENOUS AMMONIA - SERIAL CHANGES IN MEAN LEVELS

animals with lower CSF glutamine levels had donor livers of good quality, while those with grossly elevated CSF glutamine levels had poor quality donor livers. This phenomenon will be analysed and discussed in a separate study in Part III on pages 236-265.

(2) VENOUS BLOOD AMMONIA

The author found it extremely difficult to obtain arterial blood samples by means of percutaneous carotid or femoral arterial puncture in the postoperative period, and for this reason insufficient arterial blood ammonia estimations were performed, and no statistical analysis could be carried out. On the other hand, percutaneous venepuncture at the base of the neck was invariably successful, enabling one to make a detailed serial study on venous blood ammonia.

The mean venous blood ammonia levels for the four groups are illustrated and tabulated in Figure 56 on the opposite page. The significances of changes and differences in mean venous blood ammonia levels are summarised in Tables 56c and 56d respectively.

(a) Groups 1(a) and 1(b)

The mean venous ammonia levels of groups 1(a) and 1(b) did not differ significantly on days 7 and 14, as shown in Table 56d. The mean levels in group 1(a) revealed a significant, sustained elevation above normal throughout the postoperative period, as shown in Table 56c.

The mean levels in group 1(b) remained within the normal range. The serial mean levels in group 1(a) did not differ significantly from the serial mean levels in groups 2(a) and 2(b), except on day 14, as shown in Table 56d. The serial mean levels in group 1(b) were significantly lower than those seen in group 2(a), but essentially the same as those seen in group 2(b).

The combined transplant group (1(a+b)), revealed a sustained, significant elevation above normal from day 14 onwards. This elevation was significantly less than that seen in group 2(a) on days 21 and 28, and significantly higher than the mean level in group 2(b) on day 14.

(b) Group 2(a)

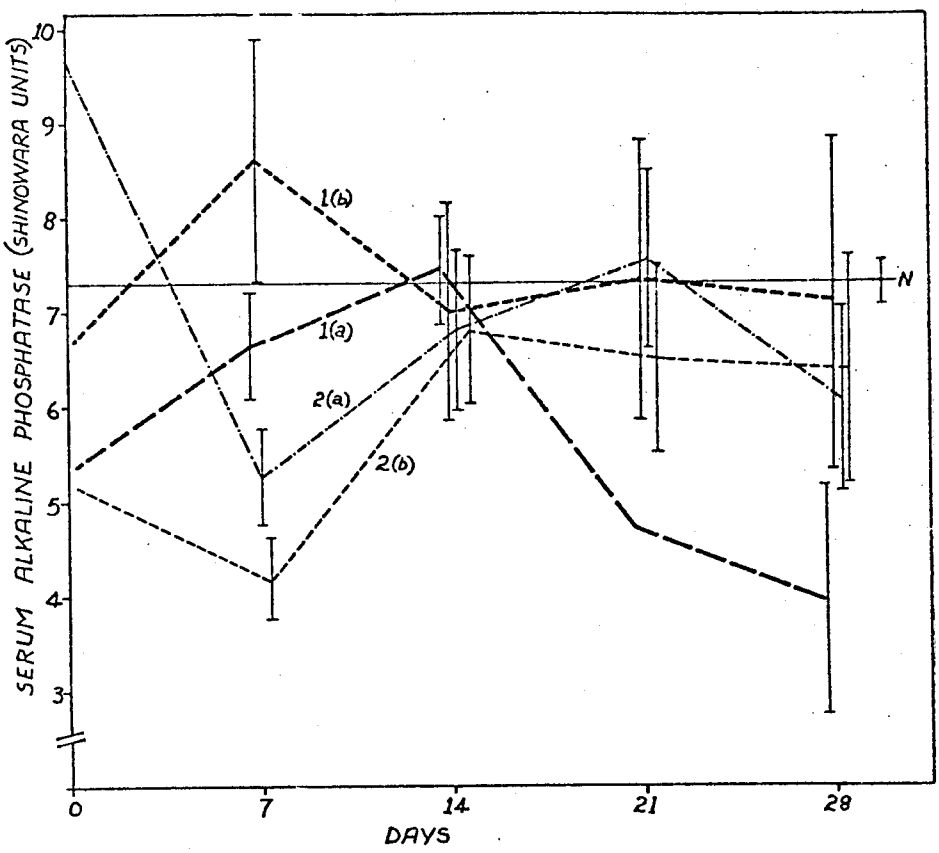
There was a significant, progressive increase in mean venous ammonia levels throughout the postoperative period. The serial mean levels were significantly higher than those seen in groups 1(b) and 2(b), as shown in Table 56d.

(c) Group 2(b)

There were no significant elevations in mean levels throughout the period of study.

Interpretation

The patterns of mean venous ammonia levels in groups 2(a) and 2(b) were significantly different throughout the postoperative period, and roughly paralleled the patterns seen in the CSF



Group 1(a) - Heterotopic allograft + cholecystocholecystostomy.
Group 1(b) - " " + cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt
Group 2(b) - Sham Laparotomy.

GRAPH 57a SERIAL CHANGES IN MEAN ALKALINE PHOSPHATASE LEVELS FOR THE GROUPS. (Mean ± 1 SEM)

Group	Day									
	OP	No	7	No	14	No	21	No	28	No
1a	5,32 ± 1,18	9	6,62 ± 1,70	9	7.43 ± 1,42	6	4,70 ±2,65	6	3,95 ±2,68	6
1b	6,70 ± 2,70	12	8,59 ± 4,53	13	7,00 ± 3,54	11	7,33 ±4,04	8	7,10 ±3,59	5
2a	9,60 ± 3,79	14	5,27 ± 1,64	14	6,80 ± 2,94	12	7,56 ±3,04	9	6,08 ±2,83	8
2b	5,18 ± 1,93	5	4,16 ± 0,98	5	6,82 ± 1,64	5	6,52 ±1,97	5	6,37 ±2,22	4
1a+1b	6,11 ± 2,29	21	7,78 ± 3,77	22	7,15 ± 2,98	17	6,20 ±3,75	14	5,38 ±3,50	11

Mean ± 1 SD No = Number of samples processed

TABLE 57b MEAN LEVELS FOR THE FOUR GROUPS AND ONE SUBGROUP

Group	Time Interval (Days)			
	0-7	0-14	0-21	0-28
1a	-	<0,01	-	-
1b	-	-	-	-
2a	<0,001	<0,05	-	<0,05
2b	-	-	-	-
1a+1b	-	-	-	-

TABLE 57c SIGNIFICANCE OF CHANGES IN MEAN LEVELS

p< = significance
- = no significant difference
0 = no or insufficient data

Groups	Date of Comparison (Days)				
	0-0	7-7	14-14	21-21	28-28
1a-1b	-	-	-	-	-
1a-2a	<0,005	-	-	-	-
1b-2a	<0,05	<0,025	-	-	-
1a-2b	-	<0,02	-	-	-
1b-2b	-	<0,05	-	-	-
2a-2b	<0,025	-	-	-	-
1a+b-2a	<0,005	<0,05	-	-	-
1a+b-2b	-	<0,05	-	-	-

TABLE 57d SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS

FIGURE 57 ALKALINE PHOSPHATASE - SERIAL CHANGES IN MEAN LEVELS

glutamine levels. Group 1(a) animals revealed significantly higher mean venous ammonia levels on days 21 and 28, compared to group 1(b) animals. As was shown in the histological analysis of the livers, the quality of the group 1(a) livers was inferior to that seen in group 1(b). The combined group 1(a+b) followed a pattern of mean venous ammonia levels significantly lower than group 2(a) and significantly higher than group 2(b), again roughly parallel to the pattern seen in the CSF glutamine levels.

The relationship of CSF glutamine to blood ammonia will be discussed further in Part III of this presentation.

(3) SERUM ALKALINE PHOSPHATASE

The serial mean alkaline phosphatase levels for the groups are illustrated and tabulated in Figure 57 on the opposite page. The significances of changes and differences in mean levels are summarised in Tables 57c and 57d respectively.

(a) Groups 1(a) and 1(b)

There were no significant differences between mean levels in groups 1(a) and 1(b) during the postoperative period, as shown in Table 57d. Group 1(a) revealed a significant elevation on day 14, (Table 57c), while group 1(b) revealed no significant serial changes (Table 57c).
The combined transplant group (1(a+b)) likewise revealed no significant changes in mean levels in the postoperative period.

(b) Group 2(a)

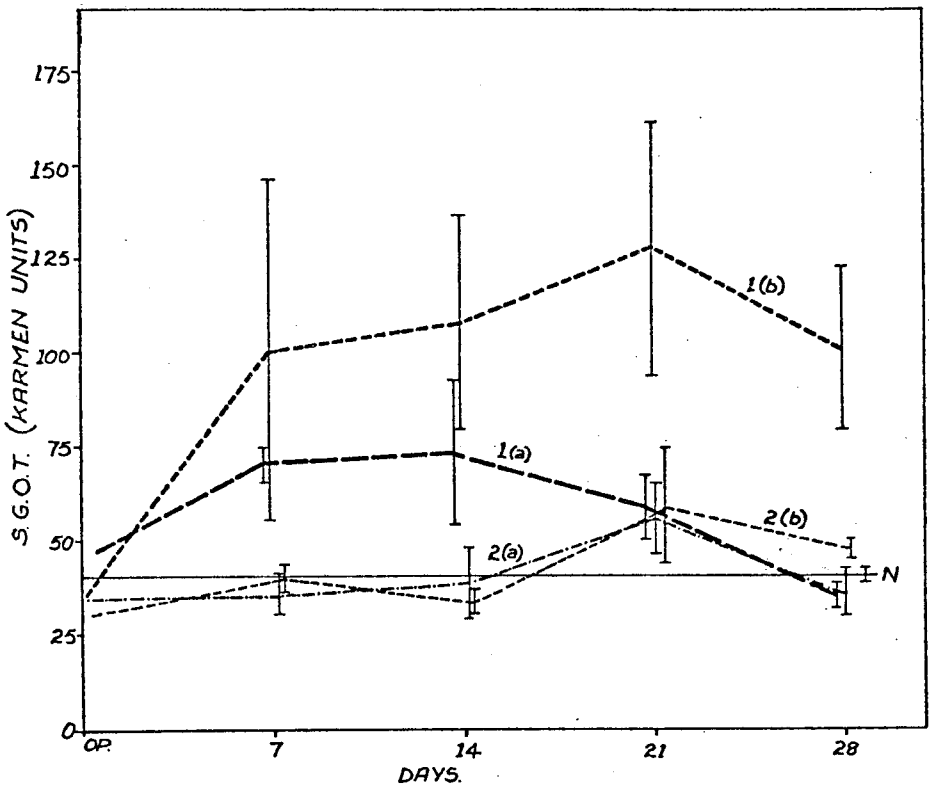
The mean pre-operative level of the group 2(a) animals was significantly higher than the laboratory normal and the mean pre-operative level of the other three experimental groups, as shown in Table 57d. The mean levels decreased significantly by day 7, and remained significantly lower than the pre-operative level, except on day 14. The mean level was significantly lower than group 1(b) on day 7 ($p < 0,025$), but thereafter the differences were not significant.

(c) Group 2(b)

There were no significant serial changes in this group - Tables 57b and 57c. On day 7, the mean level was significantly lower than that seen in groups 1(a) and 1(b), but thereafter the differences were insignificant.

Interpretation

There was no distinct, sustained pattern of changes in the postoperative period, and the mean alkaline phosphatase levels of the four main groups and 1 sub-group were comparable on days 14, 21 and 28, as shown in Table 57d on page 205F. The decrease in mean levels in groups 2(a) and 2(b) on day 7 may have been due to a decrease in bone alkaline phosphatase, as these animals were younger and smaller than most of the transplant recipients. The pattern of changes in liver alkaline phosphatase could not be determined, as the methods for iso-enzyme analysis had not been established in our laboratory at the time of doing the experiments.



GROUP 1(a) - Heterotopic allograft + cholecystocholecystostomy.
GROUP 1(b) - " " + cholecystojejunocholecystostomy.
GROUP 2(a) - End-to-side portacaval shunt.
GROUP 2(b) - Sham laparotomy.

GRAPH 58a SERIAL CHANGES IN MEAN SGOT LEVELS FOR THE GROUPS. (Mean ± 1 SEM)

Group	Day									
	OP	No	7	No	14	No	21	No	28	No
1a	45,00 ± 12,47	9	69,44 ± 16,23	9	72,83 ± 44,81	6	58,33 ± 27.29	6	35,00 ± 7,63	6
1b	35,76 ± 13,98	13	101,92 ± 156,46	13	107,72 ± 95,59	11	128,12 ± 90,27	8	101,00 ± 46,94	5
2a	34,23 ± 17,19	13	35,00 ± 21,79	14	37,91 ± 34,18	12	56,50 ± 32,25	10	35,62 ± 17,21	8
2b	30,00 ± 8,36	5	39,00 ± 8,60	5	33,00 ± 6,78	5	58,00 ± 34,43	5	47,50 ± 5,59	4
1a+1b	39,54 ± 14,13	22	88,63 ± 121,77	22	95,41 ± 83,06	17	98,21 ± 77,86	14	65,00 ± 45,97	11

Mean ± 1 SD No = Number of samples processed

TABLE 58b MEAN LEVELS FOR THE FOUR GROUPS AND ONE SUBGROUP

Group	Time Interval (Days)			
	0-7	0-14	0-21	0-28
1a	<0,005	-	-	-
1b	<0,02	<0,025	<0,001	<0,005
2a	-	-	<0,05	-
2b	-	-	-	<0,05
1a+1b	<0,001	<0,01	<0,001	-

TABLE 58c SIGNIFICANCE OF CHANGES IN MEAN LEVELS

p< = significance
- = no significant difference
0 = no or insufficient data

Groups	Date of Comparison (Days)				
	0-0	7-7	14-14	21-21	28-28
1a-1b	-	-	-	-	<0,025
1a-2a	-	<0,001	<0,05	-	-
1b-2a	-	<0,01	<0,02	<0,025	<0,02
1a-2b	<0,05	<0,005	-	-	-
1b-2b	-	-	-	-	-
2a-2b	-	-	-	-	-
1a+b-2a	-	<0,001	<0,02	-	-
1a+b-2b	-	-	-	-	-

TABLE 58d SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS

FIGURE 58 S G O T - SERIAL CHANGES IN MEAN LEVELS

(4) S.G.O.T.

The serial mean S.G.O.T. levels for the groups are illustrated and tabulated in Figure 58 on the opposite page. The standard deviations were large (Table 58b), and log transformation was used to calculate the mean and standard deviation prior to calculation of the significance of differences in mean levels, as explained in Appendix 4, page 342. The significances of changes and differences after log transformation are summarised in Tables 58c and 58d respectively.

(a) Groups 1(a) and 1(b)

There were no significant differences in the serial mean levels between groups 1(a) and 1(b), except on day 28 ($p < 0,025$), see Table 58d. The mean level in group 1(a) was significantly elevated on day 7, but not thereafter. Group 1(b) revealed a significant, sustained elevation, see Table 58c. The mean levels in group 1(a) were significantly higher than the levels in group 2(a) on days 7 and 14, and significantly higher than group 2(b) on day 7. Group 1(b) revealed a sustained, significant elevation above group 2(a), but not compared to group 2(b). The combined transplant group (1(a+b)), revealed mean levels significantly higher than those in group 2(a) on days 7 and 14. There were no significant differences compared to group 2(b), but the numbers are disparate.

(b) Group 2(a)

A significant elevation in the mean S.G.O.T. level was seen on day 21, but the pattern remained essentially normal during the postoperative period. There were no significant differences compared to group 2(b).

(c) Group 2(b)

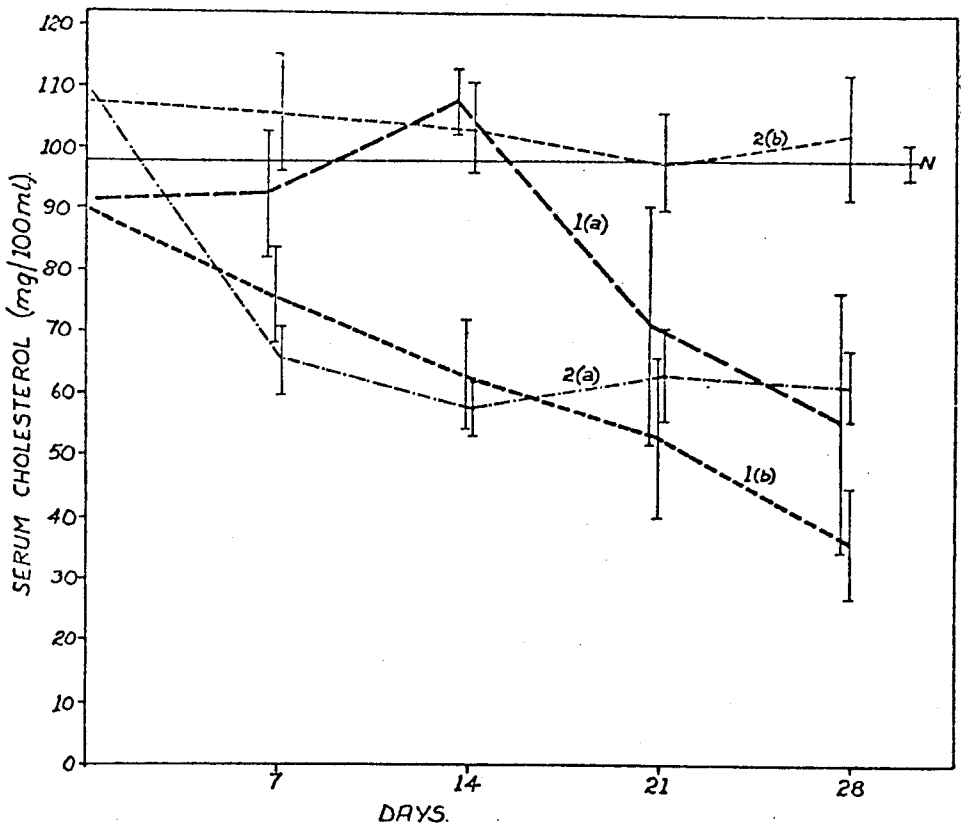
A significant elevation in the mean S.G.O.T. level was seen on day 21, while the remaining mean levels did not vary from the pre-operative normal.

Interpretation

The postoperative changes in mean S.G.O.T. levels in groups 1(a) and 1(b) were comparable except on day 28, when the group 1(b) animals exhibited mean values significantly higher than those seen in group 1(a).

The individual transplant groups 1(a) and 1(b), and the combined transplant group (1(a+b)), revealed a different pattern of mean S.G.O.T. levels in the postoperative period, when compared to the mean levels in groups 2(a) and 2(b). The range of postoperative levels varied enormously in individual animals, and also between animals in all the groups, as can be seen in the case histories on pages 269-315.

The significance of the changes seen in the levels of S.G.O.T. will be discussed in more detail in Part III of this presentation.



Group 1(a) - Heterotopic allograft + cholecystocholecystostomy.
Group 1(b) - " " + cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

GRAPH 59a SERIAL CHANGES IN MEAN CHOLESTEROL LEVELS FOR THE GROUPS. (Mean ± 1 SEM)

Group	Day									
	OP	No	7	No	14	No	21	No	28	No
1a	90,85 ± 20,97	7	92,00 ± 28,20	7	107,50 ± 11,77	6	71,40 ± 38,22	5	55,50 ± 43,72	6
1b	89,10 ± 24,91	10	75,41 ± 21,37	12	62,54 ± 29,56	11	52,85 ± 33,63	7	36,25 ± 15,80	4
2a	109,07 ± 22,11	14	65,18 ± 19,14	11	57,50 ± 16,38	12	63,00 ± 22,91	9	61,57 ± 14,62	7
2b	107,40 ± 16,24	5	105,40 ± 20,24	5	103,40 ± 15,56	5	97,40 ± 13,41	5	102,5 ± 17,52	4
1a+1b	89,82 ± 23,33	17	81,52 ± 25,40	19	78,41 ± 32,80	17	60,58 ± 36,77	12	47,80 ± 40,16	10

Mean ± 1 SD No = Number of samples processed

TABLE 59b MEAN LEVELS FOR THE FOUR GROUPS AND ONE SUBGROUP

Group	Time Interval (Days)			
	0-7	0-14	0-21	0-28
1a	-	-	-	-
1b	-	-	0,025	0,005
2a	0,001	0,001	0,001	0,001
2b	-	-	-	-
1a+1b	-	-	0,02	0,005

TABLE 59c SIGNIFICANCE OF CHANGES IN MEAN LEVELS

p< = significance
- = no significant difference
0 = no or insufficient data

Groups	Date of Comparison (Days)				
	0-0	7-7	14-14	21-21	28-28
1a-1b	-	-	0,005	-	-
1a-2a	-	0,05	0,001	-	-
1b-2a	-	-	-	-	0,025
1a-2b	-	-	-	-	-
1b-2b	-	0,02	0,02	0,025	0,005
2a-2b	-	0,005	0,001	0,02	0,005
1a+b-2a	0,05	-	-	-	-
1a+b-2b	-	-	-	-	0,025

TABLE 59d SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS

FIGURE 59 CHOLESTEROL - SERIAL CHANGES IN MEAN LEVELS

(5) SERUM CHOLESTEROL

The serial mean cholesterol levels for the groups are illustrated and tabulated in Figure 59 on the opposite page. The significances of changes and differences in mean levels are summarised in Tables 59c and 59d respectively.

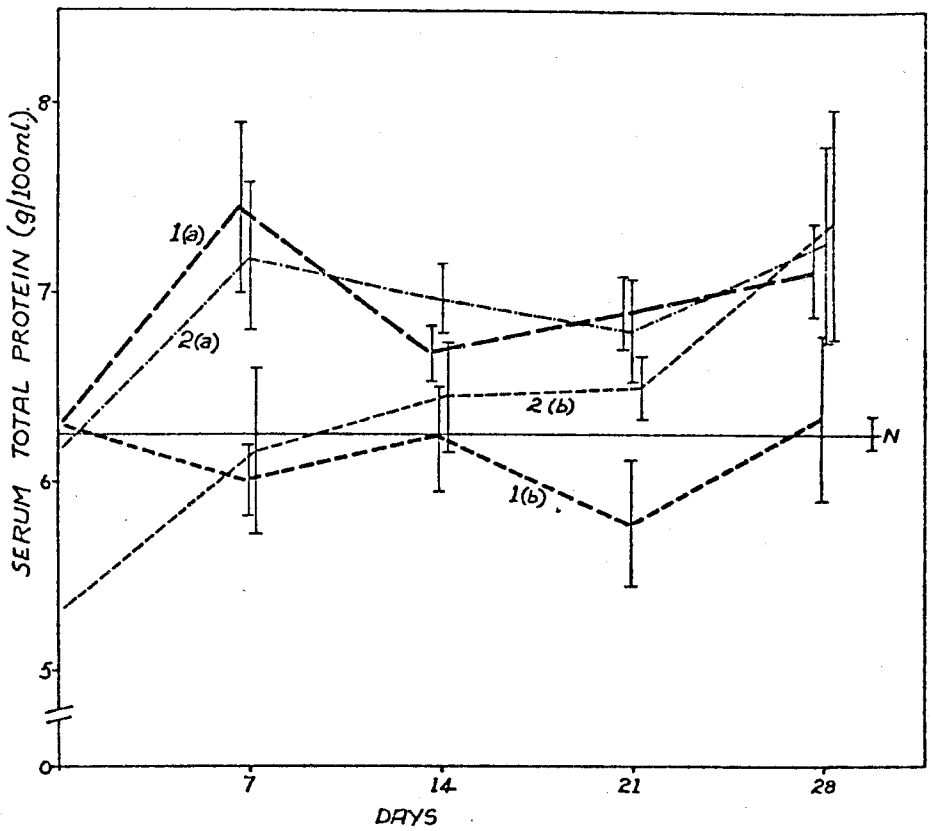
(a) Groups 1(a) and 1(b)

The mean levels in group 1(a) remained static until day 14, and then gradually fell, but the fall was not significant. Group 1(b) animals demonstrated a slow, progressive decrease in mean levels, significant on days 21 and 28. There were no significant differences between the mean levels of groups 1(a) and 1(b), except on day 14. The mean levels of the combined transplant group (1(a+b)), revealed a sustained fall, significant on days 21 and 28 - see Tables 59b and 59c.

Group 1(a) followed the pattern of group 2(b) animals for the first 14 days,and then fell to levels closer to group 2(a) animals. The mean levels of group 1(b) decreased in a manner roughly parallel to the group 2(a) animals, except for day 28 when the mean level was significantly lower.

(b) Group 2(a)

There was a progressive, highly significant decrease in mean cholesterol levels in the postoperative period. The mean levels were significantly lower than group 2(b) animals throughout the postoperative period.



Group 1(a) - Heterotopic allograft + cholecystocholecystostomy.
Group 1(b) - " " + cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

GRAPH 60a SERIAL CHANGES IN MEAN TOTAL PROTEIN LEVELS FOR THE GROUPS. (Mean ± 1 SEM)

Group	Day									
	OP	No	7	No	14	No	21	No	28	No
1a	6,31 ± 0,59	8	7,45 ± 1,07	7	6,68 ± 0,29	6	6,88 ± 0,42	6	7,11 ± 0,60	6
1b	6,31 ± 0,49	8	6,02 ± 0,71	12	6,23 ± 0,92	11	5,78 ± 0,84	7	6,33 ± 0,61	3
2a	6,18 ± 0,74	10	7,18 ± 1,18	9	6,96 ± 0,59	12	6,80 ± 0,75	8	7,26 ± 1,08	5
2b	5,32 ± 0,31	4	6,16 ± 0,94	5	6,46 ± 0,61	5	6,50 ± 0,36	5	7,37 ± 1,07	4
1a+1b	6,31 ± 0,54	16	6,55 ± 1,10	19	6,39 ± 0,79	17	6,29 ± 0,87	13	6,85 ± 0,71	9

Mean ± 1 SD No = Number of samples processed

TABLE 60b MEAN LEVELS FOR THE FOUR GROUPS AND ONE SUBGROUP

Group	Time Interval (Days)			
	0-7	0-14	0-21	0-28
1a	<0,025	-	-	0,05
1b	-	-	-	-
2a	<0,01	<0,02	-	<0,05
2b	-	<0,02	<0,005	<0,02
1a+1b	-	-	-	<0,05

TABLE 60c SIGNIFICANCE OF CHANGES IN MEAN LEVELS

p< = significance
- = no significant difference
0 = no or insufficient data

Groups	Date of Comparison (Days)				
	0-0	7-7	14-14	21-21	28-28
1a-1b	-	<0,005	-	<0,02	-
1a-2a	-	-	-	-	-
1b-2a	-	<0,02	<0,05	<0,025	-
1a-2b	<0,02	-	-	-	-
1b-2b	<0,005	-	-	-	-
2a-2b	<0,05	-	-	-	-
1a+b-2a	-	-	<0,05	-	-
1a+b-2b	<0,005	-	-	-	-

TABLE 60d SIGNIFICANCE OF DIFFERENCES

FIGURE 60 TOTAL PROTEIN - SERIAL CHANGES IN MEAN LEVELS

(c) Group 2(b)

The mean cholesterol levels remained within normal limits throughout the period of study.

Interpretation

Two distinct patterns of changes emerged in the mean post-operative cholesterol levels. Group 2(b) animals (sham laparotomy) revealed no significant changes from normal. Groups 1(a), 1(b) and 2(b) all showed a progressive decrease in mean cholesterol levels, significant in all three groups from 21 days onwards. The decrease in mean levels was more rapid in group 2(a) animals.

The pattern of postoperative changes in the transplanted animals thus roughly paralleled the patterns seen in the porta-caval-shunted animals, and were distinctly different from the pattern seen in the sham operated group.

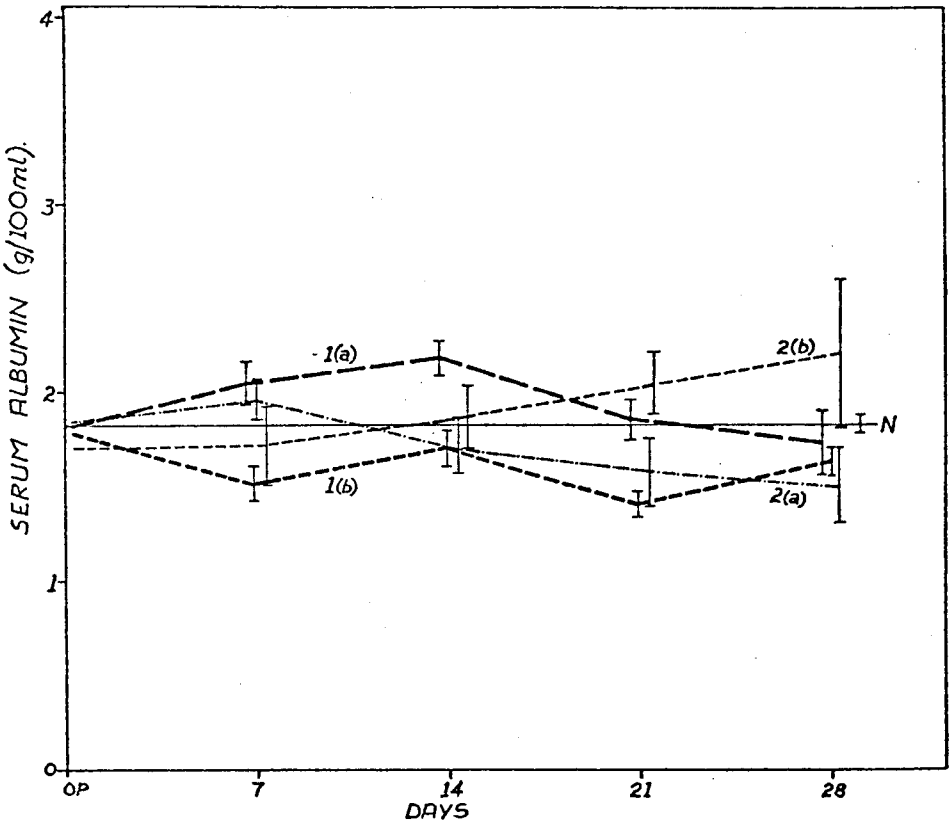
(6) SERUM TOTAL PROTEIN

The serial mean total protein levels for the groups are illustrated and tabulated in Figure 60 on the opposite page. The significances of changes and differences are summarised in Tables 60c and 60d respectively.

(a) Groups 1(a) and 1(b)

There were significant differences between groups 1(a) and 1(b) on days 7 and 21, as shown in Table 60d.

Group 1(a) showed a significant rise on days 7 and 28,



Group 1(a) - Heterotopic allograft + cholecystocholecystostomy.
Group 1(b) - " " + cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

GRAPH 61a SERIAL CHANGES IN MEAN ALBUMIN LEVELS FOR THE GROUPS. (Mean ± 1 SEM)

Group	Day									
	OP	No	7	No	14	No	21	No	28	No
1a	1,82 ± 0,32	8	2,04 ± 0,28	7	2,00 ± 0,24	6	1,85 ± 0,26	6	1,73 ± 0,47	6
1b	1,78 ± 0,16	8	1,51 ± 0,33	12	1,70 ± 0,36	11	1,41 ± 0,20	7	1,63 ± 0,12	3
2a	1,85 ± 0,40	10	1,91 ± 0,35	9	1,70 ± 0,54	12	1,58 ± 0,51	8	1,51 ± 0,37	4
2b	1,70 ± 0,33	4	1,72 ± 0,39	4	1,86 ± 0,37	5	2,04 ± 0,37	5	2,20 ± 0,69	4
1a+1b	1,80 ± 0,25	16	1,71 ± 0,40	19	1,80 ± 0,35	17	1,61 ± 0,32	13	1,70 ± 0,39	9

Mean ± 1 SD No = Number of samples processed

TABLE 61b MEAN LEVELS FOR THE FOUR GROUPS AND ONE SUBGROUP

Group	Time Interval (Days)			
	0-7	0-14	0-21	0-28
1a	-	-	-	-
1b	<0,05	-	<0,005	-
2a	-	-	-	-
2b	-	-	-	-
1a+1b	-	-	-	-

TABLE 61c SIGNIFICANCE OF CHANGES IN MEAN LEVELS

p< = significance
\- = no significant difference
0 = no or insufficient data

Groups	Date of Comparison (Days)				
	0-0	7-7	14-14	21-21	28-28
1a-1b	-	<0,025	-	<0,01	-
1a-2a	-	-	-	-	-
1b-2a	-	<0,02	-	-	-
1a-2b	<0,01	-	-	<0,025	-
1b-2b	-	-	-	<0,005	-
2a-2b	-	-	-	-	-
1a+b-2a	-	-	-	-	-
1a+b-2b	-	-	-	<0,05	-

TABLE 61d SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS

FIGURE 61 ALBUMIN - SERIAL CHANGES IN MEAN LEVELS

the increase being due to significant increases in globulin. There were no significant differences between the mean levels of groups 1(a), 2(a) and 2(b) during the postoperative period. Group 1(b) animals revealed no significant changes in mean levels, and were significantly lower than group 2(a) throughout the postoperative period. The combined transplant group (1(a+b)), revealed a pattern similar to that seen in groups 2(a) and 2(b).

(b) Group 2(a)

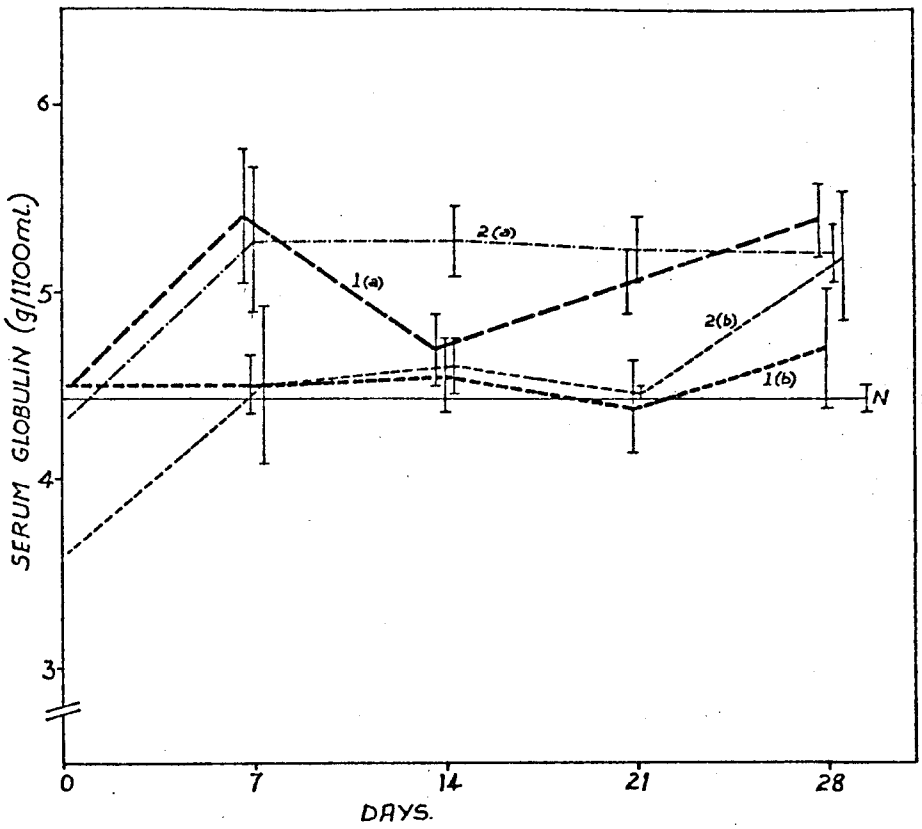
There was a sustained, significant elevation in mean total protein levels in the postoperative period. This reflected an increase in globulin.

(c) Group 2(b)

There was a sustained, significant elevation in mean total protein levels compared to the pre-operative level. On day 28 the mean level was significantly elevated above the laboratory normal. These increases reflected the sum of the increases in both albumin and globulin.

(7) SERUM ALBUMIN

The serial mean albumin levels are illustrated and tabulated in Figure 61 on the opposite page. The significances of changes and differences are summarised in Tables 61c and 61d respectively.



Group 1(a) - Heterotopic allograft + cholecystocholecystostomy
Group 1(b) - " " + cholecystojejunocholecystostomy
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

GRAPH 62a SERIAL CHANGES IN MEAN GLOBULIN LEVELS FOR THE GROUPS. (Mean ± 1 SEM)

Group	Day									
	OP	No	7	No	14	No	21	No	28	No
1a	4,48 ± 0,50	8	5,41 ± 0,92	7	4,68 ± 0,48	6	5,03 ± 0,42	6	5,38 ± 0,45	6
1b	4,52 ± 0,53	8	4,50 ± 0,57	12	4,53 ± 0,64	11	4,37 ± 0,68	7	4,70 ± 0,48	3
2a	4,33 ± 0,57	10	5,27 ± 1,20	9	5,26 ± 0,66	12	5,21 ± 0,45	8	5,22 ± 0,31	4
2b	3,62 ± 0,22	4	4,50 ± 0,94	4	4,60 ± 0,32	5	4,46 ± 0,10	5	5,17 ± 0,64	4
1a+1b	4,50 ± 0,51	16	4,84 ± 0,84	19	4,58 ± 0,59	17	4,67 ± 0,66	13	5,15 ± 0,56	9

Mean ± 1 SD No = Number of samples processed

TABLE 62b MEAN LEVELS FOR THE FOUR GROUPS AND ONE SUBGROUP

Group	Time Interval (Days)			
	0-7	0-14	0-21	0-28
1a	<0,05	-	-	<0,005
1b	-	-	-	-
2a	<0,05	<0,005	<0,005	<0,02
2b	-	<0,005	<0,001	<0,01
1a+1b	-	-	-	<0,01

TABLE 62c SIGNIFICANCE OF CHANGES IN MEAN LEVELS

p< = significance
- = no significant difference
0 = no or insufficient data

Groups	Date of Comparison (Days)				
	0-0	7-7	14-14	21-21	28-28
1a-1b	-	<0,02	-	-	-
1a-2a	-	-	-	-	-
1b-2a	-	-	<0,02	<0,02	-
1a-2b	<0,01	-	-	<0,025	-
1b-2b	<0,01	-	-	-	-
2a-2b	<0,05	-	-	<0,005	-
1a+b-2a	-	-	<0,01	-	-
1a+b-2b	<0,005	-	-	-	-

TABLE 62d SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS

FIGURE 62 GLOBULIN - SERIAL CHANGES IN MEAN LEVELS

(a) Groups 1(a) and 1(b)

There were significant differences between groups 1(a) and 1(b) on days 7 and 21. The mean levels in group 1(a) did not alter significantly during the postoperative period, and did not differ significantly from the values seen in group 2(a). Group 1(b) demonstrated a significant decrease on day 21, but not thereafter. The combined transplant group (1(a+b)), revealed a pattern similar to that seen in groups 2(a) and 2(b).

(b) Group 2(a)

There were no significantly different mean albumin levels during the postoperative period.

(c) Group 2(b)

There was a progressive elevation in mean levels, but the differences were not significant.

(8) SERUM GLOBULIN

The serial mean globulin levels are illustrated and tabulated in Figure 62 on the opposite page. The significances of changes and differences in mean levels are summarised in Tables 62c and 62d respectively.

(a) Groups 1(a) and 1(b)

There was a significant difference in mean levels between groups 1(a) and 1(b) on day 7. The mean levels in group 1(a) were significantly elevated on days 7 and

28, and were not significantly different from the mean levels in group 2(a) during the postoperative period. Group 1(b) revealed no significant changes during the postoperative period. The combined transplant group (1(a+b)), revealed an elevation in globulin on day 28 similar to that seen in groups 2(a) and 2(b).

(b) Group 2(a)

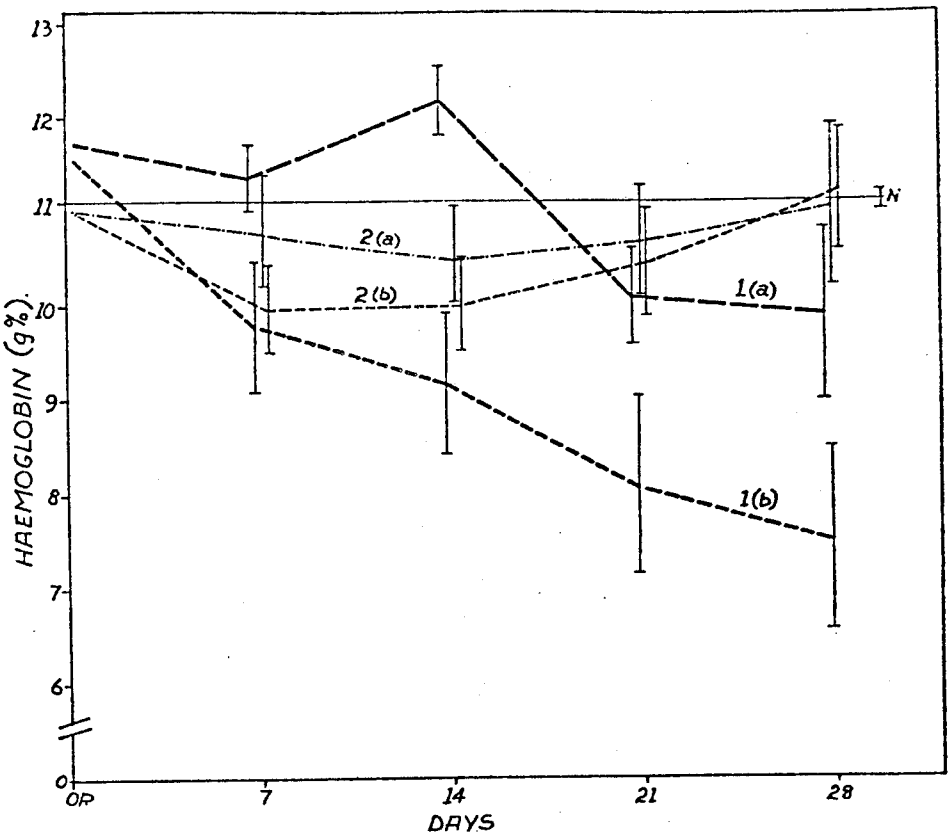
A significant, sustained elevation in mean globulin levels was seen throughout the postoperative period.

(c) Group 2(b)

The mean globulin levels in group 2(b) increased significantly in the postoperative period.

Interpretation of changes in Serum Proteins

Group 1(a) animals closely followed the pattern seen in group 2(a), and revealed significantly higher levels than group 1(b). The group 1(a) donor livers were shown to have, in general, an inferior quality compared to group 1(b) livers. Group 1(b) tended to follow the pattern of group 2(b) animals. All 4 groups revealed some elevation in globulin by day 28.



Group 1(a) - Heterotopic allograft + cholecystocholecystostomy.
Group 1(b) - " " + cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

GRAPH 63a SERIAL CHANGES IN MEAN HAEMOGLOBIN LEVELS FOR THE GROUPS. (Mean ± 1 SEM)

Group	Day									
	OP	No	7	No	14	No	21	No	28	No
1a	11,73 ± 1,49	9	11,34 ± 1,08	9	12,18 ± 0,78	6	10,08 ± 1,26	6	9,91 ± 2,15	6
1b	11,53 ± 1,34	12	9,78 ± 2,54	13	9,18 ± 2,55	11	8,08 ± 2,69	8	7,50 ± 1,96	5
2a	10,93 ± 1,19	11	10,77 ± 2,09	12	10,49 ± 1,67	10	10,69 ± 1,91	10	11,02 ± 2,39	8
2b	11,00 ± 1,04	5	9,96 ± 1,04	5	10,00 ± 1,02	5	10,44 ± 1,26	5	11,22 ± 1,19	4
1a+1b	11,61 ± 1,41	21	10,42 ± 2,21	22	10,24 ± 2,55	17	8,94 ± 2,40	14	8,81 ± 2,39	11

Mean ± 1 SD No = Number of samples processed

TABLE 63b MEAN LEVELS FOR THE FOUR GROUPS AND ONE SUBGROUP

Group	Time Interval (Days)			
	0-7	0-14	0-21	0-28
1a	-	-	<0,05	-
1b	<0,05	<0,02	<0,005	<0,001
2a	-	-	-	-
2b	-	-	-	-
1a+1b	<0,05	<0,05	<0,001	<0,001

TABLE 63c SIGNIFICANCE OF CHANGES IN MEAN LEVELS

p< = significance
\- = no significant difference
0 = no or insufficient data

Groups	Date of Comparison (Days)				
	0-0	7-7	14-14	21-21	28-28
1a-1b	-	-	<0,02	-	-
1a-2a	-	-	<0,05	-	-
1b-2a	-	-	-	<0,05	<0,02
1a-2b	-	-	<0,005	-	-
1b-2b	-	-	-	-	<0,02
2a-2b	-	-	-	-	-
1a+b-2a	-	-	-	-	-
1a+b-2b	-	-	-	-	-

TABLE 63d SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS

FIGURE 63 HAEMOGLOBIN - SERIAL CHANGES IN MEAN LEVELS

(9) HAEMOGLOBIN

The serial mean haemoglobin levels of the groups are illustrated and tabulated in Figure 63 on the opposite page. The significances of changes and differences in mean levels are summarised in Tables 63c and 63d respectively.

(a) Groups 1(a) and 1(b)

Groups 1(a) and 1(b) exhibited significantly different mean levels on day 14 only. After day 14, there was a progressive fall in mean haemoglobin levels in group 1(a) animals, being significant on day 21. Group 1(b) animals demonstrated a progressive, significant depression in levels from day 7 onwards. The combined transplant group (1(a+b)), showed a significant sustained fall in mean levels throughout the postoperative period, (Tables 63b and 63c).

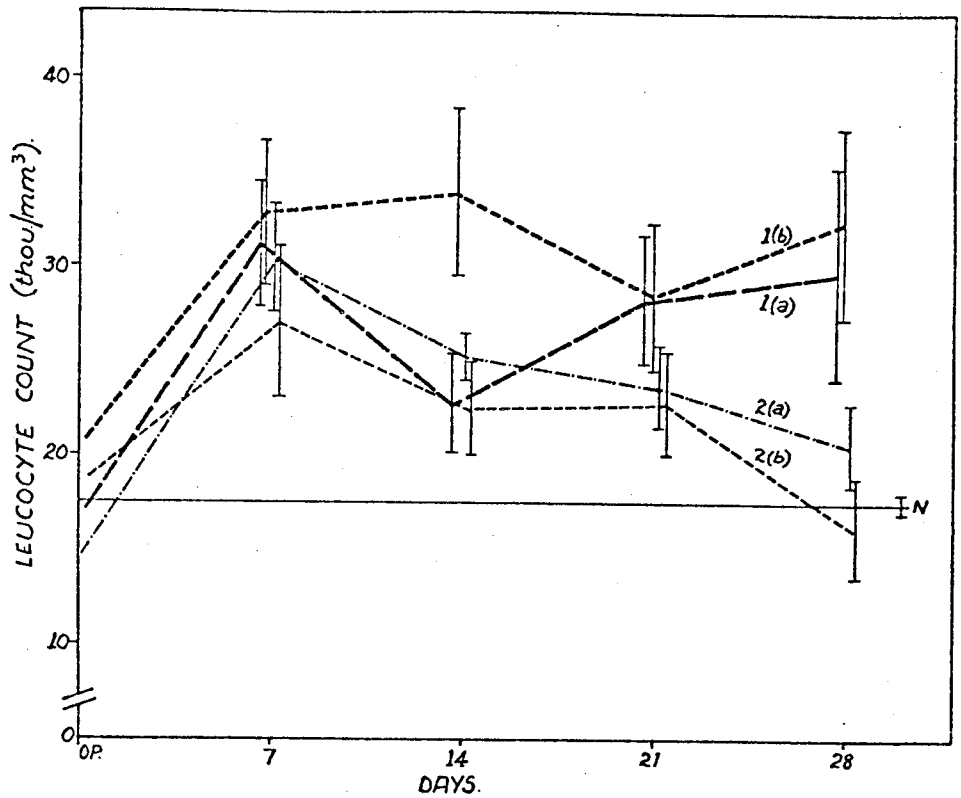
(b) Group 2(a)

There were no significant changes in mean haemoglobin levels.

(c) Group 2(b)

The mean haemoglobin levels did not change significantly, and the pattern was similar to that seen in group 2(a).

Groups 2(a) and 2(b) were not significantly different from the combined group 1(a+b).



Group 1(a) - Heterotopic allograft + cholecystocholecystostomy.
Group 1(b) - " " + cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham Laparotomy.

GRAPH 64a SERIAL CHANGES IN MEAN LEUCOCYTE LEVELS FOR THE GROUPS. (Mean ± 1 SEM)

Group	Day									
	OP	No	7	No	14	No	21	No	28	No
1a	16,95 ± 3,57	9	31,20 ± 9,92	9	22,66 ± 6,45	6	28,31 ± 8,30	6	32,95 ± 13,16	6
1b	20,51 ± 5,94	12	32,08 ± 12,96	12	33,71 ± 14,52	11	28,21 ± 11,04	8	32,20 ± 9,31	4
2a	15,04 ± 4,20	12	30,45 ± 10,33	12	25,26 ± 4,21	12	23,66 ± 7,44	10	20,56 ± 5,90	8
2b	18,80 ± 2,98	5	27,04 ± 8,28	5	22,48 ± 4,72	5	22,76 ± 5,21	5	16,02 ± 5,00	4
1a+1b	18,99 ± 5,36	21	31,70 ± 11,76	21	29,81 ± 13,38	17	28,25 ± 9,96	14	32,65 ± 11,77	10

Mean ± 1 SD No = Number of samples processed

TABLE 64b MEAN LEVELS FOR THE FOUR GROUPS AND ONE SUBGROUP

Group	Time Interval (Days)			
	0-7	0-14	0-21	0-28
1a	<0,005	-	<0,01	<0,02
1b	<0,02	<0,02	-	<0,01
2a	<0,001	<0,001	<0,005	<0,025
2b	-	-	-	-
1a+1b	<0,001	<0,005	<0,005	<0,001

TABLE 64c SIGNIFICANCE OF CHANGES IN MEAN LEVELS

p< = significance
- = no significant difference
0 = no or insufficient data

Groups	Date of Comparison (Days)				
	0-0	7-7	14-14	21-21	28-28
1a-1b	-	-	-	-	-
1a-2a	-	-	-	-	<0,05
1b-2a	<0,02	-	-	-	<0,025
1a-2b	-	-	-	-	<0,05
1b-2b	-	-	-	-	<0,025
2a-2b	-	-	-	-	-
1a+b-2a	<0,05	-	-	-	<0,02
1a+b-2b	-	-	-	-	<0,025

TABLE 64d SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS

FIGURE 64 LEUCOCYTES - SERIAL CHANGES IN MEAN LEVELS

Interpretation

Anaemia was seen in individual animals from all four groups. The degree of anaemia was most marked in group 1(b) animals. The high incidence of gastric ulcers has been discussed in Section I on page 114. Haemorrhage from the gastric ulcers contributed to the development of anaemia in the four groups, but other factors also played a part. In some of the transplanted animals, blood was entrapped in the donor livers; several animals had severe intra-operative haemorrhage which may not have been replaced fully, and the high incidence of sepsis may also have contributed to the anaemia.

There were no sustained, highly significant differences in mean haemoglobin levels during the postoperative period between groups 1(a) and 1(b), indicating that the two groups were comparable.

(10) LEUCOCYTE COUNT

The serial mean leucocyte levels for the groups are illustrated and tabulated in Figure 64 on the opposite page. The significances of changes and differences in mean levels are summarised in Tables 64c and 64d respectively.

(a) Groups 1(a) and 1(b)

There were no significant differences in mean levels between groups 1(a) and 1(b) during the postoperative period. Both groups demonstrated significant elevations during the postoperative period, when compared with the pre-operative normal. The postoperative elevations re-

vealed no significant differences compared with the elevations seen in groups 2(a) and 2(b) on days 7, 14 and 21, (see Tables 64b and 64d).

(b) Group 2(a)

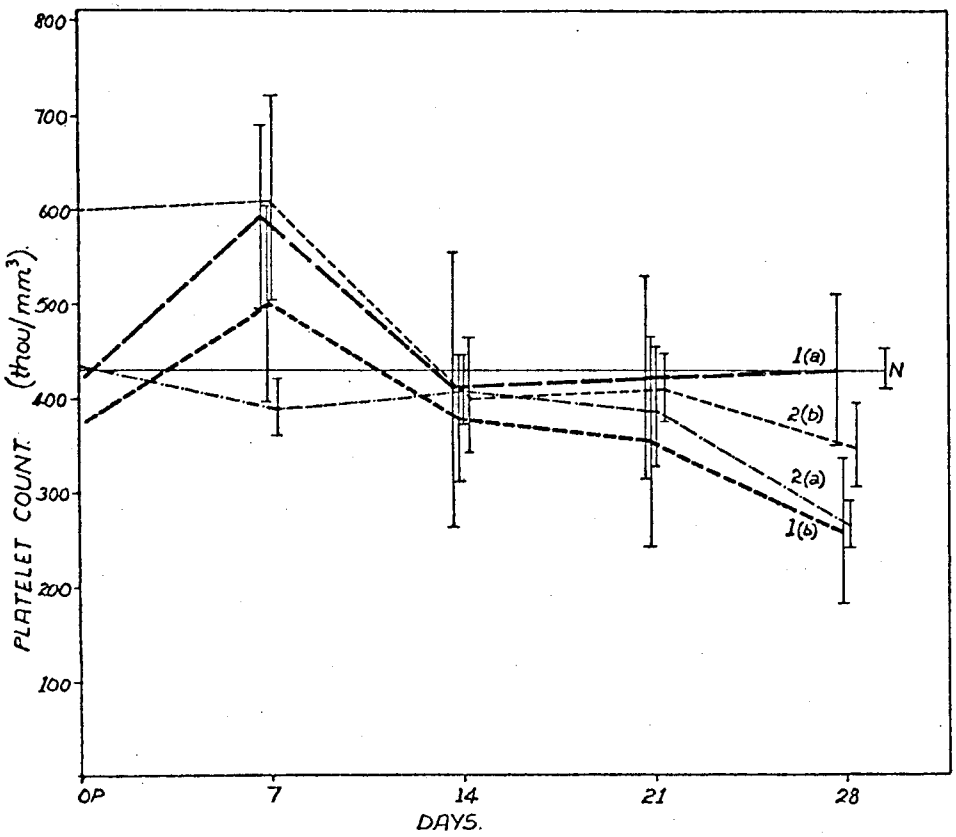
Group 2(a) animals revealed significant, sustained serial leucocyte elevations above the pre-operative normal, but on day 28, the mean levels were significantly lower than those seen in groups 1(a) and 1(b).

(c) Group 2(b)

The mean leucocyte levels in group 2(b) were elevated on days 7, 14 and 21, but not significantly so. On day 28 they were significantly lower than the mean levels in groups 1(a) and 1(b).

Interpretation

All four groups of animals exhibited leucocytosis during the postoperative period. This was significant in groups 1(a), 1(b) and 2(a). The leucocytosis in these three groups was to be expected, as the animals had all had an operation, and there was a high incidence of wound sepsis and pulmonary infection in the three groups. Group 2(b) animals had the least operative stress and revealed less sepsis and pulmonary infection, probably accounting for the lower mean levels and return to normal by day 28.



Group 1(a) - Heterotopic allograft + cholecystocholecystostomy.
Group 1(b) - " " + cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

GRAPH 65a SERIAL CHANGES IN MEAN PLATELET COUNT LEVELS FOR THE GROUPS. (Mean ± 1 SEM)

Group	Day									
	OP	No	7	No	14	No	21	No	28	No
1a	420,77 ± 126,42	9	592,37 ± 274,06	8	412,50 ± 336,37	6	422,83 ± 246,90	6	431,66 ± 186,73	6
1b	375,75 ± 156,55	12	497,15 ± 377,09	13	378,09 ± 224,42	11	353,25 ± 307,52	8	256,40 ± 160,01	5
2a	434,91 ± 147,60	12	391,23 ± 113,94	13	411,50 ± 118,05	10	387,10 ± 202,56	10	265,25 ± 71,48	8
2b	600,00 ± 163,55	5	610,00 ± 222,26	5	404,16 ± 149,59	5	410,00 ± 73,48	5	346,25 ± 75,36	4
1a+1b	395,04 ± 146,11	21	533,42 ± 344,64	21	390,23 ± 269,80	17	383,07 ± 285,22	14	352,00 ± 195,63	11

Mean ± 1 SD No = Number of samples processed

TABLE 65b MEAN LEVELS FOR THE FOUR GROUPS AND ONE SUBGROUP

FIGURE 65 PLATELETS - SERIAL CHANGES IN MEAN LEVELS

(11) PLATELET COUNT

The serial mean platelet count levels are illustrated and tabulated in Figure 65 on the opposite page. The standard deviations were extremely large, and did not allow an accurate comparative statistical analysis. The means and standard deviations were recalculated using log transformation as described on page 342 but the resultant standard deviations were still too large to permit comparative analysis.

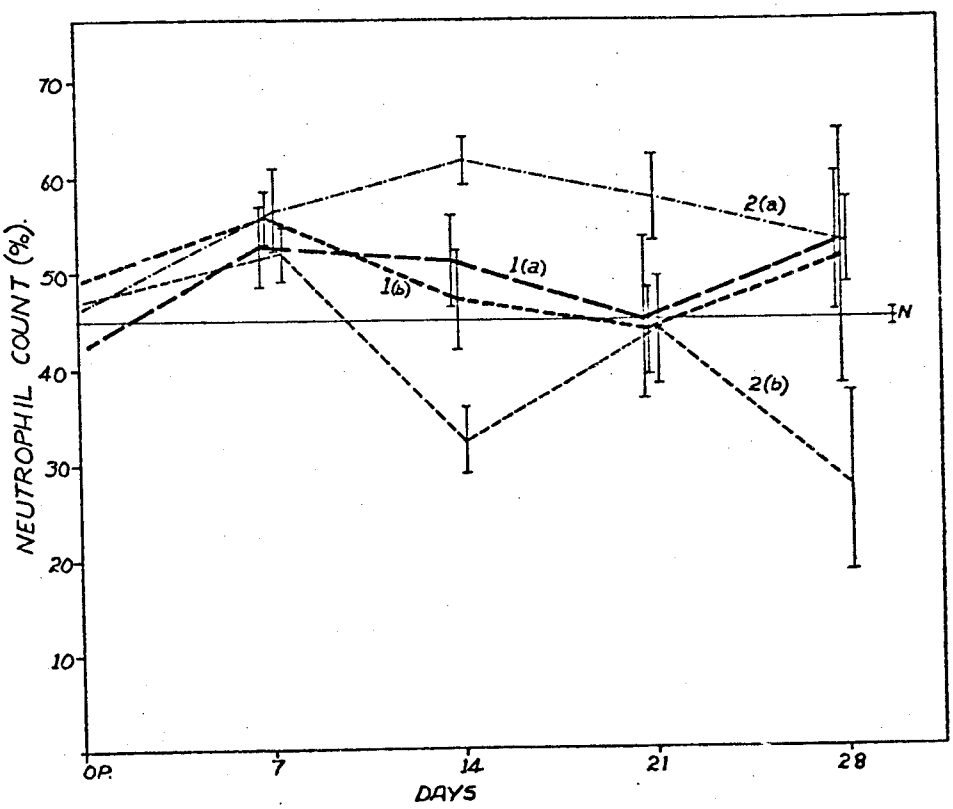
The pattern of changes in mean platelet levels is illustrated in graph 65a opposite. There were no distinct, sustained patterns of differences between the mean values of groups 1(a) and 1(b), nor between the mean levels of groups 1(a) and 1(b) compared to the control groups 2(a) and 2(b).

(12) NEUTROPHILS

The serial mean percentage neutrophil levels for the groups are illustrated and tabulated in Figure 66 on the next page. The significances of changes and differences in mean levels are summarised in Tables 66c and 66d respectively.

(a) Groups 1(a) and 1(b)

There were no significant differences between mean percentage neutrophil levels in groups 1(a) and 1(b) at any stage. Neither group 1(a) nor group 1(b) revealed changes significantly different from normal during the postoperative period. Groups 1(a) and 1(b) revealed no significant differences in mean levels on



Group 1(a) - Heterotopic allograft + cholecystocholecystostomy.
Group 1(b) - " " + cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

GRAPH 66a SERIAL CHANGES IN MEAN PERCENTAGE NEUTROPHIL LEVELS FOR THE GROUPS. (Mean ± 1 SEM)

Group	Day									
	OP	No	7	No	14	No	21	No	28	No
1a	42,44 ± 15,84	9	52,66 ± 13,14	9	51,00 ± 11,22	6	44,66 ± 19,57	6	52,83 ± 16,96	6
1b	49,16 ± 13,12	12	56,07 ± 10,45	13	47,36 ± 18,09	11	43,75 ± 13,05	8	51,40 ± 26,98	5
2a	46,46 ± 16,40	12	56,50 ± 15,15	12	61,66 ± 8,26	12	57,90 ± 13,92	10	52,75 ± 12,90	8
2b	57,00 ± 12,30	4	52,80 ± 6,24	5	32,40 ± 7,44	5	44,00 ± 10,41	4	27,75 ± 17,06	4
1a+1b	46,28 ± 14,73	21	54,68 ± 11,75	22	48,64 ± 16,10	17	44,14 ± 16,18	14	52,18 ± 22,10	11

Mean ± 1 SD No = Number of samples processed

TABLE 66b MEAN LEVELS FOR THE FOUR GROUPS AND ONE SUBGROUP

Group	Time Interval (Days)			
	0-7	0-14	0-21	0-28
1a	-	-	-	-
1b	-	-	-	-
2a	-	<0,01	-	-
2b	-	<0,01	-	<0,05
1a+1b	<0,05	-	-	-

TABLE 66c SIGNIFICANCE OF CHANGES IN MEAN LEVELS

p< = significance
- = no significant difference
0 = no or insufficient data

Groups	Date of Comparison (Days)				
	0-0	7-7	14-14	21-21	28-28
1a-1b	-	-	-	-	-
1a-2a	-	-	<0,05	-	-
1b-2a	-	-	<0,05	<0,05	-
1a-2b	-	-	<0,02	-	-
1b-2b	-	-	-	-	-
2a-2b	-	-	<0,001	-	<0,02
1a+b-2a	-	-	<0,02	<0,05	-
1a+b-2b	-	-	<0,025	-	-

TABLE 66d SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS

FIGURE 66 NEUTROPHILS - SERIAL CHANGES IN MEAN LEVELS

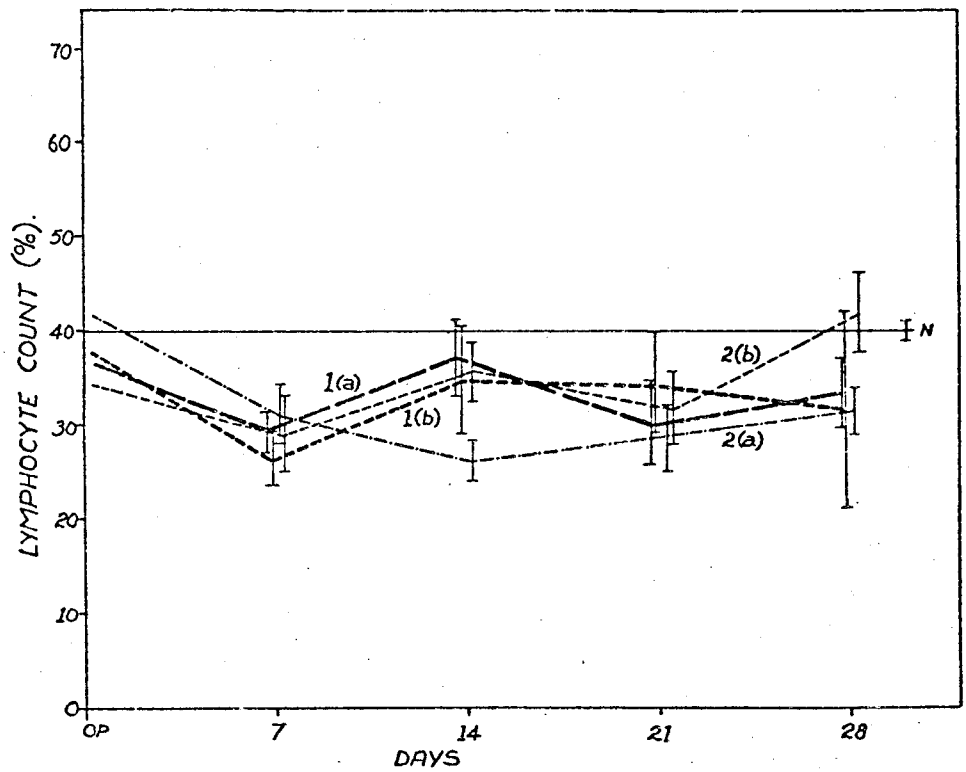
days 7 and 28 when compared to groups 2(a) and 2(b), and only minor significant differences on days 14 and 21.

(b) Group 2(a)

A significant elevation in mean neutrophil levels was seen on day 14 ($p < 0,01$). The elevation was significantly above normal, and above the mean levels in groups 1(a), 1(b), 2(b) and 1(a+b). The increase in mean percentage was accompanied by a depression in mean percentage lymphocytes of the same dimension. The mean levels were not significantly different from normal during the remainder of the postoperative period.

(c) Group 2(b)

There was significant depression in mean percentage neutrophil levels on days 14 and 28. These decreases in mean percentage levels were compensated for by gross elevations in mean monocyte levels. No significance can be read into these two dramatic decreases, and it is thought that the changes might be due to observer error as these unusual levels corresponded with the annual leave of the normal haematology technologist, and it appears that her replacement may have had difficulty in distinguishing these two types of cells.



Group 1(a) - Heterotopic allograft + cholecystocholecystostomy.
Group 1(b) - " " + cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b) - Sham laparotomy.

GRAPH 67a SERIAL CHANGES IN MEAN PERCENTAGE LYMPHOCYTE LEVELS FOR THE GROUPS. (Mean ± 1 SEM)

Group	Day									
	OP	No	7	No	14	No	21	No	28	No
1a	36,66 ± 8,37	9	29,22 ± 7,00	9	37,16 ± 9,61	6	30,16 ± 10,47	6	33,33 ± 9,21	6
1b	37,66 ± 12,37	12	26,15 ± 11,16	13	34,72 ± 19,53	11	34,12 ± 14,69	8	31,60 ± 20,94	5
2a	41,91 ± 16,92	12	31,08 ± 11,84	12	26,16 ± 7,91	12	28,80 ± 10,60	10	31,50 ± 7,66	8
2b	34,75 ± 10,98	4	29,00 ± 8,71	5	35,60 ± 6,40	5	31,75 ± 7,39	4	42,00 ± 7,44	4
1a+1b	37,23 ± 10,85	21	27,40 ± 9,79	22	35,58 ± 16,75	17	32,42 ± 13,20	14	32,54 ± 15,69	11

Mean ± 1 SD No = Number of samples processed

TABLE 67b MEAN LEVELS FOR THE FOUR GROUPS AND ONE SUBGROUP

Group	Time Interval (Days)			
	0-7	0-14	0-21	0-28
1a	-	-	-	-
1b	<0,025	-	-	-
2a	-	<0,01	<0,05	-
2b	-	-	-	-
1a+1b	<0,005	-	-	-

TABLE 67c SIGNIFICANCE OF CHANGES IN MEAN LEVELS

p< = significance
- = no significant difference
0 = no or insufficient data

Groups	Date of Comparison (Days)				
	0-0	7-7	14-14	21-21	28-28
1a-1b	-	-	-	-	-
1a-2a	-	-	<0,02	-	-
1b-2a	-	-	-	-	-
1a-2b	-	-	-	-	-
1b-2b	-	-	-	-	-
2a-2b	-	-	<0,05	-	0,05
1a+b-2a	-	-	-	-	-
1a+b-2b	-	-	-	-	-

TABLE 67d SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS

FIGURE 67 LYMPHOCYTES - SERIAL CHANGES IN MEAN LEVELS

Interpretation

There was no sustained, highly significant pattern of differences in mean percentage neutrophil levels between any of the groups in the postoperative period.

(13) LYMPHOCYTES

The serial mean percentage lymphocyte levels of the groups are illustrated and tabulated in Figure 67 on the opposite page. The significances of changes and differences in mean levels are summarised in Tables 67c and 67d respectively.

(a) Groups 1(a) and 1(b)

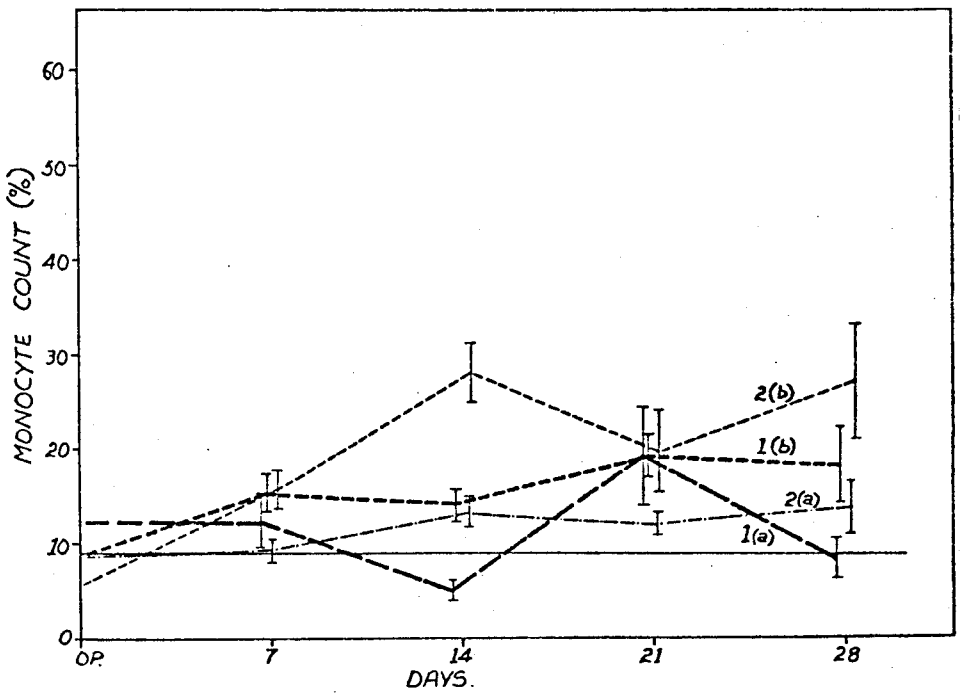
There were no significant differences in the mean percentage lymphocyte levels between the two transplant groups 1(a) and 1(b). Group 1(a) remained essentially normal, while group 1(b) exhibited a mean decrease on day 7, but returned to normal limits thereafter. Groups 1(a), 1(b) and 1(a+b) had mean levels essentially similar to groups 2(a) and 2(b) throughout the period of study.

(b) Group 2(a)

The mean percentage lymphocyte levels were significantly depressed on days 14 and 21, with compensatory neutrophilia.

(c) Group 2(b)

There were no significant changes in mean percentage lymphocyte levels during the postoperative period. The



Group 1(a) -Heterotopic allograft + cholecystocholecystostomy.
Group 1(b) - " " + cholecystojejunocholecystostomy.
Group 2(a) - End-to-side portacaval shunt.
Group 2(b)- Sham laparotomy.

GRAPH 68a SERIAL CHANGES IN MEAN PERCENTAGE MONOCYTE LEVELS FOR THE GROUPS. (Mean ± 1 SEM)

Group	Day									
	OP	No	7	No	14	No	21	No	28	No
1a	12,22 ± 6,62	9	12,22 ± 8,66	9	5,16 ± 2,26	6	19,00 ± 12,54	6	8,50 ± 5,46	6
1b	9,16 ± 3,53	12	15,15 ± 7,61	13	14,00 ± 6,14	11	19,12 ± 6,79	8	18,40 ± 8,68	5
2a	8,75 ± 3,74	12	9,91 ± 5,17	12	10,33 ± 5,72	12	10,20 ± 3,48	10	13,62 ± 7,87	8
2b	5,75 ± 1,47	4	15,60 ± 3,87	5	28,00 ± 6,78	5	19,50 ± 9,32	4	27,00 ± 11,42	4
1a+1b	10,47 ± 5,31	21	13,95 ± 8,13	22	10,38 ± 6,64	17	19,07 ± 9,68	14	13,00 ± 8,65	11

Mean ± 1 SD No = Number of samples processed

TABLE 68b MEAN LEVELS FOR THE FOUR GROUPS AND ONE SUBGROUP

Group	Time Interval (Days)			
	0-7	0-14	0-21	0-28
1a	-	<0,05	-	-
1b	<0,02	<0,05	<0,005	<0,02
2a	-	-	-	-
2b	<0,005	<0,001	<0,05	<0,05
1a+1b	-	-	<0,005	-

TABLE 68c SIGNIFICANCE OF CHANGES IN MEAN LEVELS

p< = significance
- = no significant difference
0 = no or insufficient data

Groups	Date of Comparison (Days)				
	0-0	7-7	14-14	21-21	28-28
1a-1b	-	-	<0,005	-	<0,05
1a-2a	-	-	<0,05	-	-
1b-2a	-	-	-	<0,005	-
1a-2b	-	-	<0,001	-	<0,02
1b-2b	-	-	<0,005	-	-
2a-2b	-	<0,05	<0,001	<0,01	<0,05
1a+b-2a	-	-	-	<0,02	-
1a+b-2b	-	-	0,001	-	<0,025

TABLE 68d SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS

FIGURE 68 MONOCYTES - SERIAL CHANGES IN MEAN LEVELS

levels were significantly elevated above group 2(a) mean levels on days 14 and 28.

Interpretation

There was no highly significant, sustained pattern of differences in mean percentage lymphocyte levels between any of the groups in the postoperative period.

(14) MONOCYTES

The serial mean monocyte levels of the groups are illustrated and tabulated in Figure 68 on the opposite page. The significances of changes and differences in mean levels are summarised in Tables 68c and 68d respectively.

(a) Groups 1(a) and 1(b)

There were significant differences between the mean percentage monocyte levels of groups 1(a) and 1(b) on days 14 and 28. Group 1(a) animals remained within normal limits except for day 14 when a significant decrease was seen ($p < 0,05$). Group 1(a) revealed mean percentage levels significantly lower than group 1(b) on days 14 and 28, and lower than groups 2(a), and 2(b) on day 14. Group 1(b) levels were significantly elevated above normal throughout the postoperative period. Group 1(b) mean levels were significantly elevated above group 2(a) on day 21, and were significantly lower than group 2(b) on days 14 and 28.

(b) Group 2(a)

The mean percentage monocyte levels did not change significantly during the period of study.

(c) Group 2(b)

The mean percentage monocyte levels were significantly elevated on days 14 and 28 - an elevation that may be due to observer error, as described on page 218.

Interpretation

There was no highly significant, sustained pattern of differences in mean monocyte levels between the two transplant groups 1(a) and 1(b), or between the two transplant groups and the two control groups (2(a) and 2(b)). The significant differences seen were sporadic, and may have been due, in part, to observer error.

(15&16) EOSINOPHILS AND BASOPHILS

The serial mean eosinophil and basophil levels in the four groups of animals varied by less than 5% during the postoperative period, and the small changes that occurred were considered insignificant, and no statistical analysis was performed.

The serial mean levels of the 5 groups are tabulated in Tables 54 - 58 on pages 344-348.

COLUMN	1	2	3	4	5	6	7	8
Groups Compared	1(a) - 1(b)	1(a) - 2(a)	1(a) - 2(b)	1(b) - 2(a)	1(b) - 2(b)	1(a+b) - 2(a)	1(a+b) - 2(b)	2(a) - 2(b)
CSF Glutamine	-	4	4	4	3	4	4	4
Venous NH_4	2	-	1	4	1	2	1	4
Alkaline Phos.	-	-	1	1	1	1	1	-
S G O T	1	2	1	4	-	2	-	-
Cholesterol	1	2	-	1	4	-	1	4
Total Protein	2	-	-	3	-	1	-	-
Albumin	2	-	-	1	1	-	1	-
Globulin	1	-	1	2	-	1	-	1
Haemoglobin	1	1	1	2	1	-	-	-
Leucocytes	-	1	1	1	1	1	1	-
Platelets	x	x	x	x	x	x	x	x
Neutrophils	-	1	1	2	-	2	1	2
Lymphocytes	-	1	-	-	-	-	-	2
Eosinophils	m	m	m	m	m	m	m	m
Basophils	m	m	m	m	m	m	m	m
Monocytes	2	1	2	1	1	1	2	4
pH	-	0	-	0	-	0	-	0
pCO_2	-	0	-	0	-	0	-	0
Std. Bicarb.	-	0	-	0	-	0	-	0

KEY: 1 - 4 = The number of times significant differences occurred between the mean levels of the groups compared, during the postoperative period.

- = No significant differences between mean levels of the groups during the postoperative period.

0 = No or insufficient data.

x = Unable to perform a statistical analysis, but mean levels not greatly disparate during the postoperative period.

m = Less than 5% change in mean levels during the postoperative period, and therefore differences in mean levels considered insignificant.

1(a) = Heterotopic allograft plus cholecystocholecystostomy.

1(b) = Heterotopic allograft plus cholecystojejunocholecystostomy.

2(a) = End-to-side portacaval shunt (Control).

2(b) = Sham laparotomy (Control).

1(a+b) = Combined allograft group. (The 22 animals with serial estimations of all the factors).

This Table has been compiled from Tables 64 - 71.

TABLE 38 THE FREQUENCY OF SIGNIFICANT DIFFERENCES IN MEAN LEVELS BETWEEN THE GROUPS DURING THE POSTOPERATIVE PERIOD

(17-19) ACID-BASE STATUS

The serial mean pH, pCO_2 and standard bicarbonate levels for the groups are tabulated in Tables 54-58, on pages 344-348.

The significances of changes seen in groups 1(a), 1(b), 1(a+b) and 2(b) are tabulated on Tables 59-63 in the Appendix, pages 349-353.

There were no significant differences in the postoperative acid-base status between groups 1(a), 1(b), 1(a+b) and 2(b) - see Tables 64-71 on pages 354-361.

There was insufficient data in group 2(a).

DISCUSSION IN CONTEXT

The biochemical and haematological changes that occurred during the postoperative period in the four groups of animals, and one sub-group (1(a+b)) have been presented and briefly analysed on pages 201-222. The frequencies of significant differences in mean levels between the groups, during the postoperative period, have been summarised in Table 38 on the opposite page.

There were no significant differences in mean levels between groups 1(a) and 1(b) at any time during the postoperative period for CSF glutamine, alkaline phosphatase, leucocyte count, neutrophils, lymphocytes and acid-base status (column 1 - Table 38). The platelet counts could not be analysed statistically, but the serial mean postoperative levels did not differ greatly between groups 1(a) and 1(b). The serial mean eosinophil and basophil levels in both groups remained within normal limits. Thus, in 11 of the 19 factors, groups 1(a) and 1(b) revealed comparable postoperative patterns.

There were isolated significant differences in mean SGOT, cholesterol, globulin and haemoglobin levels during the postoperative period. These differences were neither sustained nor highly significant,and groups 1(a) and 1(b) can be regarded as being comparable with regard to these 4 factors.

Significant differences were detected between the serial mean levels of venous ammonia, total protein, albumin and globulin on two occasions during the postoperative period, for each of these factors, as summarised in column 1, Table 38.

The mean venous ammonia levels for group 1(a) were elevated significantly above normal throughout the postoperative period, and closely followed the pattern of mean levels seen in the control portacaval-shunted animals (group 2(a)), while the mean postoperative levels for group 1(b) were never elevated significantly above normal, and followed the pattern seen with the sham-operated control group (2(b)). As a group, the donor livers from group 1(a) were of inferior histological quality compared to the donor livers from group 1(b), and this may have accounted for the significant differences in mean levels seen on days 21 and 28.

The differences in mean total protein and albumin levels on days 7 and 21 were not highly significant, and were not sustained. The feeding habits of individual pigs could not be established accurately. Dietary intake can be responsible for this observation, as reported by Hickman (91).

The differences in mean percentage monocyte levels on days 14 and 28 were not highly significant, and not sustained throughout the postoperative period and do not appear to be due to the types of biliary drainage used.

It has therefore been established that there were no sustained, highly significant differences between the mean postoperative levels of any of the biochemical and haematological factors tested in groups 1(a) and 1(b). Groups 1(a) and 1(b) are thus comparable biochemically and haematologically, suggesting that the type of biliary drainage did not influence the postoperative levels of these factors.

The use of the two control groups (2(a) and 2(b)), helped to clarify the importance of the changes in mean levels of the 19 factors, during the postoperative period. It can be seen, in Table 38, columns 2-7, that the frequency of significant differences in mean levels between the transplant and control groups during the postoperative period, was highest for CSF glutamine. With one exception, (group 1(b) compared to group 2(b)), there were significant differences in mean levels between the transplant and control groups at each weekly sampling period - (see Figure 55 on page 201F. The analysis also revealed three distinct patterns of serial mean CSF glutamine levels for the transplant, sham and portacaval-shunted groups, as discussed on pages 201-203.

None of the remaining 18 factors revealed a high frequency of significant differences in mean levels between the transplant and control groups during the postoperative period. The transplant group as a whole (group 1(a+b)) revealed only sporadic differences between mean levels when compared to the control groups 2(a) and 2(b) during the postoperative period - columns 6 and 7 in Table 38.

It is thus apparent that there were distinct differences in mean CSF glutamine levels between the transplant and control groups during the postoperative period, and that

there were no sustained, distinct patterns of differences in mean levels of the remaining 18 factors.

The changes that occurred in the mean levels of the 19 biochemical and haematological factors will be further analysed and discussed in Part III of this presentation.

PART II

CHAPTER 4

OVERALL DISCUSSION AND CONCLUSIONS

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OVERALL DISCUSSION AND CONCLUSIONS

The high incidence of serious and fatal complications arising directly from the reconstruction of the biliary tract in both clinical and experimental liver transplantation, has been discussed in the Main Introduction. The review of clinical and experimental experience revealed that ultimate drainage of the donor bile through the recipient's sphincter of Oddi was desirable, but that anastomoses directly involving the recipient's common bile duct had produced an unacceptably high rate of fatal complications in human transplantaion. A new technique of biliary tract reconstruction, described by Immelman (97), in which the donor and recipient gallbladders were anastomosed end-to-end was discussed. This technique allowed ultimate drainage of donor bile through the recipient's sphincter of Oddi, and avoided both direct biliary-enteric anastomosis and surgery to the recipient's common bile duct.

Immelman's technique of auxiliary transplantation and cholecystocholecystostomy was studied in Part I of this presentation, and it was shown that the donor and recipient gallbladders could be apposed comfortably in only 50% of the human cadaver transplants. A method was developed and described, whereby the gap between the donor and recipient gallbladders in the heterotopic human cadaver transplant model could be bridged by the interposition of an isolated, vascularised, isoperistaltic jejunal loop.

The main objectives in undertaking Part II of this study were to establish the technique of jejunal interposition in the heterotopic pig model, to assess the local effects, and to compare the local, regional and general effects with those seen in a parallel series of porcine transplants, in which bile drainage was created by means of cholecystocholecystostomy.

Operative techniques and times have been discussed in Part II, Chapter 2. The general, local, regional, biochemical and haematological effects have been described in detail, and briefly discussed in context, in Part II, Chapter 3, Sections I-IV respectively.

The interposition of an isolated, vascularised, isoperistaltic jejunal loop between the donor and recipient gallbladders has been shown, in the present study, to be technically feasible and successful in the heterotopic porcine model. The preparation of the isolated, vascularised jejunal loop utilised established surgical techniques (123). The gallbladder beds were not disturbed, the structures to be anastomosed were large and easily accessible, and the proximal and distal cholecystojejunal anastomoses could readily be performed. The re-establishment of jejunal continuity was complication-free.

Loop interposition added, on average, only 23 minutes to the complete transplant operation compared to cholecystocholecystostomy. Thirteen of the 14 interposed loops were viable at autopsy, and revealed minimal macro- and microscopic changes.

On comparing the local effects of loop interposition with the local effects following cholecystocholecystostomy, it

was shown that the two techniques produced comparable results. Minimal changes were detected in the recipient gallbladders of both groups, and microscopically the recipient gallbladders did not vary significantly from the respective pre-operative control biopsies, nor from the postoperative gallbladders taken from pigs subjected to portacaval shunts or sham laparotomies.

The spectra of changes seen in the donor gallbladders from the two transplant groups were similar, and were due to rejection rather than sepsis and stasis. Anastomotic leaks were infrequent with both techniques. The complications of anastomotic concretions, excessive granulation tissue and biliary sludge were comparable in the two groups, and have been reported with other techniques of biliary drainage. The bacteriological studies of the extrahepatic biliary tracts confirmed previous reports on the potential danger of introducing bowel-based organisms into the biliary tract when utilising bowel in the drainage tract, and revealed a bias in favour of cholecystocholecystostomy. Methods of decreasing the danger of bowel-based organisms in the loop technique were suggested in the discussion in Part II, Chapter 3, Section II.

The regional effects on the donor and recipient livers in the two transplant groups were essentially similar. The postoperative recipient livers in the two groups revealed minimal morphological changes compared to the pre-operative state, and compared to the postoperative livers from the animals subjected to portacaval shunts or sham laparotomy. The pattern of weight loss in the recipient livers from both groups was comparable, and similar to the pattern seen in the livers from the portacaval-shunted group, and was probably due to the recipient livers being deprived of primary access to portal blood, and not related to the type of biliary drainage used.

The morphological changes that occurred in the donor livers from the two groups were comparable, and appeared to be due mainly to rejection. The comparable low incidence and degree of cholestasis and cholangitis in the donor livers from both groups, indicated that the two methods of biliary drainage did not predispose to these complications. The direct, blind analysis of the incidence and degree of cholestasis and cholangitis in the donor livers from the present, Dent's (55) and Immelman's (97) series, revealed a comparable, low incidence and degree of these two complications in the donor livers which had been drained via the sphincter of Oddi, and a high incidence and degree of cholestasis and cholangitis in the donor livers drained by direct biliary-enteric anastomoses. These results reinforce the concept that direct biliary-enteric anastomoses should be avoided, as discussed in the Main Introduction. However, the present study was limited to 28 days, and the favourable observation with regard to cholestasis and cholangitis, does not necessarily mean that these complications will be avoided in the long term.

In the general clinico-pathological context, the two techniques of biliary drainage produced comparable results, which were essentially similar to the general effects reported with other transplantation techniques in pigs and dogs.

The pattern of changes in mean values of the biochemical and haematological factors were comparable in the two transplant groups, and with a few exceptions, similar to the pattern of changes seen in the control portacaval-shunted and sham laparotomy groups. The clinical value of the changes seen in the biochemical and haematological factors, will be discussed in Part III.

The two types of biliary drainage which have been studied in this series have practical and theoretical advantages. In principle, both techniques avoid direct biliary-enteric anastomosis, thereby decreasing the likelihood of cholangitis and cholestasis and thus creating for the donor liver, conditions more suitable for adequate function. Both methods avoid direct surgery to the recipient's common bile duct, thereby decreasing the potential hazards of leaks, common bile duct stones and strictures. Both techniques involve minimal disturbances of the donor and recipient extrahepatic biliary tracts, and leave the gallbladder beds undisturbed, thereby both preserving any blood supply from the bed, and avoiding the potential hazards of mechanical obstruction due to kinking, or tension on the cystic ducts. The structures are large and easily accessible, lending to safer anastomoses, and the avoidance of the twin dangers of leaks and strictures.

There are advantages with regard to patient management. The surgeon performing the transplant operation has the choice of two drainage techniques, allowing him to tailor the operation according to the circumstances encountered. Drainage by means of cholecystocholecystostomy would be the first choice, but the present study has shown that cholecystojejunocholecystostomy is an acceptable alternative if the two gallbladders cannot be apposed.

Should the donor liver be used as a temporary support, later donor hepatectomy could be performed, together with recipient cholecystectomy, leaving the recipient's liver and common bile duct unaffected by the previous drainage procedure. In cases where recipient hepatectomy may be indicated some time after the transplant operation, the recipient hepatectomy can be carried out, leaving the recipient's gallbladder, cystic duct and common duct in

situ, or, if indicated, the host's gallbladder can be removed, and the donor gallbladder anastomosed to either the host's common bile duct, or a loop of jejunum.

The two drainage techniques allow for revision of the biliary drainage in the event of complications. Necrosis of the donor gallbladder due to rejection or vascular accident, could be treated by anastomosing the donor common bile duct to either the recipient's gallbladder, or to a loop of jejunum. Obstruction to the recipient's cystic duct could be treated either by recipient cholecystodochostomy (as suggested by Waddell (214)) or by performing recipient cholecystectomy and creating donor bile drainage via cholecystenterostomy.

The two techniques of biliary drainage have thus been shown to have a number of theoretical, technical and patient-management advantages.

There are <u>theoretical and practical disadvantages</u> inherent in the two techniques of biliary drainage under discussion. Utilisation of the two methods of biliary drainage implies the presence of normal gallbladders in both the recipient and donor. Gallbladder disease or previous cholecystectomy in either donor or recipient, will demand a different method of bile drainage. The construction of an isolated, vascularised jejunal loop may be difficult and time-consuming in the face of established portal hypertension, or adhesions from previous abdominal surgery.

The use of two cystic ducts in the biliary drainage tract has several potential disadvantages. The donor cystic duct may become occluded during a rejection episode, or due to infection (as discussed by Martineau et al (128)).

The recipient's cystic duct may become obstructed by the type of biliary sludge that was encountered in the biliary tract in a few of the animals in this study.

The fluid-mechanics of draining donor bile from a narrow cystic duct, into a large-calibre gallbladder, and thence into a small-calibre cystic duct, may create unfavourable conditions. There may be a sump-like effect, favouring the formation of precipitates and sludge; and the likelihood of infection is increased in the face of focal stasis.

The donor biliary apparatus is denervated with the transplantation technique used. Biliary motility studies could not be performed in this series, but the denervation did not appear to be disadvantageous in the 28 day study. However, in the long term, denervation may well prove to be disadvantageous, and may contribute to some of the sludging and concretion formation that has been reported with long term human and animal survivors.

Concretions were encountered on the suture lines with both techniques in the present series, and have been reported previously with other techniques (42). These concretions may be the nidus for gallstone formation in the long term, and the two suture lines necessary when using an interposed loop, may double the hazard. Solution to the problem would probably lie in meticulous suturing technique, the use of the correct suture material and size, and the use postoperatively of agents which would oppose gallstone formation.

The present study was limited to 28 days, and in the short term the two techniques of biliary drainage have revealed promising results. However, the relative importance of the advantages and disadvantages will only be fully established with long term studies in pigs, dogs and primates.

CONCLUSIONS

The second major objective in this study was to apply any modifications found to be necessary in the human cadaver study in Part I, to a series of live porcine auxiliary liver transplant operations, and to study and compare the effects with those seen in a parallel series of transplants using Immelman's unmodified technique. Part I of the study revealed that while the subhepatic technique was feasible in the human cadaver, cholecystocholecystostomy could only be performed in 50% of the transplants carried out. Modification to the biliary drainage was thus necessary, and cholecystojejunocholecystostomy was shown to be feasible in all cases, while still adhering to the principles involved in cholecystocholecystostomy.

The modification of interposing a jejunal loop between the donor and recipient gallbladders has been developed, studied and analysed in detail in the series of heterotopic porcine allografts, as presented in Part II.

Cholecystojejunocholecystostomy is technically feasible in the pig, does not add significantly to the total operative time, and allows avoidance of both direct biliary-enteric anastomosis and direct surgery to the recipient's common bile duct. The results were satisfactory in the short term, and comparable in almost every way with those achieved in the parallel series using cholecystocholecystostomy.

Both drainage techniques produced a low incidence and degree of cholestasis and cholangitis in the donor livers, comparable with the low incidence and degree seen in porcine orthotopic allografts where choledochocholedochostomy

was used for drainage. The two techniques produced minimal effects on the donor and recipient gallbladders, and on the recipient livers.

The two techniques of biliary drainage have advantages and disadvantages, which need to be clarified in long term studies in different animals before the techniques can be considered for clinical application.

PART III

STUDY OF THE RELATIONSHIP BETWEEN DONOR LIVER QUALITY, AND BIOCHEMICAL AND HAEMATOLOGICAL CHANGES IN THE RECIPIENT

INDEX TO PART III

STUDY OF THE RELATIONSHIP BETWEEN DONOR LIVER QUALITY AND BIOCHEMICAL AND HAEMATOLOGICAL CHANGES IN THE RECIPIENT

PART III

SUMMARY

A detailed study of the relationships between the histological quality of the donor livers, and the changes that occurred in the biochemical and haematological factors in the recipient animals, will be presented.

The relationships were assessed in two ways: firstly related to time; and secondly without reference to the postoperative period. The two assessments will be presented and discussed separately.

It will be shown that the CSF glutamine levels in the recipient animals related accurately to the quality of the donor livers, in both the time-related and time-unrelated studies, and that none of the other 18 factors which were assessed, showed consistent relationships to donor liver quality.

The possible mechanisms responsible for the relationship between CSF glutamine and the donor liver are discussed, as well as the advantages and disadvantages of using CSF glutamine as a clinical diagnostic tool.

PART III

CHAPTER 1

INTRODUCTION AND AIMS

The problem of the early detection of rejection in the donor liver, and its differentiation from cholangitis, bile stasis and other complications, has been discussed in the Main Introduction. The point was stressed that in the presence of two livers in the heterotopic transplant situation, the problem of the early diagnosis of rejection is compounded, as function, or dysfunction of the host's own liver, may modify the biochemical and haematological factors which may be useful in monitoring the function of the donor liver, and thus mask changes due to dysfunction of the donor liver. Immelman (97) suggested, from a few preliminary observations, that the CSF glutamine levels might be a useful index of donor liver quality in the heterotopic porcine model under discussion in this presentation.

The third major objective of this experimental study was to establish the relationship of CSF glutamine to donor liver quality, and to compare its value with the information provided by the other biochemical and haematological factors.

The study was undertaken in two parts. In the first study, the relationship was studied on a time related basis, while in the second study, the time factor was ignored.

The recipient animals used in this part of the study consisted of 9 animals from group 1(a) and 13 from group 1(b). Both groups of animals had been subjected to auxiliary heterotopic liver transplantation as described in Part II, Chapter 2, and varied only in the type of biliary drainage which was employed. It has been shown in Part II of this presentation that the two transplant groups 1(a) and 1(b) were comparable in almost every clinical, pathological, biochemical and haematological factor assessed, and for the purposes of this part of the study, the 22 animals are regarded as a single group (the "combined transplant group" of Part II).

Serial CSF glutamine, biochemical and haematological estimations (as outlined in Table 36 on page 197F, and simultaneous serial donor and recipient liver biopsies, had been performed on the 22 recipient pigs.

PART III

CHAPTER 2

THE TIME RELATED STUDY

METHODS USED

1. To assess the quality of the donor livers

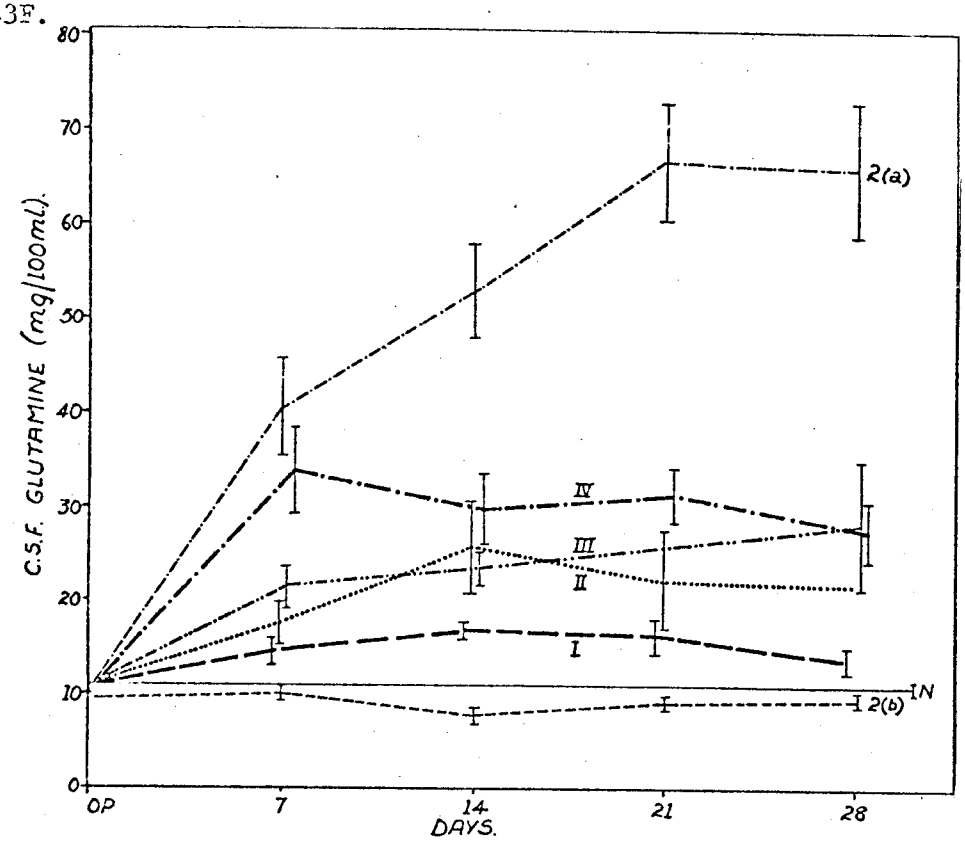
As described in Part II, Chapter 3, Section III, pages 174-176 & 183-185, the quality of the donor livers on days 0, 7, 14, 21 and 28 was established by means of two separate histological analyses on the serial liver biopsy specimens. Five grades of functional quality were established (the Functional Liver Index) and five grades of rejection (the Rejection Index). The histological grading accorded to the individual livers is detailed in the Appendix, Tables 40-45 on pages 319-324. It had been intended to investigate the relationship of cholestasis and cholangitis to the biochemical and haematological factors, but the incidence and degree of these complications were so minimal, that they could not be used in this part of the study.

2. To assess the relationship between donor liver quality, and the levels of the biochemical and haematological factors in the recipient animals - Time Related

A simple statistical analysis was used to investigate the relationship between the histological grades of the donor livers, and the levels of the simultaneously-harvested biochemical and haematological factors in the recipient animals, in relation to the post-operative period.

As described in Part II, Chapter 2, simultaneous CSF, blood and donor liver samples were obtained from the recipient animals every 7th day and at sacrifice. After the donor livers had been graded histologically as described above, the biochemical and haematological results from recipients having identical grades of donor liver were grouped together. The mean value for each factor, in each grade, was then calculated, for both the Functional Liver Index and the Rejection Index, on days 7, 14, 21 and 28. These weekly grade mean values are tabulated in the Appendix, in Table 72 for the Functional Liver Index, and in Table 73 for the Rejection Index - pages 363-366.

The mean pre-operative values of the 19 factors were calculated for the 22 recipients - these values (or levels) are included in the Tables as the pre-operative normal values.



GRAPH 69a SERIAL MEAN CSF GLUTAMINE LEVELS FOR THE FUNCTIONAL GRADES. (Mean ± 1 SEM)

PRE-OPERATIVE NORMAL (N)	POST OP DAY	FUNCTIONAL GRADES							
		I	No	II	No	III	No	IV	No
11,00 ± 2,47 (20)	7	14,56 ± 4,28	6	17,50 ± 6,40	6	21,58 ± 4,95	5	33,85 ± 8,27	4
	14	16,76 ± 1,92	5	25,57 ± 9,99	4	23,30 ± 2,20	2	29,46 ± 9,29	6
	21	16,04 ± 3,93	5	22,10 ± 8,02	3	Nil	-	31,26 ± 6,39	5
	28	13,70 ± 2,01	3	21,6	1	28,20 ± 13,01	4	27,26 ± 5,24	3

No = number of liver biopsies analysed

TABLE 69b SERIAL MEAN CSF GLUTAMINE LEVELS FOR THE DIFFERENT GRADES. (Mean ± 1 SD)

POST OP DAY	FUNCTIONAL GRADES COMPARED									
	N-I	N-II	N-III	N-IV	I-II	I-III	I-IV	II-III	II-IV	III-IV
7	<0,02	<0,01	<0,001	<0,001	-	<0,05	<0,005	-	<0,01	<0,05
14	<0,001	<0,005	0	<0,001	-	0	<0,025	0	-	0
21	<0,005	<0,02	0	<0,001	-	0	<0,005	0	-	0
28	-	0	<0,005	<0,005	0	-	<0,02	0	0	-

p< = significance. - = no significant difference. 0 = no or insufficient data.

TABLE 69c THE SIGNIFICANCE OF SERIAL CHANGES IN MEAN CSF GLUTAMINE LEVELS

FIGURE 69 SERIAL MEAN CSF GLUTAMINE LEVELS RELATED TO THE FUNCTIONAL LIVER INDEX - TIME RELATED

RESULTS

1. Mean levels related to the Functional Liver Index - Time Related

The serial mean levels of the biochemical and haematological factors, related to the Functional Liver Indices of the donor livers, are tabulated in Table 72 on pages 363-364 in the Appendix. Although the numbers for individual grades are small, a clear pattern can be seen. The mean levels for CSF glutamine reveal a step-like elevation between grades, irrespective of the postoperative period. No pattern of consistent, sustained elevation or depression between mean levels is seen in any of the other 18 factors that were tested.

The serial mean CSF glutamine levels related to the different Functional Indices (or grades) of the donor livers, are illustrated in Graph 69a and tabulated in Table 69b on the opposite page. The serial mean CSF glutamine levels of the control animals from group 2(a) (portacaval shunts) and group 2(b) (sham laparotomies), have been included in Graph 69a for easy reference. The significances of the differences in mean levels between the grades, calculated by means of a Student's t-test, are summarised in Table 69c on the opposite page.

A clear pattern can be seen, despite the small numbers. The recipient animals with Functional Grade I donor livers (0-25% loss of hepatocytes), exhibited mean CSF glutamine values elevated only slightly (but significantly) above normal, irrespective of the postoperative period - see Graph 69a and Table 69c. By contrast, the recipient animals with grade IV donor livers (75-100% loss of

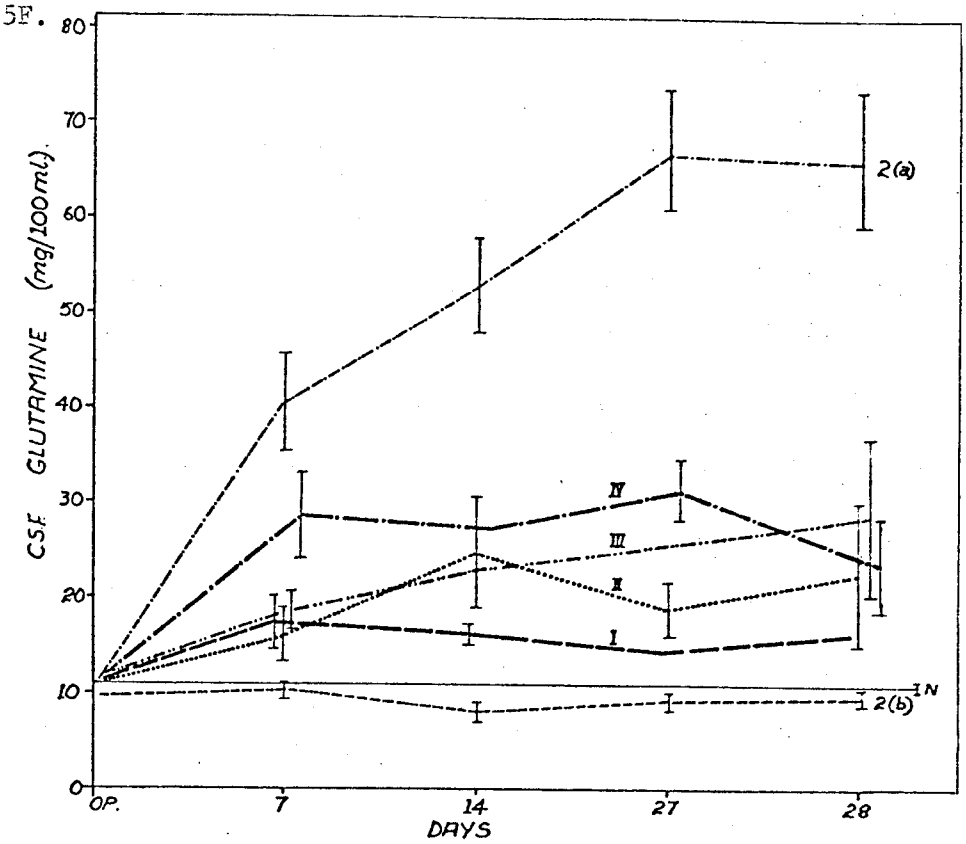
hepatocytes) exhibited mean CSF glutamine levels elevated to almost three times the pre-operative normal, and elevated significantly above both the pre-operative normal and the serial mean levels for the animals with grade I livers. The mean levels related to grade I donor livers lie nearest to the mean levels seen in the sham-operated control group (2(b)), while the mean levels related to grade IV donor livers reveal an initial elevation similar to the initial elevation seen in the portacaval-shunted control group (2(a)) - see Graph 69a.

The mean CSF glutamine levels for the recipient animals having grade II and III donor livers are elevated significantly above normal, and fall in between the mean levels for grades I and IV - see Graph 69a. The numbers in grades II and III were small, and no distinct pattern of differences emerged between the mean levels of grades II and III.

This part of the study has revealed a clear relationship between the Functional Grade or Index of the donor livers, and the CSF glutamine levels in the recipient animals.

2. Mean levels related to the Grade of Rejection - Time Related

The serial mean levels of the biochemical and haematological factors, related to the Grades of Rejection in the donor livers, are tabulated in Table 73 on pages 365-366 in the Appendix. Although the numbers for individual grades are small, a clear pattern can be seen. The mean levels for CSF glutamine reveal a step-like, significant elevation in mean levels between normal, grade I and grade IV, irrespective of the postoperative period. No pattern of consistent,



GRAPH 70a SERIAL MEAN CSF GLUTAMINE LEVELS FOR THE REJECTION GRADES. (Mean ± 1 SEM)

PRE-OPERATIVE NORMAL (N)	POST OP DAY	REJECTION GRADES							
		I	No	II	No	III	No	IV	No
11,00 ± 2,47 (20)	7	17,26 ± 4,35	3	15,76 ± 6,84	6	18,63 ± 4,90	6	28,44 ± 10,33	5
	14	16,30 ± 1,88	4	24,52 ± 10,44	4	22,80	1	27,11 ± 8,85	7
	21	14,40	1	18,87 ± 6,78	7	Nil	-	31,26 ± 6,39	5
	28	16,30	1	20,23 ± 11,10	3	28,40 ± 12,10	3	23,77 ± 7,56	4

TABLE 70b SERIAL MEAN CSF GLUTAMINE LEVELS FOR THE DIFFERENT GRADES. (Mean ± 1 SD)

POST OP DAY	REJECTION GRADES COMPARED									
	N-I	N-II	N-III	N-IV	I-II	I-III	I-IV	II-III	II-IV	III-IV
7	<0,005	<0,05	<0,005	<0,001	-	-	-	-	<0,05	-
14	<0,001	<0,01	0	<0,001	-	0	<0,05	0	-	0
21	0	<0,005	0	<0,001	0	0	0	0	<0,01	0
28	0	<0,05	<0,01	<0,005	0	0	0	-	-	-
p< = significance. - = no significant difference. 0 = no or insufficient data										

TABLE 70c THE SIGNIFICANCE OF SERIAL CHANGES IN MEAN CSF GLUTAMINE LEVELS

FIGURE 70 SERIAL MEAN CSF GLUTAMINE LEVELS RELATED TO THE REJECTION INDEX - TIME RELATED

sustained elevation or depression between mean levels is seen in any of the other 18 factors that were tested.

The serial mean CSF glutamine levels related to the different grades of rejection in the donor livers, are illustrated in Graph 70a and tabulated in Table 70b on the opposite page. The significances of the differences in mean levels between the grades, calculated by means of Student's t-tests are summarised in Table 70c. The serial mean CSF glutamine levels of the control animals (groups 2(a) and 2(b)) have been included in Graph 70a for easy reference.

A clear pattern can be seen, despite the small numbers. The recipient animals with rejection grade IV livers (gross rejection) exhibited mean CSF glutamine levels elevated two or three times the pre-operative normal. These levels were all elevated significantly above the pre-operative normal. The animals with rejection grade I donor livers, exhibited the least (but still significant) elevations in mean levels.

The pattern of differences in mean CSF glutamine levels between recipients having grades I, II or III livers were not as distinct as those seen with the Functional Liver Index, but the numbers were small for some of the rejection grades, and the histological grading had been more difficult, as explained on page 185.

DISCUSSION IN CONTEXT

The time-related study has revealed a striking relationship between the histological quality of the donor livers, and the CSF glutamine levels in the recipient animals. Sham-operated animals revealed serial glutamine levels basically within the normal range throughout the postoperative period. The recipient animals with the best quality donor livers (Functional Grade I or Rejection Grade I) exhibited small, but significant elevations in mean CSF glutamine levels, throughout the postoperative period, while the recipients with the poorest quality livers (Functional Grade IV or Rejection Grade IV) exhibited mean CSF glutamine levels that were elevated nearly threefold, and were elevated significantly above the serial mean levels seen in the animals with grade I livers.

The mean CSF glutamine levels of recipients with intermediate grade livers (grade II and III), tended to lie between the mean levels seen with grades I and IV livers. The elevation in serial mean CSF glutamine levels in the control portacaval-shunted group (2(a)) was progressive and sixfold by day 21.

Clear patterns of differences were not seen in any of the other 18 factors that were assessed in the study. CSF glutamine has thus been shown to be the best index of both functional quality and rejection in the time-related study, despite the small numbers.

The relationship between CSF glutamine and the quality of the donor liver will be further investigated in Chapter 3, and discussed in depth in Chapter 4.

PART III

CHAPTER 3

THE TIME UNRELATED STUDY

The time-related study has revealed sustained, significant differences in mean levels of CSF glutamine between recipients with good quality donor livers, and those with poor quality donor livers.

To be of use as a clinical diagnostic index of donor liver function or quality, the factor used should vary rapidly and measurably with changes in function or quality of the donor liver. In a few animals from groups 1(a), 1(b) and 2(a) it was observed that the CSF glutamine levels changed markedly in 24 hours, indicating that this was a factor that could vary rapidly and measurably.

The histological analysis of the donor livers revealed that the quality of individual livers could vary from week to week. In some donor livers, the quality became progressively worse, in others the quality was poor initially but improved with time, while some had an established pattern of quality by day 7. Dent (55) had previously reported spontaneous reversibility of the rejection pattern in porcine hepatic grafts.

Bearing these factors in mind, a second study of the relationship between the quality of the donor livers (as judged by the Functional and Rejection Indices), and the levels of the biochemical and haematological

factors in the recipients, was undertaken, with no reference to the postoperative period.

METHODS USED

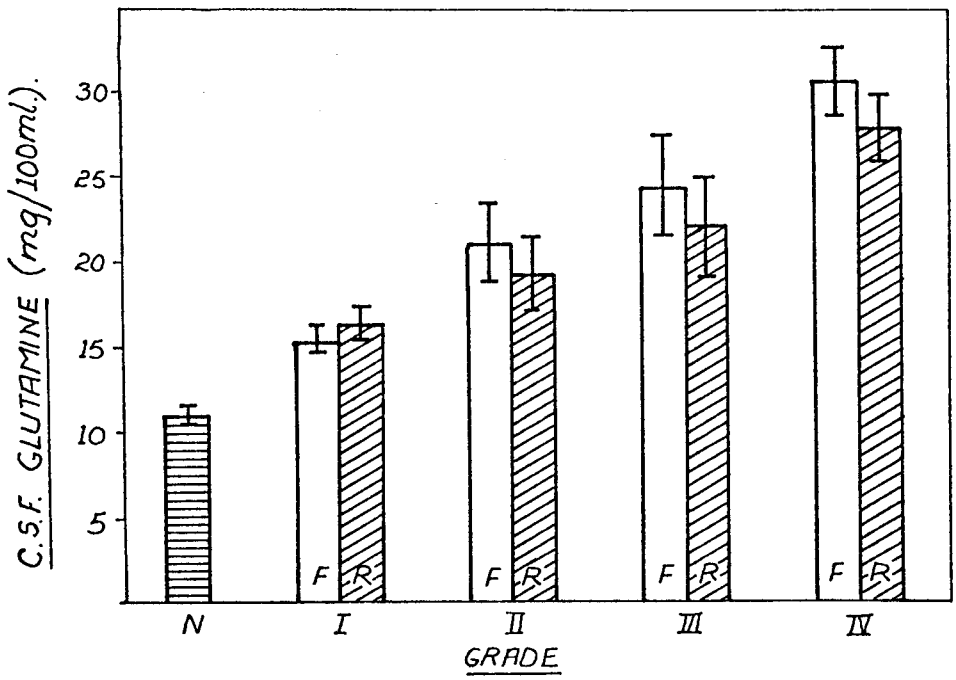
1. To assess the quality of the donor livers

All the serial donor liver biopsies were graded into 5 histological grades for each of Function and Rejection, as described in Part II, Chapter 3, Section III, pages 174-176 and 183-185. The histological grading accorded to individual livers is detailed in the Appendix, Tables 40-45 on pages 319-324. The same grading systems were used for both the time-related and the time-unrelated study.

2. To assess the relationship between donor liver quality, and the levels of the biochemical and haematological factors in the recipient animals - Time Unrelated

A simple statistical analysis was used to investigate the relationship between the histological grades of the donor livers, and the levels of the simultaneously-harvested biochemical and haematological factors in the recipient animals, without reference to the postoperative period.

As discussed on page 242 simultaneous CSF, blood and donor liver samples had been obtained from the recipient animals every 7th day and at sacrifice. Each serial donor liver was accorded histological grading, as described above. The biochemical and haematological results from recipient animals having identical grades of donor liver, were grouped together, for each grade in each of the Functional or Rejection groups, irrespective of the postopera-



F = Functional Liver index

R = Rejection index.

N = Pre-operative normal.

HISTOGRAM 71a MEAN CSF GLUTAMINE LEVELS FOR THE TWO HISTOLOGICAL GROUPS. (Mean ± 1 SEM)

HISTOLOGICAL INDEX	GRADES									
	N	No	I	No	II	No	III	No	IV	No
Functional liver	11,00 ± 2,47	20	15,39 ± 3,57	19	21,08 ± 8,45	14	24,30 ± 9,09	11	30,57 ± 8,04	18
Rejection	11,00 ± 2,47	20	16,41 ± 2,93	9	19,27 ± 8,93	20	21,98 ± 8,80	10	27,78 ± 8,85	21

No = number of liver biopsies analysed

TABLE 71b MEAN CSF GLUTAMINE LEVELS FOR THE DIFFERENT GRADES. (Mean ± 1 SD)

HISTOLOGICAL INDEX	GRADES									
	N-I	N-II	N-III	N-IV	I-II	I-III	I-IV	II-III	II-IV	III-IV
Functional liver	<0,001	<0,001	<0,001	<0,001	<0,02	<0,01	<0,001	-	<0,01	<0,05
Rejection	<0,001	<0,001	<0,001	<0,001	-	-	<0,001	-	<0,01	-

p< = significance - = no significant difference 0 = no or insufficient data

TABLE 71c THE SIGNIFICANCE OF CHANGES IN MEAN CSF GLUTAMINE LEVELS

FIGURE 71 MEAN CSF GLUTAMINE LEVELS RELATED TO DONOR LIVER QUALITY - TIME UNRELATED

tive period. The mean value for each factor for each grade, was then calculated, for both the Functional Liver Index, and the Rejection Index. The mean grade values are tabulated in the Appendix, Table 74 for values related to the Functional Liver Index, and Table 75 for values related to the Rejection Index.

The 22 transplant recipients in whom both serial liver biopsies and serial CSF glutamine levels were available (the combined transplant group of Part II) were used in this part of the study, and the mean pre-operative values of the 19 factors were used for grade N or normal.

The significances of the differences between mean levels of the grades, were calculated by means of Student's t-tests, and are summarised in Table 76 for the Functional Liver Index, and Table 77 for the Rejection Index.

In addition, the mean grade values related to the Functional and Rejection Indices, were compared by means of Student's t-tests, and are summarised in Table 78 on page 371.

RESULTS

1. CSF Glutamine

The mean recipient CSF glutamine levels related to both the Functional and Rejection Grades of the donor livers, are illustrated and tabulated in Figure 71 on the opposite page. The significances of the differences in mean levels between the grades are tabulated in Table 71c and the comparison of the mean levels for the two indices in Table 78 on page 371.

(a) Functional Liver Index

The mean CSF glutamine levels related to the Functional Grades I-IV, reveal highly significant elevations above normal (or N). Significant differences in mean levels are seen between grades I and II, I and III, I and IV, II and IV and III and IV. Thus significant differences were present between the mean levels related to all the grades of donor livers with the exception of grades II and III.

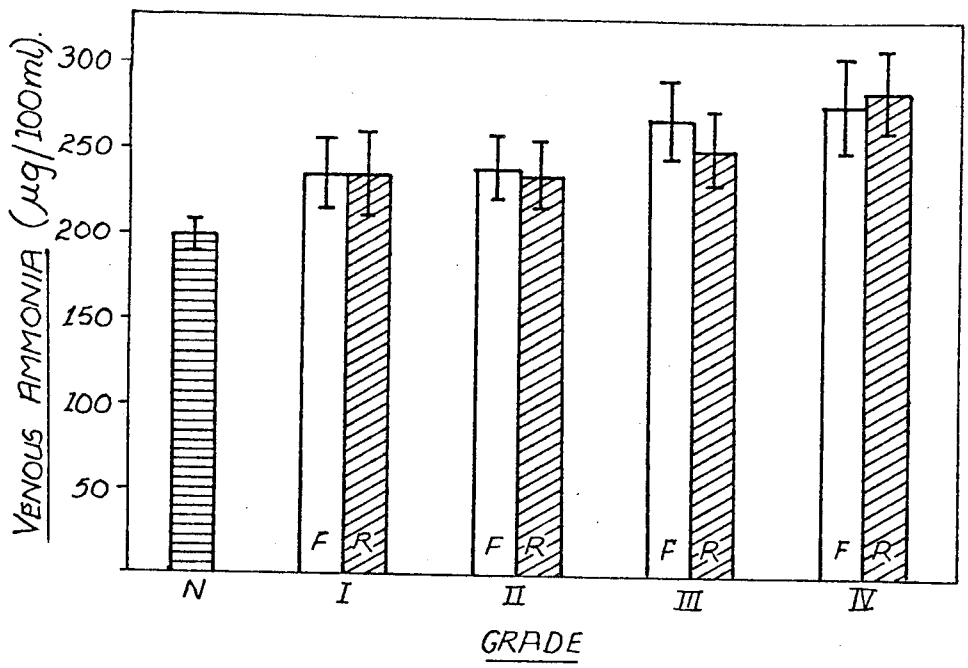
(b) Rejection Index

The mean CSF glutamine levels related to the Rejection Grades I-IV likewise reveal highly significant elevations above normal, and there was a highly significant difference in mean levels between grades I and IV. The difference between grades II and IV was significant ($p < 0,01$) but there were no significant differences between the mean levels related to the remaining grades.

(c) Interpretation

The mean CSF glutamine levels in the recipient animals related accurately to the Functional Grades of the donor livers. There was a significant, step-like elevation between all the grades with the exception of grades II and III.

The relationship of CSF glutamine to the degree of rejection reveals a similar relationship, although not as clearly defined.



F = Functional Liver index.
R = Rejection index
N = Pre-operative normal.

HISTOGRAM 72a MEAN VENOUS AMMONIA LEVELS FOR THE TWO HISTOLOGICAL GROUPS. (Mean ± 1 SEM)

HISTOLOGICAL INDEX	GRADES									
	N	No	I	No	II	No	III	No	IV	No
Functional liver	199,30 ± 39,61	20	235,52 ± 81,54	17	239,66 ± 60,88	12	269,00 ± 64,04	9	278,88 ± 109,62	17
Rejection	199,30 ± 39,61	20	235,55 ± 69,66	9	235,75 ± 76,41	16	252,00 ± 56,15	8	286,65 ± 104,98	20

TABLE 72b MEAN VENOUS AMMONIA LEVELS FOR THE DIFFERENT GRADES. (Mean ± 1 SD)

HISTOLOGICAL INDEX	GRADES							
	N-I	N-II	N-III	N-IV	I-II	I-IV	II-III	III-IV
Functional liver	-	<0,05	<0,005	<0,01	-	-	-	-
Rejection	-	-	<0,01	<0,005	-	-	-	-

p< = significance. - = no significant difference. 0 = no or insufficient data.

TABLE 72c THE SIGNIFICANCE OF CHANGES IN MEAN VENOUS AMMONIA LEVELS

FIGURE 72 MEAN VENOUS AMMONIA LEVELS RELATED TO DONOR LIVER QUALITY - TIME UNRELATED

The mean CSF glutamine levels related to each Functional Grade did not vary significantly from the mean levels related to the corresponding rejection grades, (Table 78, page 371) suggesting a relationship between the functional grades and the grades of rejection.

2. Venous Blood Ammonia

The mean venous ammonia levels related to the different grades of Function and Rejection are illustrated in Histogram 72a, and tabulated in Table 72b on the opposite page. The significances of differences in mean levels are summarised in Table 72c.

(a) Functional Liver Index

The mean recipient venous ammonia levels related to grades II, III and IV reveal elevations significantly above normal. There are, however, no significant differences between the mean levels related to grades I and IV.

(b) Rejection Index

The mean venous ammonia levels related to grades III and IV were significantly elevated above normal, but there were no significant differences between the mean levels related to grades I and IV.

(c) Interpretation

Although the mean venous ammonia levels of the recipient animals were elevated above normal with grades I and IV donor livers, for both the functional and rejection studies, there

were no clearcut, significant differences between grades I and IV. Venous blood ammonia is thus not an accurate index for distinguishing between grades of donor liver quality, when compared to CSF glutamine, where there were significant differences between grades. The problems relating to venous ammonia will be discussed in the overall discussion on pages 261-264.

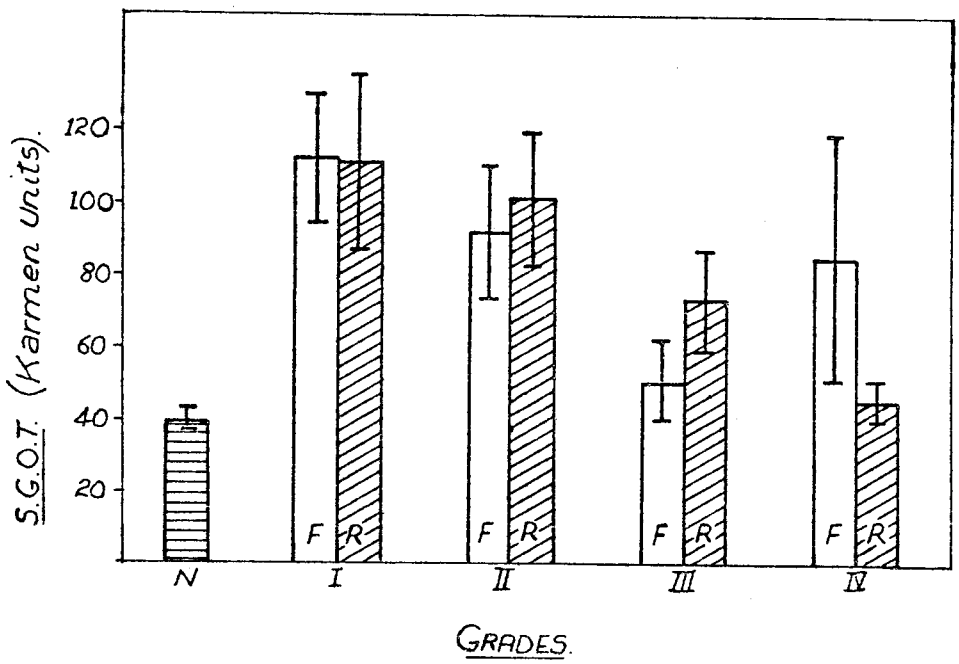
3. Alkaline Phosphatase

The mean alkaline phosphatase levels related to the different grades of function and rejection are tabulated in the Appendix, page 367 for the functional grades, and page 368 for the Rejection Index. The significances of differences in mean levels are summarised in Tables 76 and 77, pages 369-370.

The mean level related to functional grade I revealed a significant elevation above normal. The mean alkaline phosphatase levels related to all the other grades, for both the Functional Index and the Rejection Index, revealed no significant differences from normal, and no significant differences between grades. The serum alkaline phosphatase levels in the recipients have thus been shown to bear no relationship to the quality of the donor livers.

4. S.G.O.T.

The mean S.G.O.T. levels related to the different grades of function or rejection are illustrated in Histogram 73a on the following page, and tabulated in Table 73b. The significances of the differences between mean grade levels are summarised in Table 73c. Log transformation was used in the calculation of the significances, as discussed on page 207.



F = Functional Liver index.
R = Rejection index.
N = Pre-operative normal.

HISTOGRAM 73a MEAN S.G.O.T. LEVELS FOR THE TWO HISTOLOGICAL GROUPS. (Mean ± 1 SEM)

HISTOLOGICAL INDEX	GRADES									
	N	No	I	No	II	No	III	No	IV	No
Functional liver	39,54 ± 14,13	22	112,36 ± 75,22	19	91,92 ± 66,36	14	50,90 ± 34,69	11	85,27 ± 139,28	18
Rejection	39,54 ± 14,13	22	111,11 ± 67,36	9	101,50 ± 80,07	20	73,20 ± 42,21	10	45,23 ± 23,72	21

TABLE 73b MEAN S.G.O.T. LEVELS FOR THE DIFFERENT GRADES. (Mean ± 1 SD)

HISTOLOGICAL INDEX	GRADES							
	N-I	N-II	N-III	N-IV	I-II	I-IV	II-III	III-IV
Functional liver	<0,001	<0,001	-	-	-	<0,02	<0,02	-
Rejection	<0,001	<0,001	<0,005	-	-	<0,001	-	<0,025
$p_<$ = significance. - = no significant difference. 0 = no or insufficient data.								

TABLE 73c THE SIGNIFICANCE OF CHANGES IN MEAN S.G.O.T. LEVELS

FIGURE 73 MEAN S.G.O.T. LEVELS RELATED TO DONOR LIVER QUALITY - TIME UNRELATED

(a) Functional Liver Index

A highly significant elevation above normal is seen in mean levels related to grades I and II while those related to grades III and IV do not show elevations significantly different from normal.

(b) Rejection Index

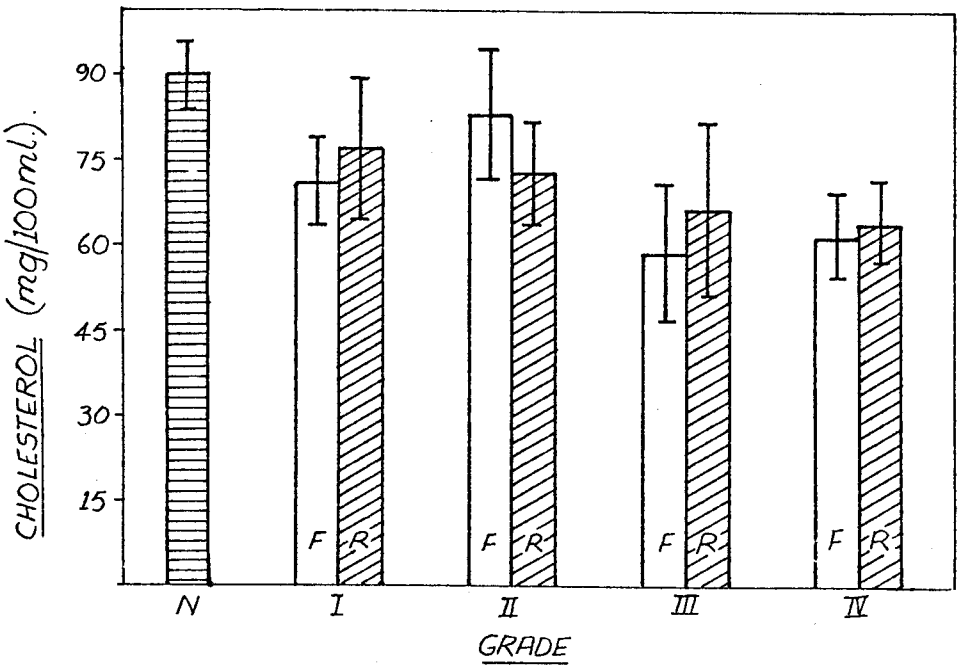
The mean levels related to grades I, II and III are elevated significantly above normal. However, the mean levels related to grades III and IV are significantly lower than the mean levels related to grade I.

(c) Interpretation

The mean S.G.O.T. levels do not relate well to donor liver quality for practical purposes. The animals with grade IV donor livers (severe rejection and/or severe hepatocyte loss) reveal mean levels which are not significantly different to the mean pre-operative levels, while those with the best quality donor livers (grade I), reveal the highest mean S.G.O.T. levels. The peaks in S.G.O.T. levels in the grade IV livers may have been missed, as the comparative analysis was carried out at 7 day intervals. S.G.O.T. could thus be misleading in the case of a patient who comes in from home some time after liver transplant, and in whom one wishes to assess donor liver function at that moment in time.

5. Serum Cholesterol

The mean cholesterol levels related to the different grades of Function and Rejection are illustrated in Histogram 74a, and tabu-



F = Functional Liver index
R = Rejection index
N = Pre-operative normal.

HISTOGRAM 74a MEAN CHOLESTEROL LEVELS FOR THE TWO HISTOLOGICAL GROUPS. (Mean ± 1 SEM)

HISTOLOGICAL INDEX	GRADES									
	N	No	I	No	II	No	III	No	IV	No
Functional liver	89,82 ± 23,38	17	71,22 ± 32,02	18	83,14 ± 41,15	14	58,88 ± 33,44	9	62,12 ± 28,93	16
Rejection	89,82 ± 23,38	17	77,11 ± 35,48	9	72,94 ± 37,78	19	66,75 ± 39,93	8	64,47 ± 29,83	19

TABLE 74b MEAN CHOLESTEROL LEVELS FOR THE DIFFERENT GRADES. (Mean ± 1 SD)

HISTOLOGICAL INDEX	GRADES							
	N-I	N-II	N-III	N-IV	I-II	I-IV	II-III	III-IV
Functional liver	-	-	<0,02	<0,005	-	-	-	-
Rejection	-	-	-	<0,01	-	-	-	-

p< = significance. - = no significant difference. 0 = no or insufficient data.

TABLE 74c THE SIGNIFICANCE OF CHANGES IN MEAN CHOLESTEROL LEVELS

FIGURE 74 MEAN CHOLESTEROL LEVELS RELATED TO DONOR LIVER QUALITY - TIME UNRELATED

lated in Table 74b on the opposite page.

(a) Functional Liver Index

The mean levels related to the functional grades III and IV were depressed significantly below normal, but there were no significant differences between mean levels related to grades I to IV.

(b) Rejection Index

The mean levels related to the rejection grades revealed no significant changes except for an isolated significant depression of the mean level related to grade IV donor livers.

(c) Interpretation

There were no significant differences between the mean serum cholesterol levels related to grades I and IV for either the Functional Index, or the Rejection Index, and cholesterol is thus not a sensitive factor for assessing the quality of the donor livers. The depression of the mean levels to below normal in the animals with grade IV donor liver biopsies, parallels the depression seen in the portacaval-shunted group 2(a), and the mean levels are significantly lower than the mean levels seen in the sham operated group 2(b). These depressed mean cholesterol levels may reflect the effects of either a dietary insufficiency, or an effective portosystemic shunt, as discussed by Hickman (91). The serum cholesterol is thus not an accurate index of donor liver quality.

6. Total Protein, Albumin and Globulin

The mean levels related to the Functional Grades are tabulated in the Appendix, page 367, and those related to the Rejection Grades on page 368. The significances of differences in the mean levels between the grades are summarised in Tables 76 and 77 on pages 369-370 respectively.

There were no sustained, significant differences in mean levels between grades in either the Functional or Rejection study, and these three factors appear to be of no value as an index of donor liver quality in the transplant model under discussion.

7. Haemoglobin

The mean haemoglobin levels related to the grades of function and rejection are tabulated in the Appendix, pages 367-368, and the significances of the differences in mean levels summarised on pages 369 and 370.

The mean haemoglobin levels were depressed significantly below normal for donor liver grades I - IV in both the functional and rejection studies. There were, however, no significant differences between the mean levels related to grades I - IV. Other factors, already discussed in Part II, may have been responsible for the anaemia seen in the transplanted animals, and the haemoglobin levels do not relate to the donor liver quality.

8. Leucocyte Count

The mean leucocyte count levels related to the different grades are tabulated in the Appendix, pages 367-368 and the significances of the differences in mean levels are summarised on pages 369-370.

For both the functional and rejection study, the mean levels related to donor liver grades I - IV were significantly elevated above normal, but there were no significant differences between the mean levels related to grades I - IV. Leucocytosis was also seen in the control animals, and other factors may have been responsible for the leucocytosis, as discussed in Part II. The leucocyte count has been shown to bear no relationship to donor liver quality.

9. Platelet Count

The mean platelet count levels, tabulated in the Appendix, pages 367-368, revealed large standard deviations, and an accurate statistical analysis could not be performed (as discussed on page 217). It can be seen, however, that the mean values do not differ greatly from normal for any of the grades, and there is no consistent pattern of changes between grades. Thus the platelet count proved to be of no diagnostic value for either the functional or rejection study. The lack of platelet change in rejection in liver transplantation has previously been reported (96).

10. Differential White Cell Count

The mean percentage levels for neutrophils, lymphocytes, eosinophils, basophils and monocytes related to the donor liver grades, are tabulated in the Appendix, on page 367 for the functional grades, and page 368 for the rejection grades. The significances of differences in mean levels between grades are summarised on pages 369-370.

There were no significant, sustained patterns of changes in the mean percentage levels for any of the cell types. The elevation in mean neutrophil levels and decrease in mean percentage monocyte levels in the animals with functional grade III livers has no practical diagnostic application.

The differential white cell count has thus been shown to be of no value in assessing either the functional or rejection quality of the donor livers.

11. Acid-Base Status

The mean pH, pCO_2 and standard bicarbonate levels related to the different grades are tabulated in the Appendix, pages 367-368, and the significances of differences between mean levels are summarised on pages 369-370.

There was no consistent, sustained pattern of changes between grades for either the functional or rejection analysis. The animals with grade IV livers exhibited metabolic acidosis, but the factors were not of value in distinguishing between grades. The acid-base status has been shown to be of no value in assessing either the functional or rejection quality of the donor livers.

DISCUSSION IN CONTEXT

The time-unrelated study of the relationship between the histological quality of the donor livers, (both on the basis of functional quality and degree of rejection) and the changes that occurred in the various biochemical and haematological factors in the recipients, has revealed that of the 19 factors assessed, only 1, CSF glutamine, showed a close relationship to the grades of donor liver quality, in the present experimental model. Changes were seen in some of the other factors, but these were either sporadic, explicable on the basis of other causes, or misleading.

In conclusion, CSF glutamine has been shown to be the only factor to relate accurately to the quality of the donor liver, in both the time-related and time-unrelated studies.

PART III

CHAPTER 4

OVERALL DISCUSSION AND CONCLUSIONS

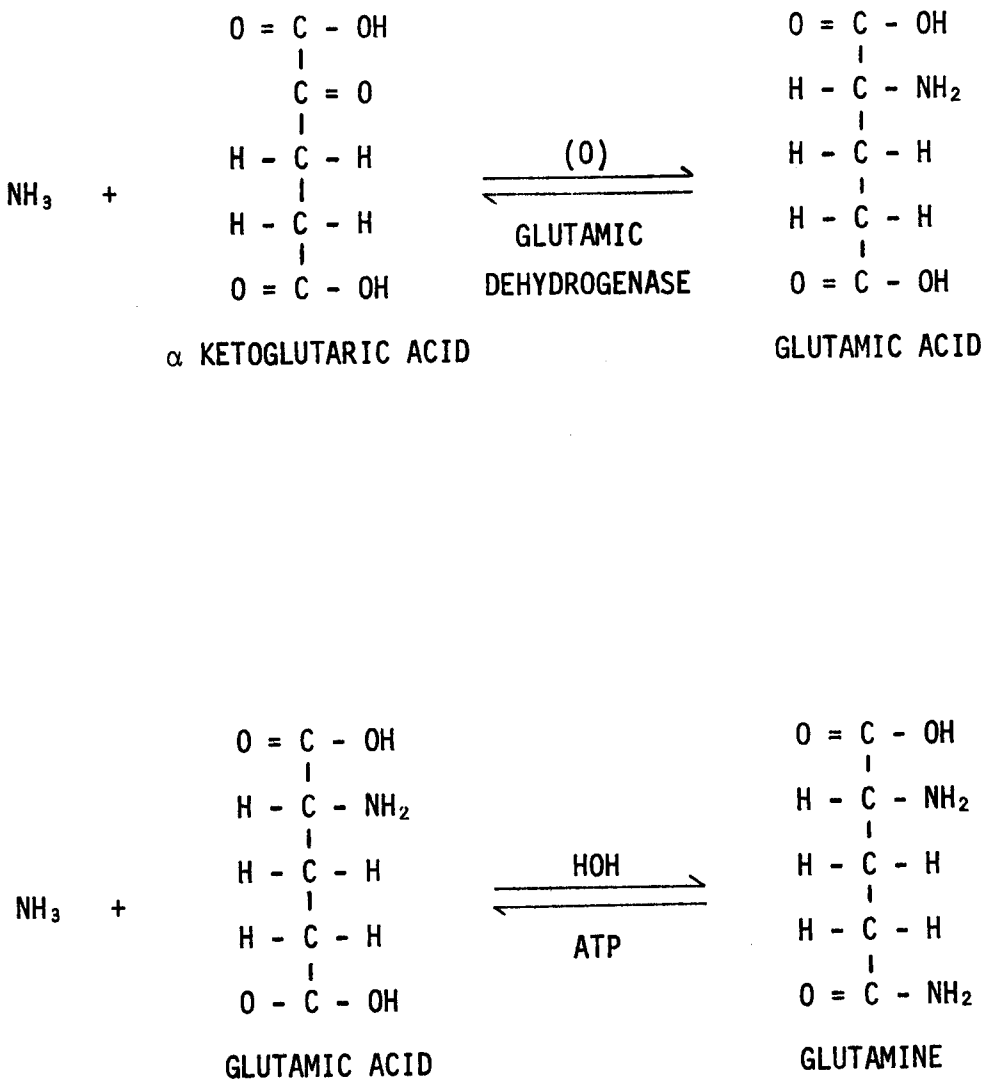


FIGURE 75 BIOSYNTHESIS OF GLUTAMINE IN THE BRAIN (26)

PART III

CHAPTER 4

OVERALL DISCUSSION AND CONCLUSIONS

OVERALL DISCUSSION

A detailed study has been performed to determine the relationship between donor liver quality, and the biochemical and haematological changes that occurred in the recipient animals, in a series of fully-portalised heterotopic porcine allografts. The relationship was investigated on both a time-related and a time-unrelated basis. Both studies revealed that CSF glutamine was the only factor, out of the 19 tested, which related accurately to the quality of the donor liver. The recipient livers of the 22 animals used in this study were shown to be essentially normal in Part II of this presentation.

CSF glutamine levels are used clinically in the differential diagnosis of the hepatic coma (93, 154), and the levels have been shown to relate well to the degree of hepatic encephalopathy (93). The glutamine levels are not elevated in non-hepatic causes of coma (29, 73, 93, 194, 219). In 1955 Bessman and Bessman (26) proposed a theory on the pathogenesis of hepatic encephalopathy. They suggested that in severe liver disease, inadequate hepatic disposal of ammonia causes excessive ammonia to pass into the central nervous system. In the central nervous system the ammonia undergoes the reactions depicted in Figure 75 on the opposite page. One molecule of

ammonia combines with alpha-ketoglutarate to form glutamic acid, which then combines with another molecule of ammonia to form glutamine. The end result of these reactions is the consumption of some ATP, and the depletion of alpha-ketoglutarate, leading to reduced activity of the Krebs cycle, and coma. Glutamine is readily diffusible into the CSF (74), making estimations practicable. The genesis of changes in CSF glutamine have been reviewed by Hourani (93), Lund (118) and Williams (220), and the original theory of Bessman has been challenged, but not disproved.

Williams (220) has shown a good correlation between the level of the plasma ammonium ion and the glutamine levels in the brain of rats following end-to-side portacaval shunts. Hickman (87) reported a significant correlation between CSF glutamine and the arterial ammonia levels in pigs following end-to-side portacaval shunts. The origin and metabolism of blood ammonia have been reviewed by Breen (30), Gips (74), Lund (118), Stahl (177) and Zimmerman (229). The main source of blood ammonia appears to be from the gastrointestinal tract, where bacterial degradation of ingested protein, and circulating urea, releases the ammonium ion, which is readily diffusible into portal blood. The portal blood has a high concentration of ammonia (118). In the presence of a normal liver, the portal blood is almost completely cleared of ammonia, mainly by the synthesis of ammonia into urea (62, 118).

In the presence of a portosystemic shunt, the portal blood bypasses the liver, and the heart receives venous blood with a high ammonia concentration, with resultant higher levels emerging on the arterial side, and with resultant higher ammonia concentrations in the arterial blood

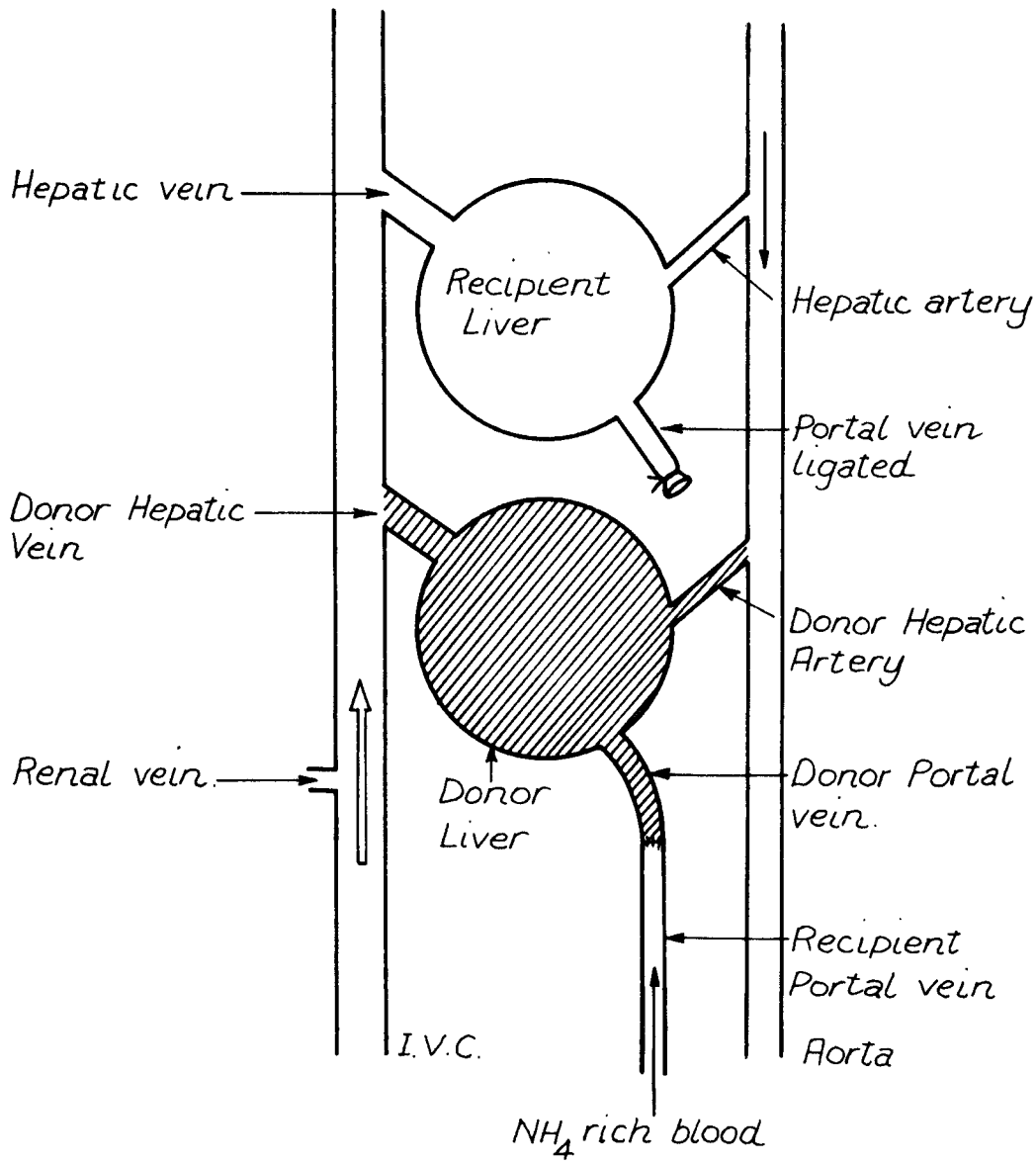


FIGURE 76

DIAGRAM OF THE ARTERIALISED DONOR LIVER INTERPOSED ACROSS A PORTACAVAL SHUNT

supplying the brain. The brain glutamine rises, and consequently the CSF glutamine. The systemic venous blood ammonia also rises, but not necessarily in proportion to the increase in arterial ammonia, as the muscles and kidneys can add or extract ammonia. This makes venous blood ammonia values less reliable in the assessment of liver function than the arterial values (Stahl, 177).

The mechanisms whereby blood ammonia is elevated in liver diseases, have been well reviewed by Stahl (177). Three mechanisms have been postulated. Firstly, there may be the formation of substantial collateral portosystemic channels, with much of the portal blood bypassing the liver, and creating a situation analogous to a direct portacaval shunt. Secondly, there may be the formation of rich anastomoses between branches of the portal and hepatic veins within the liver, deflecting a certain amount of ammonia-rich portal blood away from the liver cells, and thus creating anatomical transhepatic shunting. Thirdly, diseased liver cells may not be able to function adequately to cope with the ammonia load, thus creating a functional transhepatic portosystemic shunt. The relative importance of each of these mechanisms in the elevation of blood ammonia in liver disease is controversial (177), and remains to be fully elucidated.

The clear relationship that has been demonstrated between the histological quality of the donor livers, and the CSF glutamine levels of the recipient animals in the present series, can be explained on the basis of the principles involved in the foregoing discussion.

The technique used in the heterotopic transplant model under discussion, simply interposes an arterialised donor liver across a portacaval shunt, as illustrated in the diagram on the opposite page. The least elevation in

CSF glutamine was seen in the recipient animals in whom the donor livers revealed the lowest grade of rejection and structural damage. It appears reasonable to assume that the good quality donor livers were functioning adequately to clear the ammonia from the portal blood, and that there was minimal or no perihepatic or transhepatic shunting of ammonia-rich blood. Conversely, the grossly elevated CSF glutamine and venous ammonia levels seen in the recipient animals with gross rejection and hepatocellular destruction (grade IV) of the donor livers, reveal that the portal blood was incompletely cleared of ammonia. One or all three of the mechanisms described may have been responsible, but the relative importance of each mechanism cannot be deduced from the present study. No flow studies could be performed (45, 88) and transhepatic sampling as described by Hickman (90) was not carried out. No gross portosystemic collaterals were detected, but this does not exclude multiple small perihepatic channels.

Nevertheless, a clear relationship has been demonstrated between the histological indices of the donor livers, and the CSF glutamine. This was the only factor that related well to grades of liver quality, whatever the actual mechanism might have been.

The use of CSF glutamine as an index of donor liver quality in this model, has several advantages. The fluid is readily obtained, and the estimation was found to be quick, reliable,and the results readily reproducible. CSF glutamine elevation appears specific for hepatic dysfunction, and was the most reliable of the 19 factors tested in this experimental work. In the clinical context, there may be several disadvantages to its use. Repeated lumbar punctures for management purposes are uncomfortable for the

patient, and have the potential complications of cord damage, bleeding in the face of possible coagulation defects, and the danger of introducing infective organisms into the CSF of the immunosuppressed patients.

The exact relationship between donor hepatic venous blood ammonia, arterial ammonia, CSF glutamine, and donor liver quality in this model, needs to be investigated. Should arterial ammonia prove to be as reliable an index of donor liver quality as CSF glutamine, one would have a test in which the sampling is simple and safe, and which can be done frequently. The accuracy of blood ammonia estimations, however, leaves a lot to be desired (83) but this is a challenge to be met by the biochemists.

CONCLUSIONS

The third major objective in this experimental study, was to assess the value of CSF glutamine as an index of donor liver quality in the fully-portalised heterotopic porcine liver transplant model described in Part II, Chapter 2, and to compare its value against other biochemical and haematological factors, outlined on page 197F.

The CSF glutamine levels have been shown to be an accurate, reliable index of donor liver quality in this experimental model. None of the other 18 factors tested revealed the same reliable, consistent relationship to the quality of the donor liver.

The destruction of hepatocytes in the donor livers was shown to be due to rejection, and the CSF glutamine in this series was thus a useful index of rejection.

The principles underlying the relationship between the quality of the donor livers and the CSF glutamine levels, have been presented, and shown to be analogous to the principles involved in the changes in CSF glutamine seen in acute liver failure.

The disadvantages of the use of CSF glutamine as a clinical diagnostic tool in immunosuppressed recipients have been mentioned, and further studies of the relationship between donor hepatic venous ammonia, arterial ammonia, CSF glutamine and donor liver quality are proposed.

APPENDIX

A P P E N D I X

* Supplementary Index at the beginning of the Section.

APPENDIX 1

ABBREVIATED CASE HISTORIES OF THE ANIMALS

1. LOCATION:

2. ABBREVIATIONS USED:

NAD = No abnormality detected

NR = Not recorded

Excell = Excellent

\- = (a) Nil of note - in the clinical details

(b) No anastomosis performed - in the pathology

(c) No or insufficient data - in the Biochemistry haematology and histology.

PIG NO. 1 GROUP 1(a) SURVIVAL: 13 Days

OPERATION: Heterotopic transplant with cholecysto-cholecystostomy

	DONOR	RECIPIENT
Breed	Not recorded (N.R.)	Not recorded
Animal weight	16 kg.	35 kg.
Liver weight	582 g. (estimated)	1274 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Sacrifice - humane. Animal ill, abscess on neck. Massive abdominal wound sepsis with partial breakdown

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +++	Renal: NAD
Wound breakdown: Partial	Lymph node: N.R.
Gastric: NAD	Pancreatic: N.R.
Intestinal: NAD	Bladder: NAD
Pulmonary: Multiple abscesses right lung	Other: Gross intraperitoneal adhesions
Cardiac: NAD	

LIVER	DONOR	RECIPIENT
Appearance	"Normal". Abscess in one lobe	Normal
Weight	310 g.	920 g.
Vascular anastomoses	Adequate	-
Vessel patency	Aortic cuff thrombosed Other vessels patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal, densely adherent	Normal
Anastomoses	Intact	-
Bile stasis	Nil	Nil
Stoma size	Adequate	-
Other	Small sealed off bile leak found	

		DAY	0	7	14	21	28	
CLINICAL		Appearance	Good	Fair	Ill			
		Vigor	-	-	Fair			
		Wound Sepsis	-	++	+++			
		Weight - kg	35	NR	NR			
BIOCHEMISTRY		CSF Glutamine	-	-	-			
		Venous ammonia	-	-	-			
		Arterial ammonia	-	-	-			
		Alkaline phosphatase	-	-	-			
		S.G.O.T.	-	-	-			
		Cholesterol	-	-	-			
		Total protein	-	-	-			
		Albumin	-	-	-			
		Globulin	-	-	-			
HAEMATOLOGY		Haemoglobin	-	-	-			
		Leucocytes x 10^3	-	-	-			
		Platelets x 10^3	-	-	-			
		Neutrophils %	-	-	-			
		Lymphocytes %	-	-	-			
		Eosinophils %	-	-	-			
		Basophils %	-	-	-			
		Monocytes %	-	-	-			
ACID BASE BALANCE		pH	-	-	-			
		pCO_2	-	-	-			
		Std. Bicarbonate	-	-	-			
LIVER HISTOLOGY	DONOR	Functional Index	N	-	III			
		Rejection Degree	N	-	III			
		Cholestasis	0	-	0			
		Cholangitis	0	-	0			
	RECIPIENT	Functional Index	N	-	N			
		Rejection Degree	N	-	N			
		Cholestasis	0	-	0			
		Cholangitis	0	-	+			

PIG NO. 2 GROUP 1(a) SURVIVAL: 23 Days

OPERATION: Heterotopic transplant with cholecysto-cholecystostomy

	DONOR	RECIPIENT
Breed	White	Black
Animal weight	16 kg.	37 kg.
Liver weight	582 g. (estimated)	1346 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Found dead. No cause determined.

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +++	Renal: NAD
Wound breakdown: Nil	Lymph node: NAD
Gastric: NAD	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Mild bronchopneumonia	Other: Pelvic abscess
Cardiac: NAD	

LIVER	DONOR	RECIPIENT
Appearance	Gas gangrene. Small abscess Segmental infarction	Normal
Weight	N.R.	N.R.
Vascular anastomoses	Adequate	-
Vessel patency	All vessels patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	NAD	-
Bile stasis	Nil	Nil
Stoma size	Adequate	-
Other	No bile leaks	

		DAY	0	7	14	21	23	
CLINICAL		Appearance	Good	Good	Good	Good	Dead	
		Vigor	-	Good	Good	Good	-	
		Wound Sepsis	-	-	+	+++	+++	
		Weight - kg	37	-	-	35	35	
BIOCHEMISTRY		CSF Glutamine	-	-	-	-	-	
		Venous ammonia	-	-	-	-	-	
		Arterial ammonia	-	-	-	-	-	
		Alkaline phosphatase	5,1	9,7	4,3	-	-	
		S.G.O.T.	30	35	35	-	-	
		Cholesterol	-	-	-	-	-	
		Total protein	-	-	-	-	-	
		Albumin	-	-	-	-	-	
		Globulin	-	-	-	-	-	
HAEMATOLOGY		Haemoglobin	-	-	-	-	-	
		Leucocytes x 10^3	-	-	-	-	-	
		Platelets x 10^3	-	-	-	-	-	
		Neutrophils %	-	-	-	-	-	
		Lymphocytes %	-	-	-	-	-	
		Eosinophils %	-	-	-	-	-	
		Basophils %	-	-	-	-	-	
		Monocytes %	-	-	-	-	-	
ACID BASE BALANCE		pH	7,315	-	-	-	-	
		pCO_2	39,5	-	-	-	-	
		Std. Bicarbonate	19,5	-	-	-	-	
LIVER HISTOLOGY	DONOR	Functional Index	N	-	-	-	G	
		Rejection Degree	N	-	-	-	A	
		Cholestasis	0	-	-	-	S	
		Cholangitis	0	-	-	-		
	RECIPIENT	Functional Index	N	-	-	-	N	
		Rejection Degree	N	-	-	-	N	
		Cholestasis	0	-	-	-	0	
		Cholangitis	0	-	-	-	0	

PIG NO. 3 GROUP 1(a) SURVIVAL: 56 Days

OPERATION: Heterotopic transplant with cholecysto-cholecystostomy

	DONOR	RECIPIENT
Breed	Large White	Landrace
Animal weight	17 kg.	42 kg.
Liver weight	690 g.	1529 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Sacrifice - humane. Ill, pale, weak, unable to walk.

ASSOCIATED GENERAL PATHOLOGY

Wound sepsis: ++	Renal: NAD
Wound breakdown: Nil	Lymph node: Strikingly reactive
Gastric: Large ulcer	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Mild pneumonia and pleurisy	Other: No ascites No collateral vessels seen
Cardiac: NAD	

LIVER	DONOR	RECIPIENT
Appearance	Grey paste - total infarction	Normal
Weight	N.R.	1250 g.
Vascular anastomoses	Unable to dissect out	-
Vessel patency	All thrombosed	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Sloughed	Normal
Anastomoses		-
Bile stasis	Unable to assess	Nil
Stoma size		-
Other	No obvious bile leak	

		DAY	0	7	14	21	28	56
CLINICAL		Appearance	Good	Good	Good	Fair	Poor	Ill
		Vigor	-	Good	Good	Fair	Fair	Poor
		Wound Sepsis	-	0	0	0	0	+
		Weight - kg	42	-	-	40	38	40
BIOCHEMISTRY		CSF Glutamine	-	-	-	-	-	44,1
		Venous ammonia	-	-	-	-	-	260
		Arterial ammonia	-	-	-	-	-	-
		Alkaline phosphatase	4,7	4,3	2,5	1,6	1,8	1,3
		S.G.O.T.	20	40	20	25	20	20
		Cholesterol	76	100	88	55	48	44
		Total protein	7,6	6,2	7,5	6,8	6,8	6,4
		Albumin	2,3	1,2	2,0	1,4	0,98	0,98
		Globulin	5,3	5,0	5,5	5,4	5,8	5,4
HAEMATOLOGY		Haemoglobin	10,5	9,5	8,0	9,5	7,2	7,8
		Leucocytes x 10^3	-	-	-	31,3	33,8	27,1
		Platelets x 10^3	-	-	-	429	206	855
		Neutrophils %	-	-	-	56	56	51
		Lymphocytes %	-	-	-	23	26	27
		Eosinophils %	-	-	-	1	5	11
		Basophils %	-	-	-	0	1	2
		Monocytes %	-	-	-	20	12	9
ACID BASE BALANCE		pH	7,230	-	-	-	-	-
		pCO_2	54	-	-	-	-	-
		Std. Bicarbonate	19	-	-	-	-	-
LIVER HISTOLOGY	DONOR	Functional Index	N	-	-	-	-	IV
		Rejection Degree	N	-	-	-	-	Infarcted
		Cholestasis	0	-	-	-	-	
		Cholangitis	0	-	-	-	-	
	RECIPIENT	Functional Index	N	-	-	-	-	N
		Rejection Degree	N	-	-	-	-	N
		Cholestasis	0	-	-	-	-	0
		Cholangitis	0	-	-	-	-	0

PIG NO. 4 GROUP 1(a) SURVIVAL: 33 Days

OPERATION: Heterotopic transplant with cholecysto-cholecystostomy

	DONOR	RECIPIENT
Breed	Not recorded	Not recorded
Animal weight	23 kg.	46 kg.
Liver weight	1050 g.	1674 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Pericarditis. Sepsis

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +	Renal: Left hydronephrosis
Wound breakdown: Nil	Lymph node: Mildly reactive
Gastric: Ulcer	Pancreatic: Mild pancreatitis
Intestinal: NAD	Bladder: NAD
Pulmonary: Pleural adhesions and pneumonia	Other: Fat necrosis around pancreas Shoulder and subphrenic abscess
Cardiac: Severe pericarditis	

LIVER	DONOR	RECIPIENT
Appearance	"Normal"	Normal
Weight	850 g.	1050 g.
Vascular anastomoses	Adequate	-
Vessel patency	All patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Thickened. Dilated	Normal
Anastomoses	Unable to assess	-
Bile stasis	Inspissated bile ++++	Nil
Stoma size	Unable to determine	-
Other	Gross adhesions over conjoined gallbladders. No bile leaks. Unable to assess biliary anastomosis and stoma adequately.	

	DAY	0	7	14	21	28	33
CLINICAL	Appearance	Good	Fair	Poor	Poor	Fair	Dead
	Vigor	-	Fair	Poor	Poor	Fair	-
	Wound Sepsis	-	++	+++	++	+	+
	Weight - kg	46	43	-	44	34	30
BIOCHEMISTRY	CSF Glutamine	-	-	-	-	19	-
	Venous ammonia	-	-	-	-	226	-
	Arterial ammonia	-	-	-	-	-	-
	Alkaline phosphatase	13,2	10,1	5,8	5,6	2,9	-
	S.G.O.T.	40	80	55	55	70	-
	Cholesterol	-	66	-	53	-	-
	Total protein	-	6,6	7,0	6,6	-	-
	Albumin	-	1,6	1,0	0,9	-	-
	Globulin	-	5,0	6,0	5,7	-	-
HAEMATOLOGY	Haemoglobin	14,2	13,3	10,2	9,6	7,7	-
	Leucocytes x 10^3	18,2	32,7	17,2	65,8	40,4	-
	Platelets x 10^3	352	165	89	784	510	-
	Neutrophils %	44	52	16	77	72	-
	Lymphocytes %	41	37	48	12	19	-
	Eosinophils %	6	3	5	0	2	-
	Basophils %	1	1	3	0	1	-
	Monocytes %	8	7	28	11	6	-
ACID BASE BALANCE	pH	-	-	-	-	-	-
	pCO_2	-	-	-	-	-	-
	Std. Bicarbonate	-	-	-	-	-	-
LIVER HISTOLOGY DONOR	Functional Index	N	-	-	II	-	III
	Rejection Degree	N	-	-	II	-	III
	Cholestasis	0	-	-	0	-	0
	Cholangitis	0	-	-	0	-	+++
LIVER HISTOLOGY RECIPIENT	Functional Index	N	-	-	N	-	N
	Rejection Degree	N	-	-	N	-	N
	Cholestasis	0	-	-	0	-	0
	Cholangitis	0	-	-	0	-	0

PIG NO. 5 GROUP 1(a) SURVIVAL: 8 Days

OPERATION: Heterotopic transplant with cholecysto-cholecystostomy

	DONOR	RECIPIENT
Breed	Not recorded	Not recorded
Animal weight	14 kg.	38 kg.
Liver weight	430 g.	1383 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Sacrifice - humane. Emaciated, ill, haematuria.

ASSOCIATED GENERAL PATHOLOGY

Wound sepsis: Nil
Wound breakdown: Nil
Gastric: NAD
Intestinal: NAD
Pulmonary: Pneumonia and hamartomatous tumour
Cardiac: NAD
Renal: NAD
Lymph node: N.R.
Pancreatic: NAD
Bladder: Haemorrhagic - no mucosa
Other: Large organized intra-peritoneal haematoma

LIVER	DONOR	RECIPIENT
Appearance	"Normal". Multiple small infarcts	Normal
Weight	270 g.	800 g.
Vascular anastomoses	Adequate	-
Vessel patency	All patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	NAD	-
Bile stasis	Minimal gravel	Nil
Stoma size	Small but adequate	-
Other	No bile leaks	

		DAY	0	7	8			
CLINICAL		Appearance	Good	III	III++			
		Vigor	-	Poor	Poor			
		Wound Sepsis	-	0	-			
		Weight - kg	38	-	-			
BIOCHEMISTRY		CSF Glutamine	-	-	-			
		Venous ammonia	-	-	-			
		Arterial ammonia	-	-	-			
		Alkaline phosphatase	8,6	10,3	8,8			
		S.G.O.T.	40	45	100			
		Cholesterol	81	-	94			
		Total protein	5,8	-	6,5			
		Albumin	1,6	-	1,4			
		Globulin	4,2	-	5,1			
HAEMATOLOGY		Haemoglobin	11,5	15,2	8,2			
		Leucocytes x 10^3	20,8	11,4	30,2			
		Platelets x 10^3	425	907	390			
		Neutrophils %	54	78	82			
		Lymphocytes %	27	8	14			
		Eosinophils %	9	0	0			
		Basophils %	2	2	0			
		Monocytes %	8	12	4			
ACID BASE BALANCE		pH	7,225	-	-			
		pCO_2	46	-	-			
		Std. Bicarbonate	17	-	-			
LIVER HISTOLOGY	DONOR	Functional Index	N	-	III			
		Rejection Degree	N	-	III			
		Cholestasis	0	-	+			
		Cholangitis	0	-	0			
	RECIPIENT	Functional Index	N	-	N			
		Rejection Degree	N	-	N			
		Cholestasis	0	-	0			
		Cholangitis	0	-	0			

PIG NO. 6 GROUP 1(a) SURVIVAL: 11 Days

OPERATION: Heterotopic transplant with cholecysto-cholecystostomy

	DONOR	RECIPIENT
Breed	Red Landrace	Red Landrace
Animal weight	20 kg.	35 kg.
Liver weight	700 g.	1274 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Small bowel obstruction due to retained swab

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: Nil	Renal: NAD
Wound breakdown: Nil	Lymph node: Strikingly reactive
Gastric: Shallow ulcer	Pancreatic: NAD
Intestinal: Small bowel obstruction	Bladder: NAD
Pulmonary: Pneumonia and pulmonary oedema	Other: -
Cardiac: Excess pericardial fluid	

LIVER	DONOR	RECIPIENT
Appearance	"Normal". Patchy infarcts	Normal
Weight	525 g.	1200 g.
Vascular anastomoses	Adequate	-
Vessel patency	All patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	NAD	-
Bile stasis	Thickened bile	Thickened bile
Stoma size	Small but adequate	-
Other	No bile leaks	

		DAY	0	7	11			
CLINICAL		Appearance	Good	Good	Good			
		Vigor	-	Good	Dead			
		Wound Sepsis	-	0	0			
		Weight - kg	35	38	32			
BIOCHEMISTRY		CSF Glutamine	10,2	24	-			
		Venous ammonia	207	210	-			
		Arterial ammonia	147	-	-			
		Alkaline phosphatase	6,4	7,9	-			
		S.G.O.T.	30	80	-			
		Cholesterol	66	52	-			
		Total protein	6,4	6,5	-			
		Albumin	1,5	2,0	-			
		Globulin	4,9	4,5	-			
HAEMATOLOGY		Haemoglobin	12,1	10,1	-			
		Leucocytes x 10^3	13,3	35,3	-			
		Platelets x 10^3	457	176	-			
		Neutrophils %	53	73	-			
		Lymphocytes %	40	20	-			
		Eosinophils %	1	2	-			
		Basophils %	1	1	-			
		Monocytes %	5	4	-			
ACID BASE BALANCE		pH	7,375	7,115	-			
		pCO_2	34	55	-			
		Std. Bicarbonate	19,5	15	-			
LIVER HISTOLOGY	DONOR	Functional Index	N	IV	IV			
		Rejection Degree	N	IV	IV			
		Cholestasis	0	0	0			
		Cholangitis	0	0	0			
	RECIPIENT	Functional Index	N	N	N			
		Rejection Degree	N	N	N			
		Cholestasis	0	0	+			
		Cholangitis	0	0	0			

PIG NO. 7 GROUP 1(a) SURVIVAL: 28 Days

OPERATION: Heterotopic transplant with cholecysto-cholecystostomy and gastro-duodenostomy

	DONOR	RECIPIENT
Breed	Landrace	Black (?breed)
Animal weight	20 kg.	44 kg.
Liver weight	790 g.	1602 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +	Renal: NAD
Wound breakdown: Nil	Lymph node: Mildly reactive
Gastric: Deep penetrating ulcer	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Mild pneumonia	Other: Gastro-duodenostomy healed and widely patent Intra-peritoneal abscess at site of subcostal incision
Cardiac: NAD	

LIVER	DONOR	RECIPIENT
Appearance	"Normal"	Normal
Weight	480 g.	1020 g.
Vascular anastomoses	Adequate	-
Vessel patency	Hepatic arterial branch thrombosed Remaining vessels patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	Concretions on suture line	-
Bile stasis	Minimal	Nil
Stoma size	Large	-
Other	No bile leaks	

		DAY	0	7	14	21	28	
CLINICAL		Appearance	Good	Poor	Good	Good	Good	
		Vigor	-	Fair	Good	Good	Good	
		Wound Sepsis	-	0	0	0	+	
		Weight - kg	44	40	43	43	40	
BIOCHEMISTRY		CSF Glutamine	10,5	29,7	48,5	34,2	25,1	
		Venous ammonia	204	265	294	469	275	
		Arterial ammonia	102	-	347	331	-	
		Alkaline phosphatase	3,3	6,9	5,0	3,7	1,1	
		S.G.O.T.	65	100	35	40	35	
		Cholesterol	102	60	97	54	11	
		Total protein	6,3	9,6	6,5	7,3	6,3	
		Albumin	1,7	2,2	1,9	1,9	1,2	
		Globulin	4,6	7,4	4,6	5,4	5,1	
HAEMATOLOGY		Haemoglobin	14,1	12,7	13,0	12,3	9,0	
		Leucocytes x 10^3	19,2	26,6	13,9	18,8	58,0	
		Platelets x 10^3	321	318	200	281	207	
		Neutrophils %	43	41	36	13	51	
		Lymphocytes %	30	35	50	41	42	
		Eosinophils %	7	6	8	0	2	
		Basophils %	3	1	2	0	0	
		Monocytes %	17	17	4	46	5	
ACID BASE BALANCE		pH	7,170	-	7,325	7,275	7,185	
		pCO_2	57	-	43	38,5	52	
		Std. Bicarbonate	16,5	-	21	16,5	17,3	
LIVER HISTOLOGY	DONOR	Functional Index	N	IV	IV	IV	IV	
		Rejection Degree	N	IV	IV	IV	IV	
		Cholestasis	0	0	0	0	0	
		Cholangitis	0	+	0	0	+	
	RECIPIENT	Functional Index	N	N	N	N	N	
		Rejection Degree	N	N	N	N	N	
		Cholestasis	0	0	0	0	0	
		Cholangitis	0	0	0	0	0	

PIG NO. 8 GROUP 1(a) SURVIVAL: 28 Days

OPERATION: Heterotopic transplant with cholecysto-cholecystostomy and gastro-duodenostomy

	DONOR	RECIPIENT
Breed	Landrace	Black
Animal weight	26 kg.	36 kg.
Liver weight	830 g.	1310 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +	Renal: NAD
Wound breakdown: Nil	Lymph node: Mildly reactive
Gastric: Large ulcer	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Mild pneumonia	Other: Thyroid NAD Gastro-duodenostomy patent
Cardiac: NAD	

LIVER	DONOR	RECIPIENT
Appearance	Congested	Normal
Weight	547 g.	842 g.
Vascular anastomoses	Adequate	-
Vessel patency	All patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	Small ulcer at anastomosis (microscopic)	-
Bile stasis	Nil	Nil
Stoma size	Large	-
Other	No bile leaks	

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Good	Good	Good	Fair	Fair	
	Vigor	-	Good	Good	Fair	Fair	
	Wound Sepsis	-	+	+	+	0	
	Weight - kg	36	33	35	33	31	
BIOCHEMISTRY	CSF Glutamine	12,5	14,7	21,8	13,9	18,2	
	Venous ammonia	192	262	237	211	278	
	Arterial ammonia	134	255	-	-	-	
	Alkaline phosphatase	5,4	5,9	6,4	2,2	1,8	
	S.G.O.T.	60	75	105	75	35	
	Cholesterol	91	94	114	16	11	
	Total protein	7,4	7,1	7,2	7,2	6,4	
	Albumin	2,1	1,7	1,6	1,7	1,4	
	Globulin	5,3	5,4	5,6	5,5	5,0	
HAEMATOLOGY	Haemoglobin	13,8	10,9	12,3	9,2	7,4	
	Leucocytes x 10^3	15,4	36,0	34,0	38,3	34,1	
	Platelets x 10^3	350	764	176	427	336	
	Neutrophils %	11	28	54	49	50	
	Lymphocytes %	57	29	42	26	39	
	Eosinophils %	7	9	1	4	3	
	Basophils %	0	0	2	1	1	
	Monocytes %	25	34	2	20	7	
ACID BASE BALANCE	pH	7,055	7,325	7,290	7,140	7,275	
	pCO_2	58	33,5	33	36,5	43	
	Std. Bicarbonate	12,8	18	17	12	19	
LIVER HISTOLOGY DONOR	Functional Index	N	I	II	II	III	
	Rejection Degree	N	I	II	II	III	
	Cholestasis	0	0	0	0	0	
	Cholangitis	0	0	0	0	0	
LIVER HISTOLOGY RECIPIENT	Functional Index	N	N	N	N	N	
	Rejection Degree	N	N	N	N	N	
	Cholestasis	0	0	0	0	0	
	Cholangitis	0	0	0	0	0	

PIG NO. 9 GROUP 1(a) SURVIVAL: 28 Days

OPERATION: Heterotopic transplant with cholecysto-cholecystostomy and gastro-duodenostomy

	DONOR	RECIPIENT
Breed	Large White	Landrace
Animal weight	19 kg.	40 kg.
Liver weight	545 g.	1456 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY

Wound sepsis: +	Renal: NAD
Wound breakdown: Nil	Lymph node: Reactive
Gastric: NAD	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Pleural adhesions ++	Other: Thyroid NAD Gastro-duodenostomy patent
Cardiac: Pericarditis	

LIVER	DONOR	RECIPIENT
Appearance	Firm. Patchy infarcts	Normal
Weight	1815 g.	1220 g.
Vascular anastomoses	Adequate	-
Vessel patency	Non-occlusive arterial thrombus Organized thrombus right hepatic vein	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	Concretions on suture line	-
Bile stasis	Nil	Nil
Stoma size	Large	-
Other	No bile leaks	

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Good	Good	Good	Good	Excel	
	Vigor	-	Good	Good	Good	Excel	
	Wound Sepsis	-	0	+	+	+	
	Weight - kg	40	37	35	42	45	
BIOCHEMISTRY	CSF Glutamine	9,4	10,8	22,8	11,3	13,3	
	Venous ammonia	138	-	257	232	256	
	Arterial ammonia	128	-	-	-	-	
	Alkaline phosphatase	7,1	6,3	9,1	4,3	3,9	
	S.G.O.T.	35	75	152	95	45	
	Cholesterol	114	-	124	119	56	
	Total protein	6,7	-	6,5	6,9	7,5	
	Albumin	2,1	-	2,1	1,9	1,3	
	Globulin	4,6	-	4,4	5,0	6,2	
HAEMATOLOGY	Haemoglobin	11,2	12,4	12,8	10,1	10,3	
	Leucocytes x 10^3	12,2	23,3	18,7	28,2	33,1	
	Platelets x 10^3	256	627	211	374	750	
	Neutrophils %	23	57	63	66	78	
	Lymphocytes %	39	30	21	19	17	
	Eosinophils %	28	8	6	3	0	
	Basophils %	0	0	3	0	2	
	Monocytes %	10	5	7	12	3	
ACID BASE BALANCE	pH	7,500	7,340	7,300	7,290	7,30	
	pCO_2	30	50	50	48	57	
	Std. Bicarbonate	26,3	24,5	21,5	21	24,5	
LIVER HISTOLOGY DONOR	Functional Index	N	I	II	Too small	III	
	Rejection Degree	N	II	III		IV	
	Cholestasis	0	0	0		0	
	Cholangitis	0	0	0		++	
LIVER HISTOLOGY RECIPIENT	Functional Index	N	N	N	N	N	
	Rejection Degree	N	N	N	N	N	
	Cholestasis	0	0	0	0	0	
	Cholangitis	0	0	0	0	0	

PIG NO. 10 GROUP I(a) SURVIVAL: 7 Days

OPERATION: Heterotopic transplant with cholecysto-cholecystostomy and gastro-duodenostomy

	DONOR	RECIPIENT
Breed	Large White	Landrace
Animal weight	17 kg.	40 kg.
Liver weight	680 g.	1456 g.
Other	-	-

MAIN CAUSE OF DEATH: Iatrogenic - anaesthetic
Cardiac arrest under Halothane anaesthesia

ASSOCIATED GENERAL PATHOLOGY

Wound sepsis: ++	Renal: NAD
Wound breakdown: Partial	Lymph node: Reactive
Gastric: Large ulcer	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: NAD	Other: Gastro-duodenostomy patent
Cardiac: NAD	

LIVER	DONOR	RECIPIENT
Appearance	"Normal"	Normal
Weight	680 g.	968 g.
Vascular anastomoses	Adequate with haemorrhage at aorta-aortic anastomosis	-
Vessel patency	Organized thrombus right hepatic vein Remaining vessels patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	Granulations	-
Bile stasis	Nil	Nil
Stoma size	Large	-
Other	No bile leaks	

		DAY	0	7				
CLINICAL		Appearance	Good	Good				
		Vigor	-	Good				
		Wound Sepsis	-	++				
		Weight - kg	40	39				
BIOCHEMISTRY		CSF Glutamine	12,2	16,7				
		Venous ammonia	188	-				
		Arterial ammonia	217	-				
		Alkaline phosphatase	6,4	3,6				
		S.G.O.T.	35	50				
		Cholesterol	-	-				
		Total protein	5,7	-				
		Albumin	2,2	-				
		Globulin	3,5	-				
HAEMATOLOGY		Haemoglobin	11,4	11,0				
		Leucocytes x 10^3	22,0	44,9				
		Platelets x 10^3	243	290				
		Neutrophils %	42	57				
		Lymphocytes %	31	26				
		Eosinophils %	10	7				
		Basophils %	0	1				
		Monocytes %	17	9				
ACID BASE BALANCE		pH	7,395	-				
		pCO_2	36	-				
		Std. Bicarbonate	22,4	-				
LIVER HISTOLOGY	DONOR	Functional Index	N	III				
		Rejection Degree	N	III				
		Cholestasis	0	0				
		Cholangitis	0	0		-		
	RECIPIENT	Functional Index	N	N				
		Rejection Degree	N	N				
		Cholestasis	0	0				
		Cholangitis	0	0				

PIG NO. 11 GROUP 1(a) SURVIVAL: 8 Days

OPERATION: Heterotopic transplant with cholecysto-cholecystostomy and gastro-duodenostomy

	DONOR	RECIPIENT
Breed	Landrace	Large White
Animal weight	25 kg.	40 kg.
Liver weight	835 g.	1456 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Abdominal wound dehiscence

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +++	Renal: NAD
Wound breakdown: Dehiscence	Lymph node: Reactive
Gastric: Large ulcer	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Mild pneumonia	Other: Gastro-duodenostomy patent
Cardiac: Myocardial scarring	

LIVER	DONOR	RECIPIENT
Appearance	"Normal" with grey colour	Normal
Weight	815 g.	700 g.
Vascular anastomoses	Adequate	-
Vessel patency	All patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	Microscopic concretions	-
Bile stasis	Nil.	Nil
Stoma size	Large	-
Other	No bile leaks	

		DAY	0	7	8			
CLINICAL		Appearance	Good	Fair	Dead			
		Vigor	-	Fair	-			
		Wound Sepsis	-	+++	+++			
		Weight - kg	40	35	35			
BIOCHEMISTRY		CSF Glutamine	7,6	10,4	-			
		Venous ammonia	167	250	-			
		Arterial ammonia	223	-	-			
		Alkaline phosphatase	5,5	7,6	-			
		S.G.O.T.	45	65	-			
		Cholesterol	69	78	-			
		Total protein	5,3	6,5	-			
		Albumin	1,2	1,7	-			
		Globulin	4,1	4,8	-			
HAEMATOLOGY		Haemoglobin	9,6	10,7	-			
		Leucocytes x 10^3	15,4	29,5	-			
		Platelets x 10^3	550	-	-			
		Neutrophils %	46	69	-			
		Lymphocytes %	35	16	-			
		Eosinophils %	12	7	-			
		Basophils %	0	0	-			
		Monocytes %	7	8	-			
ACID BASE BALANCE		pH	7,340	-	-			
		pCO_2	43	-	-			
		Std. Bicarbonate	22	-	-			
LIVER HISTOLOGY	DONOR	Functional Index	N	I	I			
		Rejection Degree	N	II	II			
		Cholestasis	0	0	0			
		Cholangitis	0	0	0			
	RECIPIENT	Functional Index	N	N	N			
		Rejection Degree	N	N	N			
		Cholestasis	0	0	0			
		Cholangitis	0	0	0			

PIG NO. 12 GROUP 1(a) SURVIVAL: 28 Days

OPERATION: Heterotopic transplant with cholecysto-cholecystostomy and gastro-duodenostomy

	DONOR	RECIPIENT
Breed	Landrace	Landrace
Animal weight	16 kg.	40 kg.
Liver weight	670 g.	1456 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY

Wound sepsis: Nil
Wound breakdown: Nil
Gastric: NAD
Intestinal: NAD
Pulmonary: Pneumonia
Cardiac: Superficial myocardial fibrosis
Renal: NAD
Lymph node: NAD
Pancreatic: NAD
Bladder: Granulomata on peritoneal surface. Remainder NAD
Other: Gastro-duodenostomy patent Mild ascites

LIVER	DONOR	RECIPIENT
Appearance	"Normal"	Normal
Weight	380 g.	1050 g.
Vascular anastomoses	Adequate	-
Vessel patency	All patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Shrunken, thick walled	Normal
Anastomoses	NAD	-
Bile stasis	Soft plugs and dilated bile ducts	Soft plugs
Stoma size	Large	-
Other	Small sealed off bile leak found	

		DAY	0	7	14	21	28
CLINICAL		Appearance	Good	Good	Good	Good	Fair
		Vigor	-	Good	Good	Good	Fair
		Wound Sepsis	-	0	0	+	0
		Weight - kg	40	40	41	40	38
BIOCHEMISTRY		CSF Glutamine	11,6	14,7	21,1	37,0	35,9
		Venous ammonia	204	413	315	350	242
		Arterial ammonia	245	-	-	-	-
		Alkaline phosphatase	4,0	4,5	8,6	1,8	1,8
		S.G.O.T.	35	80	35	45	20
		Cholesterol	122	106	100	-	14
		Total protein	6,1	6,8	6,3	6,1	7,0
		Albumin	1,8	2,1	2,1	1,4	1,9
		Globulin	4,3	4,7	4,2	4,7	5,1
HAEMATOLOGY		Haemoglobin	12,4	12,0	12,7	9,0	7,6
		Leucocytes x 10^3	23,2	46,5	25,3	37,7	34,8
		Platelets x 10^3	625	823	532	192	525
		Neutrophils %	45	53	63	44	57
		Lymphocytes %	34	33	33	36	32
		Eosinophils %	3	4	1	5	3
		Basophils %	1	2	1	0	1
		Monocytes %	17	8	3	15	7
ACID BASE BALANCE		pH	7,275	7,250	7,100	7,125	7,150
		pCO_2	37	51	41	55	43
		Std. Bicarbonate	16,3	19	12	15	14
LIVER HISTOLOGY	DONOR	Functional Index	N	III	III	IV	III
		Rejection Degree	N	IV	IV	IV	II
		Cholestasis	0	0	0	0	0
		Cholangitis	0	0	0	0	0
	RECIPIENT	Functional Index	N	N	N	N	N
		Rejection Degree	N	N	N	N	N
		Cholestasis	0	0	0	0	0
		Cholangitis	0	0	0	0	0

PIG NO. 13 GROUP 1(a) SURVIVAL: 28 Days

OPERATION: Heterotopic transplant with cholecysto-cholecystostomy

	DONOR	RECIPIENT
Breed	Landrace	Landrace
Animal weight	20 kg.	35 kg.
Liver weight	655 g.	1274 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY

Wound sepsis: Nil	Renal: NAD
Wound breakdown: Nil	Lymph node: Markedly reactive
Gastric: Large ulcer	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Mild patchy pneumonia	Other: Thyroid - NAD Moderate ascites
Cardiac: NAD	

LIVER	DONOR	RECIPIENT
Appearance	"Normal"	Normal
Weight	689 g.	904 g.
Vascular anastomoses	Adequate	-
Vessel patency	All patent. Non-occlusive thrombus in aortic stump	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Shrunken, thick walled	Normal
Anastomoses	NAD	-
Bile stasis	Nil	Nil
Stoma size	Large	-
Other	No bile leaks	

		DAY	0	7	14	21	28	
CLINICAL		Appearance	Good	Good	Good	Good	Good	
		Vigor	-	Good	Good	Good	Good	
		Wound Sepsis	-	0	0	0	0	
		Weight - kg	35	37	40	42	47	
BIOCHEMISTRY		CSF Glutamine	-	30,5	15,5	19,4	16,3	
		Venous ammonia	248	244	206	369	381	
		Arterial ammonia	224	-	-	-	-	
		Alkaline phosphatase	4,2	7,3	8,4	6,8	7,7	
		S.G.O.T.	40	50	80	65	40	
		Cholesterol	72	134	118	110	128	
		Total protein	6,6	8,5	6,9	7,2	7,9	
		Albumin	2,0	2,6	1,9	1,9	2,1	
		Globulin	4,6	5,9	5,0	5,3	5,8	
HAEMATOLOGY		Haemoglobin	9,6	12,7	11,5	11,1	13,2	
		Leucocytes x 10^3	14,8	24,7	18,8	16,8	16,7	
		Platelets x 10^3	485	765	241	313	253	
		Neutrophils %	50	53	36	28	21	
		Lymphocytes %	38	36	45	43	43	
		Eosinophils %	5	0	8	14	13	
		Basophils %	0	1	3	6	3	
		Monocytes %	7	10	8	9	20	
ACID BASE BALANCE		pH	7,350	7,350	7,340	7,360	7,250	
		pCO_2	40	50	39,5	31	42	
		Std. Bicarbonate	21,5	24,5	21	20	17,4	
LIVER HISTOLOGY	DONOR	Functional Index	N	II	II	II	I	
		Rejection Degree	N	II	II	II	I	
		Cholestasis	0	0	0	0	0	
		Cholangitis	0	0	0	0	0	
	RECIPIENT	Functional Index	N	N	N	N	N	
		Rejection Degree	N	N	N	N	N	
		Cholestasis	0	0	0	0	0	
		Cholangitis	0	0	0	0	0	

PIG NO. 14 GROUP 1(a) SURVIVAL: 28 Days

OPERATION: Heterotopic transplant with cholecyste-cholecystostomy

	DONOR	RECIPIENT
Breed	Landrace	Large White
Animal weight	24 kg.	39 kg.
Liver weight	647 g.	1420 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +	Renal: NAD
Wound breakdown: Nil	Lymph node: Mild histiocyte proliferation
Gastric: Large ulcer	Pancreatic: NAD
Intestinal: Normal except for plasma cell infiltration	Bladder: NAD
Pulmonary: NAD	Other: Thyroid normal
Cardiac: NAD	

LIVER	DONOR	RECIPIENT
Appearance	"Normal"	Normal
Weight	295 g.	865 g.
Vascular anastomoses	Adequate	-
Vessel patency	All patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	NAD	-
Bile stasis	Soft plugs	Soft plugs
Stoma size	Large	-
Other	No bile leaks	

		DAY	0	7	14	21	28	
CLINICAL		Appearance	Good	Good	Good	Good	Good	
		Vigor	-	Good	Good	Good	Good	
		Wound Sepsis	-	+	0	0	+	
		Weight - kg	39	38	37	40	40	
BIOCHEMISTRY		CSF Glutamine	10,8	11,1	24,1	36,6	22,2	
		Venous ammonia	-	364	442	437	335	
		Arterial ammonia	-	-	-	-	-	
		Alkaline phosphatase	5,6	9,6	7,1	9,4	7,4	
		S.G.O.T.	60	50	30	30	35	
		Cholesterol	-	120	92	58	113	
		Total protein	-	7,2	6,7	6,6	7,6	
		Albumin	-	2,0	2,4	2,3	2,5	
		Globulin	-	5,2	4,3	4,3	5,1	
HAEMATOLOGY		Haemoglobin	11,4	9.6	10,8	8,8	12,0	
		Leucocytes x 10^3	17,1	14,0	25,3	30,1	21,0	
		Platelets x 10^3	500	976	1115	950	519	
		Neutrophils %	69	43	54	68	60	
		Lymphocytes %	26	38	32	16	27	
		Eosinophils %	0	2	4	1	2	
		Basophils %	0	2	3	3	2	
		Monocytes %	5	15	7	12	9	
ACID BASE BALANCE		pH	7,310	7,40	7,03	7,05	7,175	
		pCO_2	76	51	38	64	42	
		Std. Bicarbonate	29,4	28	12,4	13,2	14,25	
LIVER HISTOLOGY	DONOR	Functional Index	N	II	IV	IV	IV	
		Rejection Degree	N	III	IV	IV	IV	
		Cholestasis	0	0	0	0	0	
		Cholangitis	0	0	0	0	+	
	RECIPIENT	Functional Index	N	N	N	N	N	
		Rejection Degree	N	N	N	N	N	
		Cholestasis	0	0	0	0	0	
		Cholangitis	0	0	0	0	0	

PIG NO. 15 GROUP 1(b) SURVIVAL: 15 Days

OPERATION Heterotopic Transplant with cholecysto-jejuno-cholecystostomy

	DONOR	RECIPIENT
Breed	Not recorded (N.R.)	White
Animal weight	16 kg.	46 kg.
Liver weight	650 g.	1674 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Perforated gastric ulcer

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +++	Renal: NAD
Wound breakdown: Nil	Lymph node: N.R.
Gastric: Perforated ulcer	Pancreatic: N.R.
Intestinal: NAD	Bladder: NAD
Pulmonary: Mild pneumonia and pleurisy	Other: Mild ascites Peritonitis
Cardiac: NAD	

LIVER	DONOR	RECIPIENT
Appearance	"Normal"	Normal
Weight	300 g.	1100 g.
Vascular anastomoses	Adequate	-
Vessel patency	All patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Thickened	Normal
Anastomoses	NAD	NAD
Bile stasis	Nil	Nil
Stoma size	Large	Large
Interposed loop	Normal, viable	
Other	No bile leaks	

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Poor	Fair	Fair			
	Vigor	-	Fair	Good			
	Wound Sepsis	-	0	+++			
	Weight - kg	46	43	40			
BIOCHEMISTRY	CSF Glutamine	-	-	-			
	Venous ammonia	-	-	-			
	Arterial ammonia	-	-	-			
	Alkaline phosphatase	1,8	4,0	3,7			
	S.G.O.T.	20	40	25			
	Cholesterol	74	97	-			
	Total protein	7,0	6,9	-			
	Albumin	1,7	1,9	-			
	Globulin	5,3	5,0	-			
HAEMATOLOGY	Haemoglobin	-	12,8	-			
	Leucocytes x 10^3	-	35,4	-			
	Platelets x 10^3	-	340	-			
	Neutrophils %	-	57	-			
	Lymphocytes %	-	29	-			
	Eosinophils %	-	5	-			
	Basophils %	-	0	-			
	Monocytes %	-	9	-			
ACID BASE BALANCE	pH	7,34	-	-			
	pCO_2	39	-	-			
	Std. Bicarbonate	21.5	-	-			
LIVER HISTOLOGY DONOR	Functional Index	N	-	III			
	Rejection Degree	N	-	III			
	Cholestasis	0	-	0			
	Cholangitis	0	-	0			
LIVER HISTOLOGY RECIPIENT	Functional Index	N	-	N			
	Rejection Degree	N	-	N			
	Cholestasis	0	-	0			
	Cholangitis	0	-	+			

PIG NO. 16 GROUP 1(b) SURVIVAL: 14 Days

OPERATION Heterotopic transplant with cholecysto-jejuno-cholecystostomy

	DONOR	RECIPIENT
Breed	Not recorded	Not recorded
Animal weight	12 kg.	32 kg.
Liver weight	600 g.	1165 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Iatrogenic - anaesthetic. Died under Halothane anaesthesia

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis : ++	Renal: NAD
Wound breakdown: Nil	Lymph node: N.R..
Gastric: NAD	Pancreatic: N.R.
Intestinal: NAD	Bladder: NAD
Pulmonary: Pneumonia, ? pulmonary embolus	Other: Small abscess between donor and recipient livers
Cardiac: NAD	

LIVER	DONOR	RECIPIENT
Appearance	Firm. Congested	Normal
Weight	420 g.	950 g.
Vascular anastomoses	Slight stenosis cavocaval anastomosis. Others adequate	-
Vessel patency	All patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Thickened and inspissated bile	Normal
Anastomoses	NAD	NAD
Bile stasis	Yes	Nil
Stoma size	Adequate	Adequate
Interposed loop	Normal, viable	
Other	No bile leaks	

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Good	Poor	Ill			
	Vigor	-	Poor	Poor			
	Wound Sepsis	-	0	++			
	Weight - kg	32	25	30			
BIOCHEMISTRY	CSF Glutamine	20,0	27,4	22,4			
	Venous ammonia	-	198	396			
	Arterial ammonia	-	-	-			
	Alkaline phosphatase	3,9	2,7	5,2			
	S.G.O.T.	15	25	20			
	Cholesterol	-	67	92			
	Total protein	-	7,2	8,0			
	Albumin	-	1,9	2,4			
	Globulin	-	5,3	5,6			
HAEMATOLOGY	Haemoglobin	13,6	9,2	10,6			
	Leucocytes x 10^3	27,1	9,4	8,3			
	Platelets x 10^3	296	368	342			
	Neutrophils %	55	67	1			
	Lymphocytes %	22	19	87			
	Eosinophils %	8	3	4			
	Basophils %	1	2	6			
	Monocytes %	14	9	2			
ACID BASE BALANCE	pH	7,30	-	-			
	pCO_2	55	-	-			
	Std. Bicarbonate	26,4	-	-			
LIVER HISTOLOGY DONOR	Functional Index	N	III	IV			
	Rejection Degree	N	IV	IV			
	Cholestasis	0	0	0			
	Cholangitis	0	0	+++	-		
LIVER HISTOLOGY RECIPIENT	Functional Index	N	N	N			
	Rejection Degree	N	N	N			
	Cholestasis	0	0	0			
	Cholangitis	0	0	0			

PIG NO. 17 GROUP 1(b) SURVIVAL: 28 Days

OPERATION Heterotopic transplant with cholecyste-jejuno-cholecystostomy

	DONOR	RECIPIENT
Breed	Large White	Landrace
Animal weight	12 kg.	22 kg.
Liver weight	525 g.	801 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: ++	Renal: NAD
Wound breakdown: Nil	Lymph node: NAD
Gastric: Large ulcer	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Mild pneumonia	Other: NAD
Cardiac: NAD	

LIVER	DONOR	RECIPIENT
Appearance	"Normal"	Normal
Weight	340 g.	755 g.
Vascular anastomoses	Adequate	-
Vessel patency	Portal vein thrombosed. Remaining vessels patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	NAD	NAD
Bile stasis	Nil	Nil
Stoma size	Large	Large
Interposed loop	Normal, viable	
Other	No bile leaks	

		DAY	0	7	14	21	28	
CLINICAL		Appearance	Good	Good	Good	Good	Poor	
		Vigor	-	Good	Good	Good	Fair	
		Wound Sepsis	-	0	0	0	++	
		Weight - kg	22	23	20	20	23	
BIOCHEMISTRY		CSF Glutamine	8,5	20,4	22,9	20,0	34,5	
		Venous ammonia	224	285	172	184	174	
		Arterial ammonia	-	-	-	-	-	
		Alkaline phosphatase	6,2	13,8	7,4	4,9	2,0	
		S.G.O.T.	20	50	30	40	25	
		Cholesterol	112	-	72	-	43	
		Total protein	-	-	6,2	-	-	
		Albumin	-	-	1,8	-	-	
		Globulin	-	-	4,4	-	-	
HAEMATOLOGY		Haemoglobin	9,1	13,5	11,9	11,1	8,0	
		Leucocytes x 10^3	12,7	34,0	15,4	14,3	20,7	
		Platelets x 10^3	287	275	185	600	350	
		Neutrophils %	45	54	69	37	47	
		Lymphocytes %	45	18	12	32	21	
		Eosinophils %	0	2	1	2	0	
		Basophils %	0	1	0	0	0	
		Monocytes %	10	26	18	29	32	
ACID BASE BALANCE		pH	-	-	-	-	-	
		pCO_2	-	-	-	-	-	
		Std. Bicarbonate	-	-	-	-	-	
LIVER HISTOLOGY	DONOR	Functional Index	N	-	IV	IV	IV	
		Rejection Degree	N	-	IV	IV	IV	
		Cholestasis	0	-	0	0	0	
		Cholangitis	0	-	0	0	+	
	RECIPIENT	Functional Index	N	-	N	N	N	
		Rejection Degree	N	-	N	N	N	
		Cholestasis	0	-	0	0	0	
		Cholangitis	0	-	0	0	0	

PIG NO. 18 GROUP 1(b) SURVIVAL: 14 Days

OPERATION Heterotopic transplant with cholecysto-jejuno-cholecystostomy

	DONOR	RECIPIENT
Breed	Large Black	Large Black
Animal weight	17 kg.	32 kg.
Liver weight	750 g.	1165 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Sacrifice - humane. Ill, jaundiced, wound breakdown

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: ++	Renal: Mild pylonephritis
Wound breakdown: Moderate	Lymph node: Mildly reactive
Gastric: NAD	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Lung abscess and patchy interstitial fibrosis	Other: NAD
Cardiac: Excess pericardial fluid	

LIVER	DONOR	RECIPIENT
Appearance	Firm, dark. Infarcted	Normal
Weight	1000 g.	1050 g.
Vascular anastomoses	Adequate	-
Vessel patency	All vessels totally occluded with organized thrombus	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Necrotic	Normal
Anastomoses	Necrotic	NAD
Bile stasis	Yes	Yes
Stoma size	Adequate.	Adequate
Interposed loop	Necrotic	
Other	No bile leaks	

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Good	Jaundice Good	Good			
	Vigor	-	Good	Fair			
	Wound Sepsis	-	+	++			
	Weight - kg	32	29	30			
BIOCHEMISTRY	CSF Glutamine	10,8	35,3	33,6			
	Venous ammonia	237	196	200			
	Arterial ammonia	-	-	216			
	Alkaline phosphatase	5,8	20,7	10,2			
	S.G.O.T.	55	640	165			
	Cholesterol	64	84	45			
	Total protein	5,7	6,3	5,7			
	Albumin	1,8	1,6	1,4			
	Globulin	3,9	4,7	4,3			
HAEMATOLOGY	Haemoglobin	10,5	4,7	6,1			
	Leucocytes x 10^3	13,0	-	36,4			
	Platelets x 10^3	50	475	225			
	Neutrophils %	57	55	59			
	Lymphocytes %	31	22	21			
	Eosinophils %	2	4	0			
	Basophils %	0	1	0			
	Monocytes %	10	18	20			
ACID BASE BALANCE	pH	7,120	-	-			
	pCO_2	52,5	-	-			
	Std. Bicarbonate	14,2	-	-			
LIVER HISTOLOGY DONOR	Functional Index	N	IV	IV			
	Rejection Degree	N	Infarction	Infarction			
	Cholestasis	0			-		
	Cholangitis	0					
LIVER HISTOLOGY RECIPIENT	Functional Index	N	N	N			
	Rejection Degree	N	N	N			
	Cholestasis	0	++	++			
	Cholangitis	0	0	0			

PIG NO. 19 GROUP 1(b) SURVIVAL: 28 Days

OPERATION Heterotopic transplant with cholecysto-jejuno-cholecystostomy

	DONOR	RECIPIENT
Breed	Large Black	Large Black
Animal weight	23 kg.	27 kg.
Liver weight	695 g.	983 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY

Wound sepsis: ++

Wound breakdown: Partial dehiscence

Gastric: Greater curve ulcer

Intestinal: NAD

Pulmonary: Resolving haemothorax

Cardiac: Excess pericardial fluid
Myocardial parasites

Renal: NAD

Lymph node: Reactive and extra medullary haemopoeisis

Pancreatic: NAD

Bladder: NAD

Other: Moderate ascites

LIVER	DONOR	RECIPIENT
Appearance	"Normal"	Normal
Weight	517 g.	940 g.
Vascular anastomoses	Adequate	-
Vessel patency	Portal vein thromboses Non-occlusive donor vena-caval thrombus	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	2 small concretions	Intact - small concretions
Bile stasis	Minimal gravel	Minimal gravel
Stoma size	Large	Large
Interposed loop	Normal, viable	
Other	No bile leaks	

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Good	Good	Fair	Fair	Poor	
	Vigor	-	Good	Fair	Fair	Poor	
	Wound Sepsis	-	0	++	++	++	
	Weight - kg	27	30	32	31	30	
BIOCHEMISTRY	CSF Glutamine	10,4	13,7	19,3	18,6	21,6	
	Venous ammonia	234	183	243	287	185	
	Arterial ammonia	-	-	-	-	-	
	Alkaline phosphatase	6,7	6,4	3,0	5,7	3,8	
	S.G.O.T.	55	65	45	50	70	
	Cholesterol	98	68	44	28	21	
	Total protein	6,4	6,9	6,5	5,7	5,6	
	Albumin	1,9	1,9	1,7	1,4	1,5	
	Globulin	4,5	5,0	4,8	4,3	4,1	
HAEMATOLOGY	Haemoglobin	12	12,7	11	6,3	7,7	
	Leucocytes x 10^3	21,3	27,2	59,0	47,0	-	
	Platelets x 10^3	475	950	950	413	82	
	Neutrophils %	58	61	35	28	25	
	Lymphocytes %	32	34	49	54	57	
	Eosinophils %	0	0	2	1	1	
	Basophils %	0	4	1	0	0	
	Monocytes %	10	1	13	17	17	
ACID BASE BALANCE	pH	7,165	-	-	7,075	7,175	
	pCO_2	53	-	-	64	36,5	
	Std. Bicarbonate	16	-	-	18	13,2	
LIVER HISTOLOGY DONOR	Functional Index	N	I	I	I	II	
	Rejection Degree	N	I	I	II	III	
	Cholestasis	0	0	0	0	0	
	Cholangitis	0	0	0	+	++	
LIVER HISTOLOGY RECIPIENT	Functional Index	N	N	N	N	N	
	Rejection Degree	N	N	N	N	N	
	Cholestasis	0	0	0	0	0	
	Cholangitis	0	0	0	0	0	

PIG NO. 20 GROUP 1(b) SURVIVAL: 28 Days

OPERATION Heterotopic transplant with cholecysto-jejuno-cholecystostomy

	DONOR	RECIPIENT
Breed	Large Black	Large Black
Animal weight	17 kg.	23 kg.
Liver weight	725 g.	837 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +	Renal: Resolving pylonephritis
Wound breakdown: Nil	Lymph node: NAD
Gastric: NAD	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: NAD	Other: Minilap abscess extending intra-peritoneally
Cardiac: Excess pericardial fluid	

LIVER	DONOR	RECIPIENT
Appearance	"Normal"	"Normal"
Weight	895 g.	750 g.
Vascular anastomoses	Adequate	-
Vessel patency	Small branch right hepatic vein thrombosed Remaining vessels patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	NAD	NAD
Bile stasis	Slight debris	Nil
Stoma size	Large	Large
Interposed loop	Normal, viable	
Other	No bile leaks	

		DAY	0	7	14	21	28	
CLINICAL		Appearance	Good	Good	Good	Poor	Fair	
		Vigor	-	Good	Good	Poor	Fair	
		Wound Sepsis	-	0	++	+	+	
		Weight - kg	23	25	28	28	30	
BIOCHEMISTRY		CSF Glutamine	10,4	15,7	16,4	14,4	13,4	
		Venous ammonia	265	175	230	141	172	
		Arterial ammonia	-	219	-	-	255	
		Alkaline phosphatase	9,3	7,1	8,1	11,7	9,1	
		S.G.O.T.	55	75	225	185	120	
		Cholesterol	71	103	30	64	22	
		Total protein	6,7	6,5	5,8	4,9	6,3	
		Albumin	1,5	1,4	1,3	1,1	1,6	
		Globulin	5,2	5,1	4,5	3,8	4,7	
HAEMATOLOGY		Haemoglobin	-	10,9	6,7	6,9	8,0	
		Leucocytes x 10^3	-	28,7	44,2	35,0	30,6	
		Platelets x 10^3	-	500	500	1000	50	
		Neutrophils %	-	36	55	38	20	
		Lymphocytes %	-	43	23	46	56	
		Eosinophils %	-	0	0	6	0	
		Basophils %	-	0	1	1	0	
		Monocytes %	-	21	21	9	24	
ACID BASE BALANCE		pH	7,340	-	7,135	6,955	7,170	
		pCO_2	56	-	20	63	53	
		Std. Bicarbonate	26	-	14,6	13,2	16,5	
LIVER HISTOLOGY	DONOR	Functional Index	N	II	I	I	I	
		Rejection Degree	N	II	I	I	II	
		Cholestasis	0	0	0	0	0	
		Cholangitis	0	0	0	0	0	
	RECIPIENT	Functional Index	N	N	N	N	-	
		Rejection Degree	N	N	N	N	-	
		Cholestasis	0	0	0	0	-	
		Cholangitis	0	0	0	0	-	

PIG NO. 21 GROUP 1(b) SURVIVAL: 7 Days

OPERATION Heterotopic transplant with cholecysto-jejuno-cholecystostomy

	DONOR	RECIPIENT
Breed	Landrace	Landrace
Animal weight	18 kg.	31 kg.
Liver weight	761 g.	1128 g. (estimated)
Other	Temperature 41.1°C	Temperature 39.5°C

MAIN CAUSE OF DEATH: Iatrogenic - malignant hyperpyrexia (Donor and recipient developed hyperpyrexia during operation)

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +++	Renal: Focal lymphoid infiltration
Wound breakdown: Nil	Lymph node: Minimally reactive
Gastric: Large penetrating ulcer	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Severe pneumonia and pleurisy	Other: NAD
Cardiac: Gross constrictive pericarditis	

LIVER	DONOR	RECIPIENT
Appearance	Firm. Congested	Normal
Weight	655 g.	900 g.
Vascular anastomoses	Adequate	-
Vessel patency	All patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	NAD	NAD
Bile stasis	Slight debris	Nil
Stoma size	Large	Large
Interposed loop	Normal, viable	
Other	No bile leaks	

		DAY	0	7	14	21	28	
CLINICAL		Appearance	Good	Good				
		Vigor	-	Good				
		Wound Sepsis	-	+++				
		Weight - kg	31	30				
BIOCHEMISTRY		CSF Glutamine	12,1	46,4				
		Venous ammonia	151	67				
		Arterial ammonia	-	-				
		Alkaline phosphatase	7,7	8,2				
		S.G.O.T.	50	45				
		Cholesterol	49	45				
		Total protein	7,1	4,9				
		Albumin	1,7	0,7				
		Globulin	5,4	4,2				
HAEMATOLOGY		Haemoglobin	11,1	6,2				
		Leucocytes x 10^3	34,4	44				
		Platelets x 10^3	450	325				
		Neutrophils %	83	71				
		Lymphocytes %	14	17				
		Eosinophils %	0	3				
		Basophils %	0	0				
		Monocytes %	3	9				
ACID BASE BALANCE		pH	7,290	-				
		pCO_2	46	-				
		Std. Bicarbonate	20,3	-				
LIVER HISTOLOGY	DONOR	Functional Index	N	IV				
		Rejection Degree	N	IV				
		Cholestasis	0	0				
		Cholangitis	0	+		-		
	RECIPIENT	Functional Index	I	N				
		Rejection Degree	N	N				
		Cholestasis	0	0				
		Cholangitis	0	0				

PIG NO. 22 GROUP 1(b) SURVIVAL: 23 Days

OPERATION Heterotopic transplant with cholecysto-jejuno-cholecystostomy

	DONOR	RECIPIENT
Breed	Landrace	Landrace
Animal weight	16 kg.	40 kg.
Liver weight	450 g.	1456 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Not determined

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: Nil	Renal: NAD
Wound breakdown: Nil	Lymph node: Autolytic
Gastric: Large ulcer	Pancreatic: Autolytic
Intestinal: NAD	Bladder: NAD
Pulmonary: Mild pneumonia with microscopic abscesses	Other: NAD
Cardiac: NAD	

LIVER	DONOR	RECIPIENT
Appearance	"Normal"	Normal
Weight	830 g.	1000 g.
Vascular anastomoses	Adequate	-
Vessel patency	All patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	NAD	NAD
Bile stasis	Nil	Nil
Stoma size	Large	Large
Interposed loop	Normal, viable	
Other	No bile leaks	

		DAY	0	7	14	21	23	
CLINICAL		Appearance	Good	Fair	Fair	Fair	Dead	
		Vigor	-	Fair	Fair	Fair	-	
		Wound Sepsis	-	+	0	0	0	
		Weight - kg	40	35	34	35	37	
BIOCHEMISTRY		CSF Glutamine	9,9	19,8	18,6	22,2	-	
		Venous ammonia	239	207	151	248	-	
		Arterial ammonia	153	-	116	-	-	
		Alkaline phosphatase	13,7	7,6	10,0	6,4	-	
		S.G.O.T.	40	45	35	95	-	
		Cholesterol	111	41	85	86	-	
		Total protein	6,5	5,5	6,4	5,9	-	
		Albumin	1,8	1,3	1,6	1,3	-	
		Globulin	4,7	4,2	4,8	4,6	-	
HAEMATOLOGY		Haemoglobin	12,4	11,6	11,9	9,4	-	
		Leucocytes x 10^3	22,0	28,9	53,8	14,4	-	
		Platelets x 10^3	600	1100	600	200	-	
		Neutrophils %	34	40	56	27	-	
		Lymphocytes %	52	47	30	56	-	
		Eosinophils %	0	1	2	0	-	
		Basophils %	1	0	0	5	-	
		Monocytes %	13	11	12	12	-	
ACID BASE BALANCE		pH	7,045	7,360	7,532	-	-	
		pCO_2	71	46	18	-	-	
		Std. Bicarbonate	14,5	25	25,5	-	-	
LIVER HISTOLOGY	DONOR	Functional Index	N	II	I	I	II	
		Rejection Degree	N	III	II	II	II	
		Cholestasis	0	0	0	0	0	
		Cholangitis	0	0	0	0	+	
	RECIPIENT	Functional Index	N	N	N	N	N	
		Rejection Degree	N	N	N	N	N	
		Cholestasis	0	0	0	0	0	
		Cholangitis	0	0	0	0	0	

PIG NO. 23 GROUP I(b) SURVIVAL: 14 Days

OPERATION Heterotopic transplant with cholecysto-jejuno-cholecystostomy

	DONOR	RECIPIENT
Breed	Large White	Landrace
Animal weight	17 kg.	35 kg.
Liver weight	500 g.	1274 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Bleeding gastric ulcer

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +	Renal: NAD
Wound breakdown: Nil	Lymph node: Massive lymph nodes Porta hepatis
Gastric: Large ulcer - fresh bleed	Pancreatic: NAD
Intestinal: Mild cellular infiltration	Bladder: NAD
Pulmonary: Pleurisy	Other: Melaena. Small abscess lesser curve of stomach
Cardiac: Pericarditis	

LIVER	DONOR	RECIPIENT
Appearance	Congested	Normal
Weight	330 g.	1190 g.
Vascular anastomoses	Adequate	-
Vessel patency	Small non-occlusive thrombi in portal and hepatic veins	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	NAD	NAD
Bile stasis	Nil	Nil
Stoma size	Large	Large
Interposed loop	Normal, viable	
Other	No bile leaks	

		DAY	0	7	14	21	28	
CLINICAL		Appearance	Good	Good	Fair			
		Vigor	-	Excell	Good			
		Wound Sepsis	-	+	+			
		Weight - kg	35	36	33			
BIOCHEMISTRY		CSF Glutamine	8,5	24,9	25,5			
		Venous ammonia	161	-	211			
		Arterial ammonia	221	-	-			
		Alkaline phosphatase	6,8	7,7	8,6			
		S.G.O.T.	35	45	35			
		Cholesterol	125	90	31			
		Total protein	5,9	6,0	5,7			
		Albumin	1,7	1,3	1,2			
		Globulin	4,2	4,7	4,5			
HAEMATOLOGY		Haemoglobin	10,5	11,0	5,0			
		Leucocytes x 10^3	25,9	67,0	37,7			
		Platelets x 10^3	475	875	169			
		Neutrophils %	44	61	60			
		Lymphocytes %	38	31	32			
		Eosinophils %	7	0	0			
		Basophils %	1	0	0			
		Monocytes %	10	8	8			
ACID BASE BALANCE		pH	-	7,24	-			
		pCO_2	-	51	-			
		Std. Bicarbonate	-	19,5	-			
LIVER HISTOLOGY	DONOR	Functional Index	N	III	III			
		Rejection Degree	N	III	IV			
		Cholestasis	0	0	0			
		Cholangitis	0	0	0			
	RECIPIENT	Functional Index	N	N	N			
		Rejection Degree	N	N	N			
		Cholestasis	0	0	0			
		Cholangitis	0	0	0			

PIG NO. 24 GROUP 1(b) SURVIVAL: 24 Days

OPERATION Heterotopic transplant with cholecysto-jejuno-cholecystostomy

	DONOR	RECIPIENT
Breed	Large White	Landrace
Animal weight	15 kg.	32 kg.
Liver weight	510 g.	1165 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Bleeding gastric ulcer

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +	Renal: NAD
Wound breakdown: Nil	Lymph node: Reactive
Gastric: Large ulcer - fresh bleed	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: NAD	Other: -
Cardiac: NAD	

LIVER	DONOR	RECIPIENT
Appearance	"Normal"	Normal
Weight	1390 g.	800 g.
Vascular anastomoses	Adequate	-
Vessel patency	All patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	NAD	NAD
Bile stasis	Nil	Nil
Stoma size	Large	Large
Interposed loop	Normal, viable	
Other	No bile leaks	

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Good	Good	Good	Excell	III	
	Vigor	-	Good	Good	Excell	Poor	
	Wound Sepsis	-	0	+	0	0	
	Weight - kg	32	32	37	40	38	
BIOCHEMISTRY	CSF Glutamine	10,1	14,4	14,3	14,1	-	
	Venous ammonia	272	447	226	-	-	
	Arterial ammonia	230	-	-	166	-	
	Alkaline phosphatase	4,7	12,8	14,4	15,8	-	
	S.G.O.T.	35	75	200	335	-	
	Cholesterol	89	72	130	49	-	
	Total protein	6,6	5,7	7,6	7,6	-	
	Albumin	2,1	1,9	2,3	1,8	-	
	Globulin	4,5	3,8	5,3	5,8	-	
HAEMATOLOGY	Haemoglobin	11,9	11,3	12,4	13,1	-	
	Leucocytes x 10^3	18,8	31,7	32,9	29,4	-	
	Platelets x 10^3	550	1150	203	62	-	
	Neutrophils %	56	45	47	60	-	
	Lymphocytes %	26	32	37	17	-	
	Eosinophils %	4	4	6	4	-	
	Basophils %	1	0	2	1	-	
	Monocytes %	13	18	8	18	-	
ACID BASE BALANCE	pH	-	7,275	7,025	-	-	
	pCO_2	-	56	73	-	-	
	Std. Bicarbonate	-	23	14	-	-	
LIVER HISTOLOGY DONOR	Functional Index	N	I	I	I	II	
	Rejection Degree	N	II	I	II	II	
	Cholestasis	0	0	0	0	0	
	Cholangitis	0	0	0	0	0	
LIVER HISTOLOGY RECIPIENT	Functional Index	N	N	N	N	N	
	Rejection Degree	N	N	N	N	N	
	Cholestasis	0	0	0	0	0	
	Cholangitis	0	0	0	0	0	

PIG NO. 25 GROUP 1(b) SURVIVAL: 28 Days

OPERATION Heterotopic transplant with cholecysto-jejuno-cholecystostomy

	DONOR	RECIPIENT
Breed	Large Black	Large Black
Animal weight	17 kg.	32 kg.
Liver weight	545 g.	1165 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Pneumonia

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: Nil	Renal: NAD
Wound breakdown: Nil	Lymph node: Reactive
Gastric: Multiple ulcers of greater curvature	Pancreatic: Changes consistent with duct obstruction - worm in pancreatic duct
Intestinal: NAD	Bladder: NAD
Pulmonary: Gross pneumonia	Other: NAD
Cardiac: Pericarditis	

LIVER	DONOR	RECIPIENT
Appearance	"Normal"	Normal
Weight	360 g.	900 g.
Vascular anastomoses	Adequate	-
Vessel patency	Small thrombus in one branch of hepatic vein	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	4 small concretions	NAD
Bile stasis	Nil	Nil
Stoma size	Large	Large
Interposed loop	Normal, viable	
Other	No bile leaks	

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Good	Good	Fair	Poor	Dead	
	Vigor	-	Fair	Fair	Poor	-	
	Wound Sepsis	-	+	0	0	0	
	Weight - kg	32	32	30	29	27	
BIOCHEMISTRY	CSF Glutamine	9,8	24,2	25,3	28,5	-	
	Venous ammonia	185	208	275	-	-	
	Arterial ammonia	175	-	-	-	-	
	Alkaline phosphatase	7,6	5,0	2,3	3,0	-	
	S.G.O.T.	35	40	35	105	-	
	Cholesterol	61	53	72	4	-	
	Total protein	5,6	5,5	6,6	6,1	-	
	Albumin	1,8	1,5	1,9	1,5	-	
	Globulin	3,8	4,0	4,7	4,6	-	
HAEMATOLOGY	Haemoglobin	11,2	9,8	10,6	4,7	-	
	Leucocytes x 10^3	16,0	23,6	31,2	21,0	-	
	Platelets x 10^3	375	142	425	36	-	
	Neutrophils %	44	68	54	50	-	
	Lymphocytes %	48	19	24	25	-	
	Eosinophils %	3	1	9	1	-	
	Basophils %	0	0	2	0	-	
	Monocytes %	5	12	11	24	-	
ACID BASE BALANCE	pH	7,315	-	-	7,215	-	
	pCO_2	46	-	-	34,5	-	
	Std. Bicarbonate	22	-	-	14	-	
LIVER HISTOLOGY DONOR	Functional Index	N	III	IV	IV	IV	
	Rejection Degree	N	III	IV	IV	IV	
	Cholestasis	0	0	0	0	0	
	Cholangitis	0	0	0	0	0	
LIVER HISTOLOGY RECIPIENT	Functional Index	N	N	N	N	I	
	Rejection Degree	N	N	N	N	N	
	Cholestasis	0	0	0	0	+	
	Cholangitis	0	0	0	0	+	

PIG NO. 26 GROUP 1(b) SURVIVAL: 25 Days

OPERATION Heterotopic transplant with cholecysto-jejuno-cholecystostomy

	DONOR	RECIPIENT
Breed	Large Black	Large Black
Animal weight	25 kg.	33 kg.
Liver weight	885 g.	1201 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Sacrifice - humane. Animal ill, unable to walk, thin, miserable, sparse hair

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +++	Renal: NAD
Wound breakdown: Moderate	Lymph node: NAD
Gastric: Multiple superficial ulcers	Pancreatic: NAD
Intestinal: Peritonitis	Bladder: NAD
Pulmonary: Gross pneumonia	Other: Bile-stained ascites
Cardiac: Excess pericardial fluid	

LIVER	DONOR	RECIPIENT
Appearance	"Normal"	"Brown atrophy"
Weight	560 g.	960 g.
Vascular anastomoses	Adequate	-
Vessel patency	Small thrombus extending from hepatic vein to inferior vena cava	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	Concretions and ulcers	NAD
Bile stasis	Mild	Mild
Stoma size	Large	Large
Interposed loop	Dilated but normal	
Other	Recipient common bile duct kinked. Bile leak present	

		DAY	0	7	14	21	25
CLINICAL		Appearance	Good	Excell	Jaundice Poor	Poor	Ill++
		Vigor	-	Excell	Poor	Poor	Inert
		Wound Sepsis	-	0	0	++	+++
		Weight - kg	33	40	30	30	25
BIOCHEMISTRY		CSF Glutamine	11,9	15,1	42,2	33,0	45,4
		Venous ammonia	151	217	-	204	300
		Arterial ammonia	113	-	-	236	-
		Alkaline phosphatase	5,3	8,0	3,8	3,9	9,0
		S.G.O.T.	30	95	310	70	150
		Cholesterol	-	74	50	30	-
		Total protein	-	5,3	4,5	5,1	-
		Albumin	-	1,3	1,5	1,3	-
		Globulin	-	4,0	3,0	3,8	-
HAEMATOLOGY		Haemoglobin	10,6	9,1	7,4	5,7	3,9
		Leucocytes x 10^3	18,1	32,1	21,6	39,6	30,8
		Platelets x 10^3	126	37	330	410	350
		Neutrophils %	35	61	30	65	81
		Lymphocytes %	50	13	45	19	18
		Eosinophils %	4	2	5	0	2
		Basophils %	1	1	0	0	0
		Monocytes %	10	23	20	16	9
ACID BASE BALANCE		pH	7,375	-	7,290	7,170	-
		pCO_2	34,5	-	50	60	-
		Std. Bicarbonate	21	-	22	19	-
LIVER HISTOLOGY	DONOR	Functional Index	N	II	II	II	III
		Rejection Degree	N	III	II	II	III
		Cholestasis	0	0	0	0	++
		Cholangitis	0	0	0	0	+
	RECIPIENT	Functional Index	N	N	N	N	N
		Rejection Degree	N	N	N	N	N
		Cholestasis	0	0	+	+	+++
		Cholangitis	0	0	0	0	+

PIG NO. 27 GROUP 1(b) SURVIVAL: 7 Days

OPERATION Heterotopic transplant with cholecysto-jejuno-cholecystostomy

	DONOR	RECIPIENT
Breed	Landrace	Black Landrace
Animal weight	18 kg.	28 kg.
Liver weight	760 g.	1019 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Pneumonia

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: Nil	Renal: NAD
Wound breakdown: Nil	Lymph node: Mildly reactive
Gastric: NAD	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Severe pneumonia	Other: NAD
Cardiac: NAD	

LIVER	DONOR	RECIPIENT
Appearance	"Normal"	Normal
Weight	515 g.	785 g.
Vascular anastomoses	Adequate	-
Vessel patency	Small non-occlusive thrombus in portal vein	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	Small leak	NAD
Bile stasis	Nil	Nil
Stoma size	Large	Large
Interposed loop	Normal, viable	
Other	Bile leak present in proximal cholecysto-jejunal anastomosis	

		DAY	0	7	14	21	28	
CLINICAL		Appearance	Thin	Good				
		Vigor	-	Good				
		Wound Sepsis	-	0				
		Weight - kg	28	25				
BIOCHEMISTRY		CSF Glutamine	12,8	12,8				
		Venous ammonia	162	-				
		Arterial ammonia	105	214				
		Alkaline phosphatase	-	7,2				
		S.G.O.T.	20	45				
		Cholesterol	-	109				
		Total protein	-	7,0				
		Albumin	-	1,6				
		Globulin	-	5,4				
HAEMATOLOGY		Haemoglobin	11,3	11,0				
		Leucocytes x 10^3	17,9	30,3				
		Platelets x 10^3	450	210				
		Neutrophils %	37	49				
		Lymphocytes %	55	35				
		Eosinophils %	5	2				
		Basophils %	0	1				
		Monocytes %	3	13				
ACID BASE BALANCE		pH	7,252	-				
		pCO_2	44	-				
		Std. Bicarbonate	17,8	-				
LIVER HISTOLOGY	DONOR	Functional Index	N	II				
		Rejection Degree	N	II				
		Cholestasis	0	0				
		Cholangitis	0	0				
	RECIPIENT	Functional Index	N	N				
		Rejection Degree	N	N				
		Cholestasis	0	0				
		Cholangitis	0	0				

PIG NO. 28 GROUP 1(b) SURVIVAL: 28 Days

OPERATION Heterotopic transplant with cholecysto-jejuno-cholecystostomy and gastro-duodenostomy

	DONOR	RECIPIENT
Breed	White Landrace	Black Landrace
Animal weight	15 kg.	30 kg.
Liver weight	550 g.	1092 g. (estimated)
Other	-	-

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +	Renal: NAD
Wound breakdown: Nil	Lymph node: Mildly reactive
Gastric: Microscopic ulcer	Pancreatic: NAD
Intestinal: Small bowel perforation	Bladder: NAD
Pulmonary: Mild resolving pneumonia	Other: Clear ascites Gastro-duodenostomy patent
Cardiac: NAD	

LIVER	DONOR	RECIPIENT
Appearance	"Normal"	Normal
Weight	1170 g.	720 g.
Vascular anastomoses	Adequate	-
Vessel patency	All patent	All patent

BILIARY TRACT	DONOR	RECIPIENT
Appearance	Normal	Normal
Anastomoses	Abscess and concretions	Concretions
Bile stasis	Nil	Nil
Stoma size	Large	Large
Interposed loop	Normal, viable	
Other	No bile leaks	

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Thin	Good	Fair	Fair	Poor	
	Vigor	-	Good	Good	Good	Good	
	Wound Sepsis	-	0	+	+	0	
	Weight - kg	30	31	29	28	30	
BIOCHEMISTRY	CSF Glutamine	-	23,4	15,2	10,9	11,4	
	Venous ammonia	157	157	297	151	178	
	Arterial ammonia	169	151	-	-	212	
	Alkaline phosphatase	2,8	4,5	4,0	7,3	11,6	
	S.G.O.T.	20	80	85	145	140	
	Cholesterol	111	99	37	109	59	
	Total protein	-	5,5	5,6	5,2	7,1	
	Albumin	-	1,8	1,6	1,5	1,8	
	Globulin	-	3,7	4,0	3,7	5,3	
HAEMATOLOGY	Haemoglobin	14,2	6,2	7,4	7,5	9,9	
	Leucocytes x 10^3	19,0	28,1	30,4	25,0	46,7	
	Platelets x 10^3	375	56	230	105	450	
	Neutrophils %	42	61	55	45	84	
	Lymphocytes %	39	10	22	24	6	
	Eosinophils %	8	1	2	3	0	
	Basophils %	2	0	0	0	0	
	Monocytes %	9	28	21	28	10	
ACID BASE BALANCE	pH	7,430	-	7,22	-	7,33	
	pCO_2	28	-	34	-	31	
	Std. Bicarbonate	20	-	14	-	17,5	
LIVER HISTOLOGY DONOR	Functional Index	N	I	I	I	I	
	Rejection Degree	N	I	I	II	II	
	Cholestasis	0	0	0	0	0	
	Cholangitis	0	0	0	+	+	
LIVER HISTOLOGY RECIPIENT	Functional Index	N	N	N	N	N	
	Rejection Degree	N	N	N	N	N	
	Cholestasis	0	0	0	0	0	
	Cholangitis	0	0	0	0	0	

PIG NO. 29 GROUP 2(a) SURVIVAL: 38 Days

OPERATION: End-to-side porta-caval shunt

	DESCRIPTION
Breed	Not recorded (N.R.)
Animal weight	N.R.
Liver weight	(Estimated) -
Other	-

MAIN CAUSE OF DEATH: Unknown

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: ++	Renal: NAD
Wound breakdown: Nil	Lymph node: Reactive
Gastric: Multiple pinpoint erosions	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Mild pneumonia	Other: Spleen reactive
Cardiac: Mild pericardial adhesions	

LIVER	
Appearance	Normal
Weight	664 g.
Vascular anastomosis	NAD
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	-

	DAY	0	7	14	21	28	38
CLINICAL	Appearance	Good	Fair	Poor	Fair	Fair	Dead
	Vigor	Good	Fair	Poor	Fair	Fair	-
	Wound sepsis	-	+	++	++	++	++
	Weight - kg	-		25	28	29	-
BIOCHEMISTRY	CSF Glutamine	14	15	41	57,2	73	-
	Venous ammonia	-	-	-	395	720	-
	Arterial ammonia	-	-	-	-	-	-
	Alkaline phosphatase	5,6	3,5	5,0	4,3	4,9	-
	S.G.O.T.	35	20	20	70	20	-
	Cholesterol	124	100	48	84	82	-
	Total protein	6,1	8,5	7,4	-	9,3	-
	Albumin	1,7	1,9	1,6	-	-	-
	Globulin	4,4	6,6	5,8	-	-	-
HAEMATOLOGY	Haemoglobin	10,6	12	11,2	11,5	14,5	-
	Leucocytes x 10^3	13,4	50,4	23,2	14,1	29,1	-
	Platelets x 10^3	165	510	517	841	261	-
	Neutrophils %	57	52	60	29	63	-
	Lymphocytes %	27	29	25	49	30	-
	Eosinophils %	4	3	0	3	4	-
	Basophils %	2	0	0	2	0	-
	Monocytes %	10	16	15	17	3	-
ACID BASE BALANCE	pH	-	-	-	-	-	-
	pCO_2	-	-	-	-	-	-
	Std. bicarbonate	-	-	-	-	-	-
LIVER HISTOLOGY	Functional Index	N	-	-	-	-	N
	Rejection Degree	N	-	-	-	-	N
	Cholestasis	0	-	-	-	-	0
	Cholangitis	0	-	-	-	-	0

PIG NO. 30 GROUP 2(a) SURVIVAL: 38 Days

OPERATION: End-to-side porta-caval shunt

	DESCRIPTION
Breed	N.R.
Animal weight	N.R.
Liver weight	(Estimated) -
Other	-

MAIN CAUSE OF DEATH: Sacrifice - humane. Large abdominal wall abscess

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +++	Renal: Right hydronephrosis. Left NAD
Wound breakdown: Nil	Lymph node: Mildly reactive
Gastric: Superficial acute ulcer	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Pleural adhesions and focal collapse	Other: NAD
Cardiac: Excess pericardial fluid	

LIVER	
Appearance	Normal
Weight	720 g.
Vascular anastomosis	NAD
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	-

	DAY	0	7	14	21	28	35
CLINICAL	Appearance	Good	Good	Good	Good	Good	Good
	Vigor	-	Good	Good	Good	Good	Good
	Wound sepsis	-	0	0	0	0	+++
	Weight - kg	-	-	22	27	28	32
BIOCHEMISTRY	CSF Glutamine	-	-	39	86,8	77	146,2
	Venous ammonia	-	-	-	444	408	502
	Arterial ammonia	-	-	-	-	-	-
	Alkaline phosphatase	9,3	2,8	8,6	4,3	4,7	6,3
	S.G.O.T.	-	40	25	35	35	65
	Cholesterol	92	52	39	47	45	55
	Total protein	-	-	7,2	6,5	-	8,4
	Albumin	-	-	1,2	1,0	-	1,4
	Globulin	-	-	6,0	5,5	-	7,0
HAEMATOLOGY	Haemoglobin	10,4	10,8	9,3	8,6	9,4	-
	Leucocytes x 10^3	9,3	19,0	15,6	20,2	13,6	-
	Platelets x 10^3	317	233	-	610	261	-
	Neutrophils %	54	56	53	58	60	-
	Lymphocytes %	45	31	23	34	34	-
	Eosinophils %	0	1	1	3	0	-
	Basophils %	0	0	1	1	0	-
	Monocytes %	1	12	22	5	6	-
ACID BASE BALANCE	pH	-	-	-	-	-	-
	pCO_2	-	-	-	-	-	-
	Std. bicarbonate	-	-	-	-	-	-
LIVER HISTOLOGY	Functional Index	N	-	-	-	-	N
	Rejection Degree	N	-	-	-	-	N
	Cholestasis	0	-	-	-	-	0
	Cholangitis	0	-	-	-	-	0

PIG NO. 31 GROUP 2(a) SURVIVAL: 35 Days

OPERATION: End-to-side porta-caval shunt

	DESCRIPTION
Breed	Landrace
Animal weight	N.R.
Liver weight	(Estimated) -
Other	-

MAIN CAUSE OF DEATH: Sacrifice - protocol. (Weak hind legs)

ASSOCIATED GENERAL PATHOLOGY

Wound sepsis: Nil	Renal: Focal fibrosis
Wound breakdown: Nil	Lymph node: Reactive
Gastric: Ulcer	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Atelectasis	Other: Spleen mildly reactive
Cardiac: NAD	

LIVER	
Appearance	Normal
Weight	702 g.
Vascular anastomosis	NAD
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	-

	DAY	0	7	14	21	28	35
CLINICAL	Appearance	Good	Good	Good	Good	Fair	Fair
	Vigor	-	Good	Good	Good	Fair	Fair
	Wound sepsis	-	0	0	0	0	0
	Weight - kg	-	-	33	30	35	34
BIOCHEMISTRY	CSF Glutamine	8	22	70,6	93,5	99,2	52
	Venous ammonia	-	-	273	444	369	238
	Arterial ammonia	-	-	-	-	-	-
	Alkaline phosphatase	9,2	7,1	5,4	9,0	10,5	9,2
	S.G.O.T.	25	20	20	25	25	25
	Cholesterol	88	69	80	45	-	55
	Total protein	6,4	7,2	7,3	7,0	-	7,1
	Albumin	1,4	1,9	1,7	1,7	-	1,7
	Globulin	5,0	5,3	5,6	5,3	-	5,4
HAEMATOLOGY	Haemoglobin	11,4	12	12	12,3	12,9	13,2
	Leucocytes x 10^3	19,3	12,0	28,0	25,3	19,6	22,1
	Platelets x 10^3	558	453	348	495	125	250
	Neutrophils %	48	12	50	42	42	47
	Lymphocytes %	38	58	33	40	34	30
	Eosinophils %	2	10	11	0	8	12
	Basophils %	1	0	0	0	1	0
	Monocytes %	11	20	6	10	15	11
ACID BASE BALANCE	pH	-	-	-	-	-	-
	pCO_2	-	-	-	-	-	-
	Std. bicarbonate	-	-	-	-	-	-
LIVER HISTOLOGY	Functional Index	N	-	-	-	-	N
	Rejection Degree	N	-	-	-	-	N
	Cholestasis	0	-	-	-	-	0
	Cholangitis	0	-	-	-	-	0

PIG NO. 32 GROUP 2(a) SURVIVAL: 26 Days

OPERATION: End-to-side porta-caval shunt

	DESCRIPTION
Breed	Landrace
Animal weight	13 kg.
Liver weight	(Estimated) 473 g. -
Other	-

MAIN CAUSE OF DEATH: Multiple pulmonary abscesses

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +	Renal: NAD
Wound breakdown: Nil	Lymph node: Reactive
Gastric: Superficial ulcer	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Multiple small abscesses	Other: Spleen mildly reactive
Cardiac: NAD	

LIVER	
Appearance	Normal
Weight	420 g.
Vascular anastomosis	NAD
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	-

	DAY	0	7	14	21	26	
CLINICAL	Appearance	Good	Fair	Poor	Ill++	Dead	
	Vigor	-	Poor	Poor	Poor	-	
	Wound sepsis	-	0	0	0	+	
	Weight - kg	13	12	-	15	12	
BIOCHEMISTRY	CSF Glutamine	16	40	80,7	-	-	
	Venous ammonia	-	257	306	374	-	
	Arterial ammonia	-	-	-	-	-	
	Alkaline phosphatase	14,4	3,4	8,4	-	-	
	S.G.O.T.	50	105	25	30	-	
	Cholesterol	104	-	45	-	-	
	Total protein	-	-	7,6	-	-	
	Albumin	-	-	2,2	-	-	
	Globulin	-	-	5.4	-	-	
HAEMATOLOGY	Haemoglobin	-	11,2	13,1	11,8	-	
	Leucocytes x 10^3	7,2	30,4	22,3	24,8	-	
	Platelets x 10^3	343	595	405	200	-	
	Neutrophils %	7	71	59	79	-	
	Lymphocytes %	78	20	35	11	-	
	Eosinophils %	2	2	0	1	-	
	Basophils %	1	1	0	1	-	
	Monocytes %	12	6	6	8	-	
ACID BASE BALANCE	pH	-	-	-	-	-	
	pCO_2	-	-	-	-	-	
	Std. bicarbonate	-	-	-	-	-	
LIVER HISTOLOGY	Functional Index	N	-	-	-	N	
	Rejection Degree	N	-	-	-	N	
	Cholestasis	0	-	-	-	0	
	Cholangitis	0	-	-	-	0	

PIG NO. 33 GROUP 2(a) SURVIVAL: 28 Days

OPERATION: End-to-side porta-caval shunt

	DESCRIPTION
Breed	Landrace
Animal weight	N.R.
Liver weight	(Estimated) -
Other	-

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: Nil	Renal: NAD
Wound breakdown: Nil	Lymph node: Reactive
Gastric: Large ulcer	Pancreatic: NAD
Intestinal: Small colonic macules	Bladder: NAD
Pulmonary: Mild broncho-pneumonia	Other: Extra medullary haemopoeisis present in spleen
Cardiac: NAD	

LIVER	
Appearance	Normal
Weight	640 g.
Vascular anastomosis	NAD
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	-

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Good	Good	Fair	Fair	Fair	
	Vigor	-	Good	Fair	Fair	Fair	
	Wound sepsis	-	0	0	0	0	
	Weight - kg	-	25	23	25	25	
BIOCHEMISTRY	CSF Glutamine	7	29,1	30,3	68,7	37,4	
	Venous ammonia	-	252	210	311	214	
	Arterial ammonia	-	-	-	-	-	
	Alkaline phosphatase	18,5	4,8	5,4	10,6	6,5	
	S.G.O.T.	30	50	25	25	20	
	Cholesterol	122	86	57	91	81	
	Total protein	6,9	-	5,6	5,3	6,2	
	Albumin	2,4	-	1,1	0,69	0,87	
	Globulin	4,5	-	4,5	4,61	5,33	
HAEMATOLOGY	Haemoglobin	12,9	10,5	10,2	6,6	5,9	
	Leucocytes x 10^3	16,2	27,7	28,3	13,1	28,0	
	Platelets x 10^3	524	512	720	425	225	
	Neutrophils %	17	59	60	71	66	
	Lymphocytes %	73	22	32	20	26	
	Eosinophils %	0	5	1	4	0	
	Basophils %	3	1	1	0	0	
	Monocytes %	7	13	6	5	8	
ACID BASE BALANCE	pH	-	-	-	-	-	
	pCO_2	-	-	-	-	-	
	Std. bicarbonate	-	-	-	-	-	
LIVER HISTOLOGY	Functional Index	N	-	-	-	N	
	Rejection Degree	N	-	-	-	N	
	Cholestasis	0	-	-	-	0	
	Cholangitis	0	-	-	-	0	

PIG NO. 34 GROUP 2(a) SURVIVAL: 28 Days

OPERATION: End-to-side porta-caval shunt

	DESCRIPTION
Breed	N.R.
Animal weight	16 kg.
Liver weight	(Estimated) 582 g.
Other	-

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +	Renal: NAD
Wound breakdown: Nil	Lymph node: Reactive
Gastric: Small ulcer	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Mild pneumonia and atelectasis	Other: -
Cardiac: NAD	

LIVER	
Appearance	Normal
Weight	460 g.
Vascular anastomosis	NAD
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	-

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Good	Good	Good	Good	Good	
	Vigor	-	Good	Good	Good	Good	
	Wound sepsis	-	0	0	0	+	
	Weight - kg	16	16	20	20	22	
BIOCHEMISTRY	CSF Glutamine	17,7	32	43,9	32	63,8	
	Venous ammonia	170	306	339	321	299	
	Arterial ammonia	-	-	-	-	-	
	Alkaline phosphatase	12,4	5,9	12	11,8	10,8	
	S.G.O.T.	40	25	35	65	40	
	Cholesterol	127	52	55	104	66	
	Total protein	-	6,1	6,8	7,8	6,7	
	Albumin	-	1,7	2,2	2,0	1,8	
	Globulin	-	4,4	4,6	5,8	4,9	
HAEMATOLOGY	Haemoglobin	10,3	-	10,7	12,2	11,3	
	Leucocytes x 10^3	14,5	-	24,3	27,6	25,0	
	Platelets x 10^3	455	313	425	300	350	
	Neutrophils %	39	-	55	48	51	
	Lymphocytes %	51	-	40	36	34	
	Eosinophils %	1	-	2	3	0	
	Basophils %	1	-	0	1	0	
	Monocytes %	8	-	3	12	15	
ACID BASE BALANCE	pH	-	-	-	-	-	
	pCO_2	-	-	-	-	-	
	Std. bicarbonate	-	-	-	-	-	
LIVER HISTOLOGY	Functional Index	N	-	-	-	N	
	Rejection Degree	N	-	-	-	N	
	Cholestasis	0	-	-	-	0	
	Cholangitis	0	-	-	-	+	

PIG NO. 35 GROUP 2(a) SURVIVAL: 16 Days

OPERATION: End-to-side porta-caval shunt

	DESCRIPTION
Breed	Large White
Animal weight	15 kg.
Liver weight	(Estimated) 546 g.
Other	-

MAIN CAUSE OF DEATH: Pericarditis. Pneumonia

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +	Renal: NAD
Wound breakdown: Nil	Lymph node: NAD
Gastric: NAD	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Acute and chronic inflammatory changes	Other: -
Cardiac: Gross pericarditis	

LIVER	
Appearance	Normal
Weight	400 g.
Vascular anastomosis	NAD
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	-

	DAY	0	7	14	16		
CLINICAL	Appearance	Good	Fair	Fair	Poor		
	Vigor	-	Fair	Fair	Dead		
	Wound sepsis	-	+	+	+		
	Weight - kg	15	15	11	12		
BIOCHEMISTRY	CSF Glutamine	8,4	76,8	55,7	-		
	Venous ammonia	216	383	416	-		
	Arterial ammonia	-	-	-	-		
	Alkaline phosphatase	10,2	5,5	3,2	-		
	S.G.O.T.	35	35	35	-		
	Cholesterol	106	66	90	-		
	Total protein	6,4	7,4	7,9	-		
	Albumin	2,4	1,8	1,6	-		
	Globulin	4,0	5,6	6,3	-		
HAEMATOLOGY	Haemoglobin	12,0	-	12,2	-		
	Leucocytes x 10^3	18,5	-	22,6	-		
	Platelets x 10^3	701	-	350	-		
	Neutrophils %	48	-	63	-		
	Lymphocytes %	40	-	20	-		
	Eosinophils %	3	-	3	-		
	Basophils %	0	-	0	-		
	Monocytes %	9	-	14	-		
ACID BASE BALANCE	pH	-	-	-	-		
	pCO_2	-	-	-	-		
	Std. bicarbonate	-	-	-	-		
LIVER HISTOLOGY	Functional Index	N	-	-	N		
	Rejection Degree	N	-	-	N		
	Cholestasis	0	-	-	0		
	Cholangitis	0	-	-	0		

PIG NO. 36 GROUP 2(a) SURVIVAL: 22 Days

OPERATION: End-to-side porta-caval shunt

	DESCRIPTION
Breed	Landrace
Animal weight	13 kg.
Liver weight	(Estimated) 473 g.
Other	-

MAIN CAUSE OF DEATH: Iatrogenic - left haemothorax. Died approximately 12 hours after venepuncture at base of neck

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: ++	Renal: NAD
Wound breakdown: Nil	Lymph node: Reactive
Gastric: Small ulcer	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Fresh haemothorax. Mild acute and chronic inflammation	Other: Spleen mildly reactive
Cardiac: Pericarditis	

LIVER	
Appearance	Normal
Weight	380 g.
Vascular anastomosis	NAD
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	-

	DAY	0	7	14	21	22	
CLINICAL	Appearance	Good	Poor	Poor	Ill	Dead	
	Vigor	-	Poor	Poor	Poor	-	
	Wound sepsis	-	0	++	++	++	
	Weight - kg	13	15	14	13	13	
BIOCHEMISTRY	CSF Glutamine	11,6	79,1	76,6	77	-	
	Venous ammonia	212	320	327	468	-	
	Arterial ammonia	-	-	-	-	-	
	Alkaline phosphatase	7,2	4,2	4,9	4,6	-	
	S.G.O.T.	85	35	35	80	-	
	Cholesterol	95	44	48	47	-	
	Total protein	6,7	6,1	6,2	6,6	-	
	Albumin	1,7	1,1	1,2	1,2	-	
	Globulin	5,0	5,0	5,0	5,4	-	
HAEMATOLOGY	Haemoglobin	11,2	9,8	10,1	8,5	-	
	Leucocytes x 10^3	19,5	27,8	30,5	30,0	-	
	Platelets x 10^3	610	360	325	200	-	
	Neutrophils %	65	65	73	67	-	
	Lymphocytes %	25	24	20	21	-	
	Eosinophils %	0	1	0	0	-	
	Basophils %	2	0	0	0	-	
	Monocytes %	8	10	7	12	-	
ACID BASE BALANCE	pH	-	-	-	-	-	
	pCO_2	-	-	-	-	-	
	Std. bicarbonate	-	-	-	-	-	
LIVER HISTOLOGY	Functional Index	N	-	-	N	N	
	Rejection Degree	N	-	-	N	N	
	Cholestasis	0	-	-	0	0	
	Cholangitis	0	-	-	+	+	

PIG NO. 37 GROUP 2(a) SURVIVAL: 13 Days

OPERATION: End-to-side porta-caval shunt

	DESCRIPTION
Breed	Landrace
Animal weight	20 kg.
Liver weight	(Estimated) 728 g.
Other	-

MAIN CAUSE OF DEATH: Small bowel obstruction due to adhesive band

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +	Renal: NAD
Wound breakdown: Nil	Lymph node: NAD
Gastric: Ulcer with fresh bleed	Pancreatic: NAD
Intestinal: Small bowel obstruction	Bladder: NAD
Pulmonary: Multiple pustules	Other: -
Cardiac: NAD	

LIVER	
Appearance	Normal
Weight	720 g.
Vascular anastomosis	NAD
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	-

	DAY	0	7	13			
CLINICAL	Appearance	Fair	Good	Dead			
	Vigor	-	Good	-			
	Wound sepsis	-	0	+			
	Weight - kg	20	20	-			
BIOCHEMISTRY	CSF Glutamine	12,3	40,5	-			
	Venous ammonia	189	276	-			
	Arterial ammonia	-	-	-			
	Alkaline phosphatase	8,6	4,8	-			
	S.G.O.T.	15	20	-			
	Cholesterol	73	37	-			
	Total protein	4,7	7,7	-			
	Albumin	1,4	2,1	-			
	Globulin	3,3	5,6	-			
HAEMATOLOGY	Haemoglobin	-	11,4	-			
	Leucocytes x 10^3	-	49,0	-			
	Platelets x 10^3	-	245	-			
	Neutrophils %	-	71	-			
	Lymphocytes %	-	19	-			
	Eosinophils %	-	0	-			
	Basophils %	-	0	-			
	Monocytes %	-	10	-			
ACID BASE BALANCE	pH	-	-	-			
	pCO_2	-	-	-			
	Std. bicarbonate	-	-	-			
LIVER HISTOLOGY	Functional Index	N	-	N			
	Rejection Degree	N	-	N			
	Cholestasis	0	-	0			
	Cholangitis	0	-	0			

PIG NO. 38 GROUP 2(a) SURVIVAL: 28 Days

OPERATION: End-to-side porta-caval shunt

	DESCRIPTION
Breed	Landrace
Animal weight	17 kg.
Liver weight	(Estimated) 619 g.
Other	-

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +	Renal: NAD
Wound breakdown: Nil	Lymph node: Mildly reactive
Gastric: NAD	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Mild pneumonia	Other: -
Cardiac: Excessive pericardial fluid	

LIVER	
Appearance	Normal
Weight	653 g.
Vascular anastomosis	NAD
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	-

DAY		0	7	14	21	28	
CLINICAL	Appearance	Fair	Fair	Fair	Good	Fair	
	Vigor	-	Fair	Fair	Good	Fair	
	Wound sepsis	-	+++	+	+	+	
	Weight - kg	17	19	-	24	25	
BIOCHEMISTRY	CSF Glutamine	8,9	39,3	32,7	75,4	37,7	
	Venous ammonia	366	274	280	380	389	
	Arterial ammonia	-	-	-	-	392	
	Alkaline phosphatase	6,8	6,0	12,9	11,7	3,7	
	S.G.O.T.	20	20	30	30	45	
	Cholesterol	167	49	34	33	50	
	Total protein	6,0	6,6	6,9	7,0	7,4	
	Albumin	1,5	2,3	2,5	1,9	1,7	
	Globulin	4,5	4,3	4,4	5,1	5,7	
HAEMATOLOGY	Haemoglobin	-	10,4	-	12,0	10,9	
	Leucocytes x 10^3	-	31,4	22,5	21,0	13,1	
	Platelets x 10^3	-	390	300	150	275	
	Neutrophils %	-	60	56	59	26	
	Lymphocytes %	-	33	30	30	48	
	Eosinophils %	-	1	1	0	0	
	Basophils %	-	0	0	0	1	
	Monocytes %	-	6	13	11	25	
ACID BASE BALANCE	pH	-	-	-	-	7,315	
	pCO_2	-	-	-	-	52	
	Std. bicarbonate	-	-	-	-	24	
LIVER HISTOLOGY	Functional Index	N	-	-	N	N	
	Rejection Degree	N	-	-	N	N	
	Cholestasis	0	-	-	0	0	
	Cholangitis	0	-	-	0	+	

PIG NO. 39 GROUP 2(a) SURVIVAL: 28 Days

OPERATION: End-to-side porta-caval shunt

	DESCRIPTION
Breed	Landrace
Animal weight	21 kg.
Liver weight	(Estimated) 764 g.
Other	Intestinal worms. Milk spots on liver

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +	Renal: NAD
Wound breakdown: Nil	Lymph node: Acute lymphadenitis
Gastric: Superficial ulcer	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Mild pneumonia	Other: -
Cardiac: Excess pericardial fluid	

LIVER	
Appearance	Normal
Weight	390 g.
Vascular anastomosis	NAD
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	-

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Poor	Poor	Fair	Fair	Ill	
	Vigor	-	Poor	Fair	Poor	Poor	
	Wound sepsis	-	++	+	+	+	
	Weight - kg	21	22	20	20	20	
BIOCHEMISTRY	CSF Glutamine	10,8	34,5	44	45	57,8	
	Venous ammonia	173	313	400	375	530	
	Arterial ammonia	-	-	-	-	-	
	Alkaline phospnatase	6	4,8	4,1	5	2,6	
	S.G.O.T.	30	20	30	75	25	
	Cholesterol	123	-	58	62	45	
	Total protein	7,4	-	6,7	7,8	-	
	Albumin	2,5	-	1,4	2,2	-	
	Globulin	4,9	-	5,3	5,6	-	
HAEMATOLOGY	Haemoglobin	11,9	15,6	9,0	11,5	12,2	
	Leucocytes x 10^3	16,1	32,9	31,0	20,6	15,5	
	Platelets x 10^3	288	450	350	325	250	
	Neutrophils %	48	66	77	63	65	
	Lymphocytes %	32	23	20	24	22	
	Eosinophils %	12	1	0	4	0	
	Basophils %	2	0	0	0	2	
	Monocytes %	7	10	3	9	11	
ACID BASE BALANCE	pH	-	-	-	-	-	
	pCO_2	-	-	-	-	-	
	Std. bicarbonate	-	-	-	-	-	
LIVER HISTOLOGY	Functional Index	N	-	N	N	N	
	Rejection Degree	N	-	N	N	N	
	Cholestasis	0	-	0	0	0	
	Cholangitis	0	-	0	0	0	

PIG NO. 40 GROUP 2(a) SURVIVAL: 28 Days

OPERATION: End-to-side porta-caval shunt

	DESCRIPTION
Breed	Landrace
Animal weight	15 kg.
Liver weight	(Estimated) 546 g.
Other	-

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: ++	Renal: NAD
Wound breakdown: Nil	Lymph node: Reactive
Gastric: NAD	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Atelectasis	Other: Spleen mildly reactive
Cardiac: NAD	

LIVER	
Appearance	Normal
Weight	360 g.
Vascular anastomosis	NAD
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	-

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Fair	Good	Fair	Poor	Ill	
	Vigor	-	Good	Fair	Poor	Poor	
	Wound sepsis	-	0	++	++	++	
	Weight - kg	15	15	15	15	15	
BIOCHEMISTRY	CSF Glutamine	-	55,2	66	65	82,6	
	Venous ammonia	126	324	258	389	504	
	Arterial ammonia	-	-	-	-	437	
	Alkaline phosphatase	11,4	5,5	6,6	6,8	5,0	
	S.G.O.T.	25	40	25	130	75	
	Cholesterol	104	84	78	54	62	
	Total protein	-	5,6	7,0	6,4	6,7	
	Albumin	-	2,4	2,7	2,0	1,7	
	Globulin	-	3,2	4,3	4,4	5,0	
HAEMATOLOGY	Haemoglobin	11,4	11,0	-	11,9	11,1	
	Leucocytes x 10^3	10,1	23,6	25,9	39,9	20,6	
	Platelets x 10^3	333	200	-	325	375	
	Neutrophils %	53	57	74	63	49	
	Lymphocytes %	26	29	10	23	24	
	Eosinophils %	3	2	0	0	0	
	Basophils %	1	0	0	1	1	
	Monocytes %	17	12	16	13	26	
ACID BASE BALANCE	pH	-	-	-	-	-	
	pCO_2	-	-	-	-	-	
	Std. bicarbonate	-	-	-	-	-	
LIVER HISTOLOGY	Functional Index	N	-	N	N	N	
	Rejection Degree	N	-	N	N	N	
	Cholestasis	0	-	0	0	0	
	Cholangitis	0	-	0	0	0	

PIG NO. 41 GROUP 2(a) SURVIVAL: 18 Days

OPERATION: End-to-side porta-caval shunt

	DESCRIPTION
Breed	Landrace
Animal weight	17 kg.
Liver weight	(Estimated) 619 g.
Other	-

MAIN CAUSE OF DEATH: Cause not determined. Autolysis too far advanced for reasonable postmortem assessment

ASSOCIATED GENERAL PATHOLOGY			
Wound sepsis:	++	Renal:	
Wound breakdown:	Nil	Lymph node:	
Gastric:		Pancreatic:	Autolytic
Intestinal:		Bladder:	
Pulmonary:	Autolytic	Other:	
Cardiac:			

LIVER	
Appearance	Gas gangrene - autolytic
Weight	
Vascular anastomosis	Autolytic
Vessel patency	
BILIARY TRACT	
Appearance	
Bile stasis	
Bile leaks	Autolytic
Other	

	DAY	0	7	14	18		
CLINICAL	Appearance	Ill	Good	Fair	Dead		
	Vigor	-	Good	Fair	-		
	Wound sepsis	-	0	++	++		
	Weight - kg	17	17	17	-		
BIOCHEMISTRY	CSF Glutamine	9,1	35	55	-		
	Venous ammonia	376	242	318	-		
	Arterial ammonia	-	-	-	-		
	Alkaline phosphatase	11,6	5,9	5,1	-		
	S.G.O.T.	20	40	150	-		
	Cholesterol	110	78	58	-		
	Total protein	6,0	9,5	7,0	-		
	Albumin	1,7	2,0	1,0	-		
	Globulin	4,3	7,5	6,0	-		
HAEMATOLOGY	Haemoglobin	8,2	7,7	7,1	-		
	Leucocytes x 10^3	21,2	30,4	29,0	-		
	Platelets x 10^3	400	400	375	-		
	Neutrophils %	46	63	60	-		
	Lymphocytes %	40	32	26	-		
	Eosinophils %	3	0	1	-		
	Basophils %	1	0	0	-		
	Monocytes %	10	4	13	-		
ACID BASE BALANCE	pH	-	-	-	-		
	pCO_2	-	-	-	-		
	Std. bicarbonate	-	-	-	-		
LIVER HISTOLOGY	Functional Index	N	N	N	G		
	Rejection Degree	N	N	N	A		
	Cholestasis	0	0	0	S		
	Cholangitis	0	0	0			

PIG NO. 42 GROUP 2(a) SURVIVAL: 7 Days

OPERATION: End-to-side porta-caval shunt

	DESCRIPTION
Breed	Large White
Animal weight	15 kg.
Liver weight	(Estimated) 546 g.
Other	-

MAIN CAUSE OF DEATH: Iatrogenic - right haemothorax. Died one hour after venepuncture at base of neck

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: Nil	Renal: NAD
Wound breakdown: Nil	Lymph node: Haemorrhagic
Gastric: Large ulcer	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Fresh haemothorax Lungs - NAD	Other: NAD
Cardiac: NAD	

LIVER	
Appearance	Normal
Weight	450 g.
Vascular anastomosis	NAD
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	-

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Fair	Good				
	Vigor	-	Good				
	Wound sepsis	-	0				
	Weight - kg	15	15				
BIOCHEMISTRY	CSF Glutamine	4,1	28,3				
	Venous ammonia	217	263				
	Arterial ammonia	-	-				
	Alkaline phosphatase	3,3	9,6				
	S.G.O.T.	35	20				
	Cholesterol	92	-				
	Total protein	5,2	-				
	Albumin	1,8	-				
	Globulin	3,4	-				
HAEMATOLOGY	Haemoglobin	10,0	6,9				
	Leucocytes x 10^3	15,2	30,9				
	Platelets x 10^3	525	425				
	Neutrophils %	65	46				
	Lymphocytes %	28	53				
	Eosinophils %	2	1				
	Basophils %	0	0				
	Monocytes %	5	0				
ACID BASE BALANCE	pH	-	-				
	pCO_2	-	-				
	Std. bicarbonate	-	-				
LIVER HISTOLOGY	Functional Index	N	N				
	Rejection Degree	N	N				
	Cholestasis	0	0				
	Cholangitis	0	0				

PIG NO. 43 GROUP 2(b) SURVIVAL: 21 Days

OPERATION: Sham laparotomy

	DESCRIPTION
Breed	Large White
Animal weight	21 kg.
Liver weight	(Estimated) 764 g.
Other	Gross ascaris infestation of intestine

MAIN CAUSE OF DEATH: Iatrogenic - malignant hyperpyrexia. Died under Halothane anaesthesia - rectal temperature above 43° C.

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +	Renal: NAD
Wound breakdown: Nil	Lymph node: Reactive
Gastric: NAD	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: Mild patchy pneumonia	Other: Thyroid NAD
Cardiac: NAD	

LIVER	
Appearance	Normal
Weight	1000 g.
Vascular anastomosis	-
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	-

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Good	Good	Good	Good		
	Vigor	-	Good	Good	Good		
	Wound sepsis	-	0	++	+		
	Weight - kg	21	22	23	26		
BIOCHEMISTRY	CSF Glutamine	10,2	11,1	8,9	11,8		
	Venous ammonia	157	256	228	-		
	Arterial ammonia	175	-	-	-		
	Alkaline phosphatase	5,3	5,3	4,4	4,6		
	S.G.O.T.	35	45	40	125		
	Cholesterol	125	106	99	111		
	Total protein	5,6	6,0	7,0	6,6		
	Albumin	1,6	1,2	2,1	2,3		
	Globulin	4,0	4,8	4,9	4,3		
HAEMATOLOGY	Haemoglobin	10,5	10,4	10,6	10,8		
	Leucocytes x 10^3	17,9	25,3	23,2	31,8		
	Platelets x 10^3	650	350	375	500		
	Neutrophils %	50	55	24	-		
	Lymphocytes %	44	26	38	-		
	Eosinophils %	0	0	0	-		
	Basophils %	0	0	2	-		
	Monocytes %	6	19	36	-		
ACID BASE BALANCE	pH	7,410	-	7,000	-		
	pCO_2	30	-	37	-		
	Std. bicarbonate	20,5	-	8	-		
LIVER HISTOLOGY	Functional Index	N	N	N	N		
	Rejection Degree	N	N	N	N		
	Cholestasis	0	0	0	0		
	Cholangitis	0	0	0	0		

PIG NO. 44 GROUP 2(b) SURVIVAL: 28 Days

OPERATION: Sham laparotomy

	DESCRIPTION
Breed	Landrace
Animal weight	15 kg.
Liver weight	(Estimated) 546 g.
Other	Gross ascaris infestation of intestines

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: ++	Renal: NAD
Wound breakdown: Nil	Lymph node: Reactive
Gastric: NAD	Pancreatic: NAD
Intestinal: NAD	Bladder: NAD
Pulmonary: NAD	Other: -
Cardiac: NAD	

LIVER	
Appearance	Normal
Weight	998 g.
Vascular anastomosis	-
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	-

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Fair	Good	Good	Good	Good	
	Vigor	-	Good	Good	Good	Good	
	Wound sepsis	-	+	++	+	++	
	Weight - kg	15	20	19	24	27	
BIOCHEMISTRY	CSF Glutamine	12,5	9,5	7,2	10,6	10,1	
	Venous ammonia	181	-	144	155	272	
	Arterial ammonia	211	178	-	-	-	
	Alkaline phosphatase	3,2	4,0	7,0	6,4	4,6	
	S.G.O.T.	20	25	25	30	40	
	Cholesterol	128	85	116	98	101	
	Total protein	4,8	5,0	5,6	6,3	6,4	
	Albumin	1,2	1,5	1,4	1,9	2,2	
	Globulin	3,6	3,5	4,2	4,4	4,2	
HAEMATOLOGY	Haemoglobin	9,6	7,9	8,4	8,9	9,8	
	Leucocytes x 10^3	21,3	21,7	23,5	25,3	24,6	
	Platelets x 10^3	875	750	700	500	405	
	Neutrophils %	-	60	32	55	56	
	Lymphocytes %	-	26	40	28	32	
	Eosinophils %	-	2	0	0	1	
	Basophils %	-	0	0	0	0	
	Monocytes %	-	12	28	17	11	
ACID BASE BALANCE	pH	7,355	-	7,215	-	7,300	
	pCO_2	38	-	49	-	35	
	Std. bicarbonate	21	-	17,5	-	17,5	
LIVER HISTOLOGY	Functional Index	N	N	N	N	N	
	Rejection Degree	N	N	N	N	N	
	Cholestasis	0	0	0	0	0	
	Cholangitis	0	0	0	0	0	

PIG NO. 45 GROUP 2(b) SURVIVAL: 28 Days

OPERATION: Sham laparotomy

	DESCRIPTION
Breed	Landrace
Animal weight	20 kg.
Liver weight	(Estimated) 728 g.
Other	

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: Nil	Renal: Minimal lymphoid infiltration
Wound breakdown: Nil	Lymph node: Reactive
Gastric: NAD	Pancreatic: NAD
Intestinal: Small colonic macules	Bladder: NAD
Pulmonary: Severe pneumonia	Other: -
Cardiac: NAD	

LIVER	
Appearance	Normal
Weight	890 g.
Vascular anastomosis	-
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	-

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Fair	Good	Good	Good	Good	
	Vigor	-	Good	Good	Good	Good	
	Wound sepsis	-	+	+	0	0	
	Weight - kg	20	24	24	27	31	
BIOCHEMISTRY	CSF Glutamine	8,8	11,6	8,9	10,9	11,5	
	Venous ammonia	-	204	180	185	256	
	Arterial ammonia	229	-	-	-	256	
	Alkaline phosphatase	8,8	4,0	5,6	4,3	6,4	
	S.G.O.T.	35	40	25	55	55	
	Cholesterol	88	81	76	62	75	
	Total protein	5,5	6,0	6,3	6,0	7,0	
	Albumin	2,1	2,2	1,5	1,5	1,4	
	Globulin	3,4	3,8	4,8	4,5	5,6	
HAEMATOLOGY	Haemoglobin	11,1	10,2	9,4	9,0	10,3	
	Leucocytes x 10^3	13,7	40,3	28,0	20,3	13,4	
	Platelets x 10^3	600	350	450	350	235	
	Neutrophils %	53	58	45	53	12	
	Lymphocytes %	39	16	23	23	38	
	Eosinophils %	4	4	14	7	7	
	Basophils %	0	1	1	0	1	
	Monocytes %	4	21	17	7	42	
ACID BASE BALANCE	pH	7,435	-	7,155	-	7,310	
	pCO_2	41	-	52	-	52	
	Std. bicarbonate	21	-	16,8	-	22,5	
LIVER HISTOLOGY	Functional Index	N	N	N	N	N	
	Rejection Degree	N	N	N	N	N	
	Cholestasis	0	0	0	0	0	
	Cholangitis	0	0	0	0	0	

PIG NO. 46 GROUP 2(b) SURVIVAL: 28 Days

OPERATION: Sham laparotomy

	DESCRIPTION
Breed	Landrace
Animal weight	21 kg.
Liver weight	(Estimated) 764 g.
Other	Gross ascaris infestation of intestines

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: Nil.	Renal: NAD
Wound breakdown: Nil	Lymph node: -
Gastric: Subacute shallow ulcer	Pancreatic: Autolysed
Intestinal: NAD	Bladder: NAD
Pulmonary: NAD	Other: -
Cardiac: NAD	

LIVER	
Appearance	Normal
Weight	1535 g.
Vascular anastomosis	-
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Fair	Good	Excell	Excell	Excell	
	Vigor	-	Good	Excell	Excell	Excell	
	Wound sepsis	-	+	+	0	0	
	Weight - kg	21	29	35	41	47	
BIOCHEMISTRY	CSF Glutamine	9,5	7,2	7,2	7,3	8,2	
	Venous ammonia	223	216	141	380	236	
	Arterial ammonia	250	-	-	-	-	
	Alkaline phosphatase	4,1	2,5	8,2	7,7	10,0	
	S.G.O.T.	40	35	35	40	45	
	Cholesterol	102	133	120	112	122	
	Total protein	-	7,9	7,3	7,1	9,2	
	Albumin	-	2,0	2,4	2,6	3,3	
	Globulin	-	5,9	4,9	4,5	5,9	
HAEMATOLOGY	Haemoglobin	12,8	10,5	10,2	11,6	12,6	
	Leucocytes x 10^3	18,9	31,6	13,7	18,2	14,1	
	Platelets x 10^3	475	700	225	350	425	
	Neutrophils %	47	44	26	30	26	
	Lymphocytes %	40	36	37	43	47	
	Eosinophils %	3	5	3	0	4	
	Basophils %	3	0	0	1	0	
	Monocytes %	8	15	34	26	23	
ACID BASE BALANCE	pH	7,315	-	7,180	-	7,165	
	pCO_2	32,5	-	60	-	55	
	Std. bicarbonate	17,5	-	18	-	16,3	
LIVER HISTOLOGY	Functional Index	N	N	N	N	N	
	Rejection Degree	N	N	N	N	N	
	Cholestasis	0	0	0	0	0	
	Cholangitis	0	0	0	+	0	

PIG NO. 47 GROUP 2(b) SURVIVAL: 28 Days

OPERATION: Sham laparotomy

	DESCRIPTION
Breed	Landrace
Animal weight	15 kg.
Liver weight	(Estimated) 546 g.
Other	Gross ascaris infestation of intestines

MAIN CAUSE OF DEATH: Sacrifice - protocol

ASSOCIATED GENERAL PATHOLOGY	
Wound sepsis: +	Renal: NAD
Wound breakdown: Nil	Lymph node: Reactive
Gastric: NAD	Pancreatic: NAD
Intestinal: Small colonic macules	Bladder: NAD
Pulmonary: Pleural adhesions	Other: -
Cardiac: Mild pericarditis	

LIVER	
Appearance	Normal
Weight	960 g.
Vascular anastomosis	-
Vessel patency	All vessels patent

BILIARY TRACT	
Appearance	Normal
Bile stasis	Nil
Bile leaks	Nil
Other	-

	DAY	0	7	14	21	28	
CLINICAL	Appearance	Poor	Good	Good	Fair	Excell	
	Vigor	-	Good	Good	Good	Excell	
	Wound sepsis	-	+	+	+	0	
	Weight - kg	15	17	18	24	30	
BIOCHEMISTRY	CSF Glutamine	8,4	11,9	6,7	6,5	9,4	
	Venous ammonia	229	233	147	163	232	
	Arterial ammonia	205	-	-	-	-	
	Alkaline phospnatase	4,5	5,0	8,9	9,6	4,5	
	S.G.O.T.	20	50	40	40	50	
	Cholesterol	94	122	106	104	112	
	Total protein	5,4	5,9	6,1	6,5	6,9	
	Albumin	1,9	-	1,9	1,9	1,9	
	Globulin	3,5	-	4,2	4,6	5,0	
HAEMATOLOGY	Haemoglobin	11,0	10,8	11,4	11,9	12,2	
	Leucocytes x 10^3	22,2	16,3	24,0	18,2	12,0	
	Platelets x 10^3	400	900	300	350	320	
	Neutrophils %	78	47	35	38	17	
	Lymphocytes %	16	41	40	33	51	
	Eosinophils %	0	1	0	1	0	
	Basophils %	1	0	0	0	0	
	Monocytes %	5	11	25	28	32	
ACID BASE BALANCE	pH	7,485	-	7,240	-	7,320	
	pCO_2	25,5	-	57	-	48	
	Std. bicarbonate	22,8	-	19,5	-	22	
LIVER HISTOLOGY	Functional Index	N	N	N	N	N	
	Rejection Degree	N	N	N	N	N	
	Cholestasis	0	0	0	0	0	
	Cholangitis	0	0	0	0	0	

APPENDIX 2

HISTOLOGICAL ANALYSES OF THE LIVERS

IDENTIFICATION OF HISTOLOGICAL MATERIAL

The numbers allocated to the pigs in the operative laboratory differed from the numbers used in the histology laboratory and in the text. The operative, text and histology numbers of the animals from the present series are detailed in Table 39, page 317.

The identification of the liver histology from the previous two series (Dent and Immelman), is detailed at the foot of Table 39.

ANALYSES OF LIVER HISTOLOGY

1. The criteria, methods and symbols used in the analyses are explained in the text:

   (1) Assessment of the degree of Cholestasis and Cholangitis pages 162-165.

   (2) Assessment of the Functional Liver Index - pages 174-176.

   (3) Assessment of the Degree of Rejection - pages 183-185.

2. The results of the analyses are detailed:

   (1) Present Series:

   (a) Groups 1(a) and 1(b) - Tables 40-43 - pages 319-322
   (b) Group 2(a) - Table 44 - page 323
   (c) Group 2(b) - Table 45 - page 324

   (2) Previous Series:

   (a) Groups 3(a) and 3(b) (Dent) - Table 46 - page 325
   (b) Groups 4(a), 4(b) and 4(c) (Immelman) - Table 47 - page 326

ANIMAL NUMBERS		HISTOLOGY NUMBERS					Survival Days
Script No	Laboratory No	Days					
		Op	7	14	21	Autopsy	
1	JH 4/5	84/73	-	†	†	113/73	13
2	JH B1	190/73	-	-	-	252/73	23
3	JH 100	218/73	-	-	-	339/73	56
4	106	269/73	-	-	314/73	336/73	33
5	110	281/73	-	†	†	298/73	8
6	149	455/73	471/73	†	†	493/73	11
7	162	525/73	536/73	556/73	576/73	600/73	28
8	166	557/73	578/73	602/73	622/73	636/73	28
9	167	565/73	580/73	604/73	624/73	638/73	28
10	168	568/73	586/73	†	†	586/73	7
11	169	569/73	594/73	†	†	597/73	8
12	170	572/73	599/73	616/73	632/73	656/73	28
13	171	577/73	601/73	621/73	637/73	657/73	28
14	172	582/73	603/73	625/73	639/73	659/73	28
15	105	251/73	-	-	†	282/73	15
16	114	305/73	320/73	337/73	†	337/73	14
17	124	338/73	-	369/73	393/73	411/73	28
18	136	394/73	409/73	432/73	†	432/73	14
19	137	405/73	414/73	435/73	456/73	472/73	28
20	138	404/73	425/73	437/73	458/73	474/73	28
21	141	415/73	436/73	†	†	436/73	7
22	143	424/73	440/73	461/73	476/73	495/73	23
23	144	433/73	449/73	463/73	†	468/73	14
24	145	434/73	457/73	470/73	497/73	504/73	24
25	148	450/73	464/73	494/73	517/73	533/73	28
26	152	465/73	492/73	518/73	532/73	551/73	25
27	154	475/73	496/73	†	†	501/73	7
28	155	477/73	505/73	524/73	535/73	558/73	28
29	108	279/73	-	-	-	358/73	38
30	109	278/73	-	-	-	359/73	38
31	113	294/73	-	-	-	370/73	35
32	115	306/73	-	-	-	365/73	26
33	116	307/73	-	-	-	376/73	28

TABLE 39 IDENTIFICATION OF THE HISTOLOGICAL MATERIAL

(Continued on the following page)

ANIMAL NUMBERS		HISTOLOGY NUMBERS					Survival Days
Script No	Laboratory No	Days					
		0p	7	14	21	Autopsy	
34	121	332/73	-	-	-	398/73	28
35	122	333/73	-	-	†	364/73	16
36	123	334/73	-	-	381/73	385/73	22
37	126	340/73	-	†	†	377/73	13
38	127	341/73	-	-	400/73	423/73	28
39	128	343/73	-	382/73	403/73	427/73	28
40	129	344/73	-	383/73	402/73	426/73	28
41	130	360/73	384/73	401/73	†	-	18
42	132	375/73	399/73	†	†	399/73	7
43	156	491/73	506/73	526/73	539/73	540/73	21
44	157	490/73	507/73	527/73	541/73	562/73	28
45	158	489/73	508/73	528/73	542/73	564/73	28
46	159	488/73	509/73	529/73	543/73	563/73	28
47	160	487/73	510/73	530/73	544/73	561/73	28

- = No or inadequate biopsy material. † = Animal dead.

DENT'S SERIES				IMMELMAN'S SERIES			
ANIMAL NUMBERS				ANIMAL NUMBERS			
Script No	Laboratory No	Script No	Laboratory No	Script No	Laboratory No	Script No	Laboratory No
01	WD	011	45b	H1	K2	H10	K44
02	C	012	47b	H2	7J	H11	56M
03	S	013	48b	H3	94J	H12	92J
04	513	014	67b	H4	44M	H13	73J
05	49a	015	63b	H5	27J	H14	54J
06	55a	016	61b	H6	99I	H15	45J
07	74a	017	59b	H7	K83	H16	64J
08	89a	018	99b	H8	K71	H17	62J
09	91a	019	96b	H9	42M	H18	65J
010	94a	020	100b				

TABLE 39 IDENTIFICATION OF THE HISTOLOGICAL MATERIAL

(Continued from the previous page)

Pig No	Survival (Days)	Operation				7 Days				14 Days				21 Days				28 Days				35 Days				Longer			
		I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG
1	13	N	N	0	0	-	-	-	-	III	III	0	0																
2	23	N	N	0	0	-	-	-	-	-	-	-	-	Gas															
3	56	N	N	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IV	Infarct		
4	33	N	N	0	0	-	-	-	-	-	-	-	-	II	II	0	0	-	-	-	-	III	III	0	+++				
5	8	N	N	0	0	III	III	+	0																				
6	11	N	N	0	0	IV	IV	0	0	IV	IV	0	0																
7	28	N	N	0	0	IV	IV	0	+	IV	IV	0	0	IV	IV	0	0	IV	IV	0	+								
8	28	N	N	0	0	I	I	0	0	II	II	0	0	II	II	0	0	III	III	0	0								
9	28	N	N	0	0	I	II	0	0	II	III	0	0	Too Small				III	IV	0	++								
10	7	N	N	0	0	III	III	0	0																				
11	8	N	N	0	0	I	II	0	0																				
12	28	N	N	0	0	III	IV	0	0	III	IV	0	0	IV	IV	0	0	III	II	0	0								
13	28	N	N	0	0	II	II	0	0	II	II	0	0	II	II	0	0	I	I	0	0								
14	28	N	N	0	0	II	III	0	0	IV	IV	0	0	IV	IV	0	0	IV	IV	0	+								

Group 1(a) Heterotopic allograft + cholecystocholecystostomy

Analysis of DONOR livers

KEY: I - functional liver index
R - degree of rejection
CS - degree of cholestasis
CG - degree of cholangitis
* - inadequate or no specimens
—— - autopsy specimens

TABLE 40 ANALYSIS OF LIVER HISTOLOGY - PRESENT SERIES

Pig No	Survival (Days)	Operation				7 Days				14 Days				21 Days				28 Days				35 Days				Longer			
		I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG
15	15	N	N	0	0	-	-	-	-	III	III	0	0																
16	14	N	N	0	0	III	IV	0	0	IV	IV	0	+++																
17	28	N	N	0	0	-	-	-	-	IV	IV	0	0	IV	IV	0	0	IV	IV	0	+								
18	14	N	N	0	0	IV	Infarct			IV	Infarct																		
19	28	N	N	0	0	I	I	0	0	I	I	0	0	I	II	0	+	II	III	0	++								
20	28	N	N	0	0	II	II	0	0	I	I	0	0	I	I	0	0	I	II	0	0								
21	7	N	N	0	0	IV	IV	0	+																				
22	23	N	N	0	0	II	III	0	0	I	II	0	0	I	II	0	0	II	II	0	0								
23	14	N	N	0	0	III	III	0	0	III	IV	0	0																
24	24	N	N	0	0	I	II	0	0	I	I	0	0	I	II	0	0	II	II	0	0								
25	28	N	N	0	0	III	III	0	0	IV	IV	0	0	IV	IV	0	0	IV	IV	0	0								
26	25	N	N	0	0	II	III	0	0	II	II	0	0	II	II	0	0	III	III	++	+								
27	7	N	N	0	0	II	II	0	0																				
28	28	N	N	0	0	I	I	0	0	I	I	0	0	I	II	0	+	I	II	0	+								

Group 1(b) Heterotopic allograft + cholecystojejunocholecystostomy

Analysis of DONOR livers

KEY: I - functional liver index
R - degree of rejection
CS - degree of cholestasis
CG - degree of cholangitis
* - inadequate or no specimens
—— - autopsy specimens

TABLE 41 ANALYSIS OF LIVER HISTOLOGY - PRESENT SERIES

Pig No	Survival (Days)	Operation				7 Days				14 Days				21 Days				28 Days				35 Days				Longer			
		I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG
1	13	N	N	0	0	-	-	-	-	N	N	0	+																
2	23	N	N	0	0	-	-	-	-	-	-	-	-	N	N	0	0												
3	56	N	N	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N	0	0
4	33	N	N	0	0	-	-	-	-	-	-	-	-	N	N	0	0	-	-	-	-	N	N	0	0				
5	8	N	N	0	0	N	N	0	0																				
6	11	N	N	0	0	N	N	0	0	N	N	+	0																
7	28	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0								
8	28	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0								
9	28	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0								
10	7	N	N	0	0	N	N	0	0																				
11	8	N	N	0	0	N	N	0	0																				
12	28	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0								
13	28	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0								
14	28	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0								

Group 1(a) Heterotopic allograft + cholecystocholecystostomy

Analysis of RECIPIENT livers

KEY: I - functional liver index
R - degree of rejection
CS - degree of cholestasis
CG - degree of cholangitis
\- - inadequate or no specimens
—— - autopsy specimens

TABLE 42 ANALYSIS OF LIVER HISTOLOGY - PRESENT SERIES

Pig No	Survival (Days)	Operation				7 Days				14 Days				21 Days				28 Days				35 Days				Longer			
		I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG
15	15	N	N	0	0	-	-	-	-	N	N	0	+																
16	14	N	N	0	0	N	N	0	0	N	N	0	0																
17	28	N	N	0	0	-	-	-	-	N	N	0	0	N	N	0	0	N	N	0	0								
18	14	N	N	0	0	N	N	++	0	N	N	++	0																
19	28	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0								
20	28	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0	-	-	-	-								
21	7	I	N	0	0	N	N	0	0																				
22	23	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0												
23	14	N	N	0	0	N	N	0	0	N	N	0	0																
24	24	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0												
25	28	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0	I	N	+	+								
26	25	N	N	0	0	N	N	0	0	N	N	+	0	N	N	+	0	N	N	+++	+								
27	7	N	N	0	0	N	N	0	0																				
29	28	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0								

Group 1(b) Heterotopic allograft + cholecystojejunocholecystostomy

Analysis of RECIPIENT livers

KEY: I - functional liver index
R - degree of rejection
CS - degree of cholestasis
CG - degree of cholangitis
\- - inadequate or no specimens
—— - autopsy specimens

TABLE 43 ANALYSIS OF LIVER HISTOLOGY - PRESENT SERIES

Pig No	Survival (Days)	Operation				7 Days				14 Days				21 Days				28 Days				35 Days				Longer			
		I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG
29	38	N	N	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N	0	0				
30	38	N	N	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N	0	0				
31	35	N	N	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	N	N	0	0				
32	26	N	N	0	0	-	-	-	-	-	-	-	-	-	-	-	-	N	N	0	0								
33	29	N	N	0	0	-	-	-	-	-	-	-	-	-	-	-	-	N	N	0	0								
34	28	N	N	0	0	-	-	-	-	-	-	-	-	-	-	-	-	N	N	0	+								
35	16	N	N	0	0	-	-	-	-	-	-	-	-	N	N	0	0												
36	22	N	N	0	0	-	-	-	-	-	-	-	-	N	N	0	+												
37	13	N	N	0	0	-	-	-	-	N	N	0	0				-												
38	28	N	N	0	0	-	-	-	-	-	-	-	-	N	N	0	0	N	N	0	+								
39	28	N	N	0	0	-	-	-	-	N	N	0	0	N	N	0	0	N	N	0	0								
40	28	N	N	0	0	-	-	-	-	N	N	0	0	N	N	0	0	N	N	0	0								
41	18	N	N	0	0	N	N	0	0	N	N	0	0	Gas															
42	7	N	N	0	0	N	N	0	0																				

Group 2(a) End-to-side portacaval shunt

KEY: I - functional liver index
R - degree of rejection
CS - degree of cholestasis
CG - degree of cholangitis
- - inadequate or no specimens
—— - autopsy specimens

TABLE 44 ANALYSIS OF LIVER HISTOLOGY - PRESENT SERIES

Pig No	Survival (Days)	Operation				7 Days				14 Days				21 Days				28 Days				35 Days				Longer			
		I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG
43	21	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0												
44	28	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0								
45	28	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0								
46	28	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	+	N	N	0	0								
47	28	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0	N	N	0	0								

Group 2(b) Sham laparotomy

KEY I - functional liver index
R - degree of rejection
CS - degree of cholestasis
CG - degree of cholangitis
- - inadequate or no specimens
——— - autopsy specimens

TABLE 45 ANALYSIS OF LIVER HISTOLOGY - PRESENT SERIES

	Pig No.	Survival (days)	Operation				7 Days				14 Days				21 Days				28 Days				35 Days				Longer			
			I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG
Group 3(a)	01	51	N	N	0	0	N	I	0	0	I	I	++	ins	I	I	+++	ins	-	-	-	-	I	N	+++	ins	I	I	++++	0
	02	5	-	-	-	-																								
	03	46	N	N	0	0	ins	N	0	ins	ins	I	++	ins	I	I	+++	ins	ins	N	0	ins	-	-	-	-	II	II	+++	0
	04	6	N	N	0	0	II	III	0	++																				
	05	21	N	N	0	0	ins	I	+	ins	-	-	-	-	II	II	+++	+++												
	06	7	-	-	-	-	N	I	+++	+																				
	07	13	N	N	0	0	ins	I	0	ins	III	III	++	+++																
	08	28	-	-	-	-	-	-	-	-	ins	I	0	ins	ins	I	0	ins	II	I	0	+								
	09	8	N	N	0	0	III	II	0	0																				
	010	7	N	N	0	0	II	II	0	+																				
Group 3(b)	011	13	N	N	0	0	ins	N	0	ins	III	III	+	+																
	012	33	N	N	0	0	ins	N	0	ins	ins	I	0	ins	-	-	-	-	ins	I	0	ins	II	I	0	0				
	013	18	N	N	0	0	ins	I	0	ins	ins	I	0	ins	I	I	0	0												
	014	15	-	-	-	-	ins	N	0	ins																				
	015	13	-	-	-	-	ins	N	0	ins																				
	016	7	-	-	-	-	I	I	+	0																				
	017	23	-	-	-	-	ins	II	0	ins	ins	III	++	ins	I	I	++	0												
	018	24	-	-	-	-	ins	N	0	ins	ins	N	0	ins																
	019	38	-	-	-	-	-	-	-	-	-	-	-	-	ins	N	0	ins	ins	N	0	ins	-	-	-	-	II	II	0	0
	020	15	-	-	-	-	-	-	-	-	I	I	+++	0																

Group 3(a) Orthotopic allograft + cholecystoduodenostomy

Group 3(b) Orthotopic allograft + choledochocholedochostomy

Key: ins = section too small for accurate grading

Other symbols - same as for group 1(a)

TABLE 46 ANALYSIS OF LIVER HISTOLOGY - PREVIOUS SERIES (DENT)

	Pig No.	Survival (days)	Operation				7 Days				14 Days				21 Days				28 Days				35 Days				Longer			
			I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG	I	R	CS	CG
Group 4(a)	H1	14	-	-	-	-	-	-	-	-	I	I	+	0																
	H2	24	-	-	-	-	-	-	-	-	-	-	-	-	I	II	0	+												
	H3	28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	0	0								
	H4	36	-	-	-	-	-	-	-	-	I	II	0	0	II	II	+	+	-	-	-	-	IV	III	0	+				
	H5	64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	II	II	0	+
	H6	91	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	0	0
	H7	105	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	0	0
	H8	143	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IV	IV	0	0
	H9	43	-	-	-	-	-	-	-	-	I	II	0	0	II	III	0	+	-	-	-	-	II	III	0	0	III	III	0	0
	H10	45	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I	I	0	0
	H11	36	-	-	-	-	I	II	0	0	I	II	0	0	I	I	0	0	-	-	-	-	N	N	0	0				
Group 4(b)	H12	8	-	-	-	-	III	III	+	++																				
	H13	24	-	-	-	-	-	-	-	-	-	-	-	-	I	II	+	+												
	H14	33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	III	III	++	+++				
Group 4(c)	H15	5	-	-	-	-	I	II	++	++																				
	H16	5	-	-	-	-	II	III	++	+++																				
	H17	11	-	-	-	-	-	-	-	-	I	II	++	++																
	H18	47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	IV	IV	++	+++

Group 4(a) Heterotopic allograft + cholecystocholecystostomy

Group 4(b) Heterotopic allograft + cholecystojejunostomy-en-y

Group 4(c) Heterotopic allograft + cholecystostomy

KEY: Same as for group 1(a)

TABLE 47 ANALYSIS OF LIVER HISTOLOGY - PREVIOUS SERIES (IMMELMAN)

THE ESTIMATION OF LIVER CELL SIZES

During the course of the experimental study, it was noted that:

(1) The livers of the portacaval-shunted animals at autopsy, were lighter in weight than the estimated weight at initial operation, and lighter than the predicted weight at autopsy - see pages 157–158.

(2) The liver cell sizes appeared to be smaller 28 days after portacaval shunt, compared to the corresponding biopsies at initial operation, and smaller than the cells seen 28 days after sham laparotomy.

The question arose as to whether the loss in liver weight was due to loss of cell size, a decrease in the number of hepatocytes, or a combination of the two. A study was undertaken to try to assess the relationships between liver cell size and liver weight.

METHOD

The method used was similar to that alluded to by Starzl (185).

1. Liver biopsies had been obtained from both the portacaval-shunted (group 2(a)), and sham laparotomy (group 2(b)) animals at initial operation and at autopsy. The biopsies had been fixed in formol saline and embedded in Paraplast as discussed on page 161. Thin sections (2-4μ) were cut, and stained with Haematoxylin and Eosin.

2. The outlines of 50 hepatocytes, selected at random, were drawn onto standard thickness paper, using a light microscope with a drawing tube attachment (Sankei). An oil emersion lens was used for maximum magnification. Fifty cell outlines were drawn for each pre- and postoperative liver biopsy specimen, by each of 2 independent observers. Forty randomly-selected cell outlines were cut out of each sheet of paper and weighed on a chemical balance by a third observer. The change in weight between the 40 pre-operative "cells" and the 40 postoperative "cells" was taken as representative of the change in liver cell size.

	LIVER CELL OUTLINE WEIGHTS AT AUTOPSY *				
	Observer 1			Observer 2	
	Mean SD	No		Mean SD	No
Group 2(b)	103,06 ± 16,95	3		119,90 ± 5,23	3
Group 2(a)	77,12 ± 18,89	5		79,32 ± 18,83	5
Significance	p = < 0,05			p = < 0,01	

* Expressed as a percentage compared to the weight at initial operation

TABLE 48 THE SIGNIFICANCE OF THE DIFFERENCES IN MEAN "LIVER CELL" WEIGHTS AT AUTOPSY

Group 2(b) - Sham laparotomy
Group 2(a) - End-to-side portacaval shunt

The animals were all sacrificed at 28 days and liver biopsy taken immediately after death.

RESULTS

1. The "liver cells" for the livers from group 2(a) showed a significant loss in mean weight at autopsy, compared to the mean pre-operative weight ($p < 0,001$). The "liver cells" from the group 2(b) animals showed no significant change in mean weights.

2. On comparing the mean "liver cell" weights in groups 2(a) and 2(b) at autopsy, significant differences were recorded by both observers - see Table 48 opposite. The numbers are, however, too small to be of real statistical value.

DISCUSSION

The liver cell walls were often extremely difficult to demarcate. This prevented true randomisation in drawing the cell outlines, as the observers had to select cells with clear outlines. Polarising light and phase contrast microscopy provided no advantage over ordinary light microscopy, while the use of diastase-digested cells did not improve resolution. Attempts to find a cell wall stain suitable for the material available, were fruitless.

The cell outlines obtained with this method were small (1-1,5 cms. in diameter) and the margin of error in drawing and cutting out such small outlines appeared significant. Attempts to obtain larger outlines by projection of the specimen onto a screen failed on account of the poor resolution which was obtained.

The changes and differences in "liver cell" weights were statistically significant, but the method was considered too inaccurate to provide meaningful results, as mentioned above, and the study was abandoned.

APPENDIX 3

ANALYSIS OF THE TIME TAKEN TO PERFORM THE DIFFERENT STAGES OF THE OPERATIONS

PIG NUMBER	DONOR OPERATION			RECIPIENT OPERATION							DONOR LIVER INSULT				ANAESTHETIC TIME-TOTAL		VASCULAR ANASTOMOSES			Gastro-duodenostomy
	Preparation	Exsanguination and hepatectomy	Total operative time	Preparation	Full implantation	Closure	Total operative time	Bypass time	Implant to full revascularisation	Cholecysto-cholecystostomy	Cold ischaemia	Warm ischaemia	Total period ischaemia	Period to full revascularisation	Donor	Recipient	Cavo-caval	Porto-portal	Aorto-aortic	
1	60	25	85	55	70	20	145	20	60	10	20	40	60	80	135	210	20	20	20	-
2	50	13	63	50	54	30	134	5	39	5	18	25	43	63	140	180	11	5	15	-
3	75	15	90	45	NR	NR	105	7	40	NR	16	22	38	53	140	125	12	7	8	-
4	70	7	77	60	NR	NR	115	7	39	NR	20	25	45	58	147	145	10	7	8	-
5	45	10	55	35	NR	NR	105	5	37	NR	17	20	37	54	100	135	10	5	10	-
6	60	15	75	30	76	25	131	10	48	8	21	28	49	64	150	180	17	10	10	-
7	60	17	77	NR	67	15	NR	8	50	10	26	27	53	73	122	228	19	8	12	20
8	52	12	64	60	71	16	147	15	55	13	51	32	83	102	72	205	16	15	9	13
9	60	22	82	35	70	12	117	12	50	15	24	28	52	68	132	170	17	12	10	15
10	35	17	52	45	62	22	129	11	45	16	23	26	49	64	102	185	15	11	11	15
11	47	27	74	31	59	18	108	7	39	12	24	21	45	61	110	165	14	7	12	11
12	43	12	55	44	51	20	115	8	36	12	20	20	40	53	97	160	11	8	9	15
13	35	11	46	35	42	35	112	Nil	28	7	18	16	34	44	96	140	8	6	7	-
14	40	18	58	36	76	30	140	15	46	15	24	27	51	66	110	176	10	12	10	-

TABLE 49 GROUP 1(a) - TIME REQUIRED FOR THE DIFFERENT PROCEDURES (MINUTES)

PIG NUMBER	DONOR OPERATION			RECIPIENT OPERATION							DONOR LIVER INSULT				ANAESTHETIC TIME-TOTAL		VASCULAR ANASTOMOSES			Gastro-duodenostomy
	Preparation	Exsanguination and hepatectomy	Total operative time	Preparation	Full implantation	Closure	Total operative time	Bypass time	Implant to full revascularisation	Cholecysto-jejuno-cholecystostomy and jejuno-jejunostomy	Cold ischaemia	Warm ischaemia	Total period ischaemia	Period to full revascularisation	Donor	Recipient	Cavo-caval	Porto-portal	Aorto-aortic	
15	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	-
16	55	16	71	45	98	8	151	10	46	45	15	30	45	61	126	178	15	10	10	-
17	40	13	53	52	90	18	160	13	45	22	20	25	45	60	130	187	14	13	13	-
18	30	15	45	63	90	13	166	17	48	33	16	23	39	57	110	200	15	10	13	-
19	50	16	66	45	85	15	145	12	39	27	18	22	40	54	124	180	11	12	12	-
20	NR	18	NR	50	93	15	158	10	40	49	20	24	44	61	115	195	14	10	15	-
21	50	11	61	50	82	19	151	15	43	32	15	27	42	57	127	180	12	11	11	-
22	40	13	53	50	98	17	165	9	50	45	18	30	48	65	124	195	20	9	12	-
23	50	14	64	33	95	14	142	13	49	38	30	33	63	77	125	183	20	14	9	-
24	50	17	67	34	81	10	125	11	43	32	22	26	48	63	105	159	15	9	11	-
25	36	10	46	30	90	15	135	9	50	35	35	23	58	75	85	180	13	9	15	-
26	45	15	60	35	71	25	131	8	39	25	18	20	38	55	100	172	10	6	13	-
27	40	18	58	40	90	15	145	17	50	25	21	32	53	68	98	185	13	17	12	-
28	40	12	52	42	95	30	167	13	47	37	26	24	50	66	96	202	12	12	9	NR

TABLE 50 GROUP 1(b) - TIME REQUIRED FOR THE DIFFERENT PROCEDURES (MINUTES)

	GROUPS				Significance
	1(a)		1(b)		
DONOR OPERATION	Mean ± 1 SD	No	Mean ± 1 SD	No	
Preparation	52,28 ± 11,91	14	43,83 ± 7,00	12	<0,05
Exsanguination and hepatectomy	15,78 ± 5,53	14	14,46 ± 2,46	13	-
Total donor anaesthetic time	118,07 ± 22,49	14	112,69 ± 14,09	13	-
RECIPIENT OPERATION					
Preparation	43,15 ± 10,12	13	43,76 ± 8,99	13	-
Full implantation	63,45 ± 10,43	11	89,07 ± 7,37	13	<0,001
Closure	22,09 ± 6,84	11	16,46 ± 5,58	13	<0,05
Total recipient operative time	123,30 ± 14,48	13	149,30 ± 13,16	13	<0,001
Porto-systemic bypass time	10,00 ± 4,29	13	12,07 ± 2,84	13	-
Implantation to full revascularisation	43,71 ± 8,14	14	45,30 ± 4,00	13	-
Cholecysto-cholecystostomy	11,18 ± 3,37	11	-		
Cholecysto-jejuno-cholecystostomy and jejuno-jejunostomy	-		34,23 ± 8,11	13	< 0,001
DONOR LIVER INSULT					
Cold ischaemic time	23,00 ± 8,28	14	21,07 ± 5,74	13	-
Warm ischaemic time	25,50 ± 5,69	14	26,07 ± 3,89	13	-
Period to full revascularisation	64,50 ± 13,58	14	63,00 ± 6,86	13	-
TOTAL ANAESTHETIC TIMES					
Donor animal	118,07 ± 22,49	14	112,69 ± 14,09	13	-
Recipient animal	171,71 ± 28,67	14	184,30 ± 11,38	13	-
VASCULAR ANASTOMOTIC TIMES					
Cavo-caval	13,57 ± 3,65	14	14,15 ± 2,90	13	-
Porto-portal	9,50 ± 4,06	14	10,92 ± 2,64	13	-
Aorto-aortic	10,78 ± 3,21	14	11,92 ± 1,85	13	-
ADDITIONAL PROCEDURES					
Gastro-duodenostomy	14,83 ± 2,73	6	NR	1	0

TABLE 51 COMPARISON OF THE TWO TRANSPLANT TECHNIQUES - TIME (MINUTES)

GROUP 2(a).

Pig No	Total Portal Occlusion Time (Minutes)	Total Anaesthetic Time (Minutes)
29	12	65
30	12	50
31	15	-
32	14	70
33	11	-
34	10	50
35	12	-
36	7	56
37	11	-
38	14	63
39	12	-
40	10	45
41	11	80
42	14	85
Average	11,78	62,66

GROUP 2(b)

Pig No	Total Portal Occlusion Time (Minutes)	Total Anaesthetic Time (Minutes)
43	12	70
44	12	60
45	11	67
46	12	60
47	12	75
Average	11,8	66,4

TABLE 52 OPERATIVE TIMES FOR THE TWO CONTROL GROUPS

APPENDIX 4

APPENDIX OF METHODS

BIOCHEMICAL ESTIMATIONS

The estimations were all performed in the biochemical research laboratories of the Department of Surgery, University of Cape Town. The author frequently submitted duplicated specimens, unknown to the laboratory staff, as an additional control on the routine laboratory control of techniques.

1. Cerebrospinal Fluid Glutamine

CSF glutamine estimations were performed by the hot acid hydrolysis method of Whitehead and Whittaker (219).

In view of the importance of CSF glutamine in this experimental project, the accuracy and precision of the estimations was checked in a number of ways. A number of duplicate samples were submitted, under code names, to the laboratory at different intervals during the project - with results varying by less than 10% in all cases. Duplicate samples were deep-frozen and submitted for estimation at a later date - the results did not differ materially from the original. Whenever CSF was sampled, it was noted whether the fluid was clear or bloodstained - there was no appreciable difference in the pre-operative levels between samples which were clear or bloodstained.

Finally, amino-acid chromatographic estimations were performed on a number of random duplicate CSF samples, using the method described by de Wet (56). The amino-acid chromatography was performed in the Department of Chemical Pathology by Mr. P. de Wet (Dip. Med. Tech. (Chem. Path.)), under the direction of Professor M. Berman.

The comparative results of the glutamine estimations are listed in Table 53 below.

Sample No	CSF GLUTAMINE LEVELS	
	Amino-acid Chromatography	Hot acid Hydrolysis
1	9,2mg/100ml	10,9mg/100ml
2	9,7mg/100ml	10,9mg/100ml
3	29,2mg/100ml	26,4mg/100ml
4	36,9mg/100ml	48,5mg/100ml
5	46,9mg/100ml	45,4mg/100ml

TABLE 53 COMPARISON OF THE RESULTS FROM TWO METHODS OF CSF GLUTAMINE ESTIMATION

In addition, the chromatographs revealed that glutamine was the only amino-acid which peaked in the three samples of cerebrospinal fluid with elevated glutamine levels.

All the animals had been starved of food for 24 hours prior to sampling of CSF and blood, thus creating standard conditions. To eliminate the effect of diet, CSF glutamine estimations were performed on 6 stock pigs in a separate control study. The CSF was taken after the pigs had had free access to food and water for 36 hours, and then a few days later, after 24 hours starvation. The glutamine levels ranged from 9,0 - 11,3mg/100ml, and it was concluded that starvation did not affect the levels in the normal pigs.

In 30 animals, blood taken simultaneously with the CSF, was submitted for blood urea determinations as described by Chaney and Marbach (40). The urea was elevated in only 1 of the 30 specimens submitted.

The author is satisfied that the method and technique used in the CSF glutamine estimations was accurate and reliable.

2. Blood Ammonia

Plasma ammonia was measured using a combination of the methods of Kaplan (102), and Chaney and Marbach (40). Under anaerobic conditions, the blood samples were added to equal volumes of 15% trichloro-acetic acid for protein precipitation. After centrifugation, 0,5 ml supernatant was mixed with 0,5 ml phenol/sodium nitroprusside reagent, and 1,0 ml alkaline hypochlorite. (The commercial bleach, Nomisol, was found to contain the correct concentration of hypochlorite). Following a ten minute incubation period in a water-bath at 37°C, the absorbance was read spectrophotometrically at 625µ. Final calculation was made from a standard curve.

3. Alkaline Phosphatase

Alkaline phosphatase determinations were performed using phenolphthalein monophosphate as the substrate (Babson (16)), the method being modified for use on a Technicon Auto-Analyser. The results were expressed as Shinowara-Jones-Reinhart units.

4. Serum Glutamic Oxaloacetic Transaminase (SGOT)

The SGOT assays were performed according to the method of Karmen (103).

5. Serum Cholesterol

Cholesterol estimations utilised the Liebermann-Burchard reaction (Pearson (142)).

6. Serum Proteins

Total protein and albumin were measured by the biuret method before and after precipitation with sodium sulphite, and the globulin calculated (Wolfson (227)).

7. Acid-Base Determinations

The Astrup estimations were made on a Radiometer apparatus with derivation of the partial pressure of carbon dioxide, the bicarbonate and the base excess from samples equilibrated with known concentrations of carbon dioxide (4% and 8%) using the Siggaard-Anderson nomogram (167).

HAEMATOLOGICAL INVESTIGATIONS

These estimations were all performed in the routine diagnostic laboratories of the Department of Haematology, Groote Schuur Hospital. Coded duplicate samples were submitted at intervals as a personal check on the accuracy and precision of the laboratory, and the author was satisfied that there were no significant discrepancies in the results.

The blood samples were placed into sequestrine tubes, and delivered to the laboratory within 2 hours of being taken.

1. Haemoglobin and white cell counts

   Haemoglobin and total leucocyte counts were performed on a Coulter counter. (Coulter Counter - Model S - Coulter Electronics - Hialeah, Florida).

2. Platelet counts

   The platelet counts were performed using a Thrombocounter. (Thrombocounter - C, Coulter Electronics, Dunstable). Each platelet count obtained from the thrombocounter was checked by means of a smear estimation, and in a number of cases, platelet-clumping had resulted in incorrect counts on the thrombocounter.

3. Differential white cell count and blood smear report

   The blood smears were stained with May-Grünwald-Giemsa stains, and examined under light microscopy. With only a few exceptions, the differential counts and smear reports were all performed by the same technologist - Miss H.A. Clark (Dip. Med. Tech. (Haem.)), senior technologist in the Department of Haematology.

STATISTICAL METHODS

1. CALCULATION OF THE MEAN, STANDARD DEVIATION, AND STANDARD ERROR OF THE MEAN

The calculations were all performed on an Olivetti Calculator (P203) in the Liver Research Laboratories of the Department of Medicine, University of Cape Town. The programs had been prepared by the research staff of the Liver Laboratories.

The author performed most of the calculations, while some of the earlier calculations were performed by the research staff of the Department of Surgery. Complete print-outs were obtained for each calculation, and all entries double checked. In addition, approximately 10% of the calculations, randomly selected, were re-calculated at a later date as a check on the function of the calculator.

The formulae used were:-

(1) Mean

$$\bar{\chi} = \frac{\chi_1 + \chi_2 + \ldots + \chi_n}{n}$$

Where χ is the variable, and n the number of observations (8).

(2) Standard deviation

$$s = \sqrt{\left(\frac{\Sigma(\chi i - \bar{\chi})^2}{n - 1}\right)}$$

Where $\bar{\chi}$ is the mean (9).

(3) Standard error of the mean

$$S.E.M. = \frac{s}{\sqrt{n}}$$

Where s^2 = (standard deviation)^2 = Variance (10)

2. COMPARISON OF TWO MEANS

The t and F tests were all calculated by Miss C.G. Vader, statistician to the Department of Obstetrics and Gynaecology, University of Cape Town, and the author, with the aid of two Hewlett-Packard calculators (models 9100B and 55A), the use of two calculators being dictated by logistics. The programs were prepared by Miss C. Vader. The precision of each calculator was checked by repeating approximately 10% of the calculations, randomly selected, on each calculator, and, in addition, the calculators were checked by repeating approximately 10% of the calculations from the one calculator on the other. The author was satisfied that the programs were identical, and that the calculators functioned correctly.

(1) The t test for the comparison of two means where the variances are equal

(a) The F test for equal variances

$$F = s_1^2/s_2^2$$

Where s_1^2 is the larger of the two variances (12).

Significance levels of F were determined from tables (14).

(b) The t test (11)

$$t = \frac{(\bar{\chi}_1 - \bar{\chi}_2)}{\sqrt{s^2(\frac{1}{n_1} + \frac{1}{n_2})}}$$

Where $s^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}$

and where $\bar{\chi}_1$ and $\bar{\chi}_2$ are the means of the two samples, s_1 and s_2 are the standard deviations, and n_1 and n_2 the sample sizes. Degrees of freedom (df) = $n_1 + n_2 - 2$. Significance levels of t were determined from tables (57). Where the distributions of the variables were markedly skewed, as in SGOT, log transformation was applied to the variable before calculating F and t (13).

(2) <u>The t test for the comparison of the means where the variances are unequal</u>

Where the F test showed that the variances were significantly different, Dixon and Massey's formula (52) was used to modify the degrees of freedom, and the t test described above was then applied.

$$df = \frac{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)^2}{\frac{(s_1^2/n_1)^2}{n_1} + \frac{(s_2^2/n_2)^2}{n_2}}$$

Where s_1 and s_2 are the standard deviations and n_1 and n_2 are the sample sizes.

APPENDIX 5

STATISTICAL ANALYSIS OF THE SERIAL CHANGES IN THE BIOCHEMICAL AND HAEMATOLOGICAL FACTORS IN THE FOUR GROUPS - RELEVANT TO PART II

SERIAL MEAN VALUES FOR THE GROUPS (Mean ± 1 SEM)

THE SIGNIFICANCE OF SERIAL CHANGES IN MEAN VALUES FOR EACH GROUP

THE SIGNIFICANCE OF THE DIFFERENCES IN MEAN VALUES BETWEEN GROUPS AT WEEKLY INTERVALS

TEST	DAY											
	OP	NA 9 No	7	NA 9 No	14	NA 6 No	21	NA 6 No	28	NA 6 No
CSF Glutamine	10,60 ± 0,56	8	18,06 ± 2,66	9	25,63 ± 4,73	6	25,40 ± 4,84	6	21,83 ± 3,29	6
Venous NH_4	193,50 ± 11,32	8	286,85 ± 27,62	7	291,83 ± 33,99	6	344,66 ± 42,88	6	294,50 ± 21,61	6
Alkaline Phos.	5,32 ± 0,41	9	6,62 ± 0,60	9	7,43 ± 0,63	6	4,70 ± 1,18	6	3,95 ± 1,20	6
S.G.O.T.	45,00 ± 4,40	9	69,44 ± 5,73	9	72,83 ± 20,04	6	58,33 ± 9,97	6	35,00 ± 3,41	6
Cholesterol	90,85 ± 8,56	7	92,00 ± 11,51	7	107,50 ± 5,26	6	71,40 ± 19,11	5	55,50 ± 21,79	6
Total protein	6,31 ± 0,22	8	7,45 ± 0,44	7	6,68 ± 0,13	6	6,88 ± 0,18	6	7,11 ± 0,27	6
Albumin	1,82 ± 0,12	8	2,04 ± 0,11	7	2,00 ± 0,10	6	1,85 ± 0,12	6	1,73 ± 0,21	6
Globulin	4,48 ± 0,19	8	5,41 ± 0,37	7	4,68 ± 0,21	6	5,03 ± 0,18	6	5,38 ± 0,20	6
Haemoglobin	11,73 ± 0,52	9	11,34 ± 0,38	9	12,18 ± 0,35	6	10,08 ± 0,56	6	9,91 ± 0,96	6
Leucocytes x 10^3	16,95 ± 1,26	9	31,20 ± 3,50	9	22,66 ± 2,88	6	28,31 ± 3,71	6	32,95 ± 5,88	6
Platelets x 10^3	420,77 ± 44,69	9	592,37 ±103,58	8	412,50 ±150,43	6	422,83 ±110,41	6	431,66 ± 83,57	6
Neutrophils %	42,44 ± 5,60	9	52,66 ± 4,64	9	51,00 ± 5,01	6	44,66 ± 8,75	6	52,83 ± 7,58	6
Lymphocytes %	36,66 ± 2,96	9	29,22 ± 2,47	9	37,16 ± 4,30	6	30,16 ± 4,68	6	33,33 ± 4,12	6
Eosinophils %	8,11 ± 2,81	9	5,00 ± 1,04	9	4,66 ± 1,30	6	4,50 ± 2,04	6	3,83 ± 1,88	6
Basophils %	0,55 ± 0,33	9	0,88 ± 0,26	9	2,33 ± 0,33	6	1,66 ± 0,98	6	1,50 ± 0,42	6
Monocytes %	12,22 ± 2,34	9	12,22 ± 3,06	9	5,16 ± 1,01	6	19,00 ± 5,60	6	8,50 ± 2,44	6
pH	7,30 ± 0,04	9	7,29 ± 0,04	6	7,23 ± 0,05	6	7,20 ± 0,04	6	7,22 ± 0,02	6
pCO_2	45,66 ± 4,98	9	48,41 ± 3,07	6	40,75 ± 2,30	6	45,50 ± 5,09	6	46,50 ± 2,61	6
Std. bicarbonate	20,74 ± 1,72	9	21,50 ± 2,00	6	17,48 ± 1,79	6	16,28 ± 1,47	6	17,74 ± 1,56	6

Mean ± 1 Sem NA = Number of animals No = Number of samples processed

TABLE 54 SERIAL MEAN LEVELS - GROUP 1(a) (Heterotopic allograft plus cholecystocholecystostomy)

TEST	DAY													
	OP	NA 13 No	7	NA 13 No	14	NA 11 No	21	NA 8 No	28	NA 6 No
CSF Glutamine	11,26 ± 0,87	12	22,57 ± 2,67	13	23,24 ± 2,53	11	20,21 ± 2,66	8	25,26 ± 6,46	5
Venous NH_4	203,16 ± 13,41	12	212,72 ± 28,17	11	240,10 ± 22,13	10	202,50 ± 23,08	6	201,80 ± 24,65	5
Alkaline Phos.	6,70 ± 0,81	12	8,59 ± 1,30	13	7,00 ± 1,12	11	7,33 ± 1,52	8	7,10 ± 1,79	5
S.G.O.T.	35,76 ± 4,03	13	101,92 ± 45,16	13	107,72 ± 30,22	11	128,12 ± 34,12	8	101,00 ± 23,47	5
Cholesterol	89,10 ± 8,30	10	75,41 ± 6,44	12	62,54 ± 9,35	11	52,85 ± 13,72	7	36,25 ± 9,12	4
Total protein	6,31 ± 0,18	8	6,02 ± 0,21	12	6,23 ± 0,29	11	5,78 ± 0,34	7	6,33 ± 0,43	3
Albumin	1,78 ± 0,06	8	1,51 ± 0,10	12	1,70 ± 0,11	11	1,41 ± 0,08	7	1,63 ± 0,08	3
Globulin	4,52 ± 0,20	8	4,50 ± 0,17	12	4,53 ± 0,20	11	4,37 ± 0,27	7	4,70 ± 0,34	3
Haemoglobin	11,53 ± 0,40	12	9,78 ± 0,73	13	9,18 ± 0,80	11	8,08 ± 1,01	8	7,50 ± 0,98	5
Leucocytes x 10^3	20,51 ± 1,79	12	32,08 ± 3,90	12	33,71 ± 4,59	11	28,21 ± 4,17	8	32,20 ± 5,37	4
Platelets x 10^3	375,75 ± 47,20	12	497,15 ± 108,85	13	378,09 ± 70,96	11	353,25 ± 116,23	8	256,40 ± 80,00	5
Neutrophils %	49,16 ± 3,95	12	56,07 ± 3,01	13	47,36 ± 5,72	11	43,75 ± 4,93	8	51,40 ± 13,49	5
Lymphocytes %	37,66 ± 3,73	12	26,15 ± 3,22	13	34,72 ± 6,17	11	34,12 ± 5,55	8	31,60 ± 10,47	5
Eosinophils %	3,41 ± 0,89	12	1,76 ± 0,39	13	2,81 ± 0,87	11	2,12 ± 0,74	8	0,60 ± 0,40	5
Basophils %	0,58 ± 0,19	12	0,76 ± 0,32	13	1,09 ± 0,54	11	0,87 ± 0,61	8	0	5
Monocytes %	9,16 ± 1,06	12	15,15 ± 2,19	13	14,00 ± 1,94	11	19,12 ± 2,56	8	18,40 ± 4,34	5
pH	7,26 ± 0,03	10	7,29 ± 0,03	3	7,23 ± 0,08	5	7,10 ± 0,05	4	7,22 ± 0,05	3
pCO_2	48,70 ± 3,77	10	51,00 ± 2,88	3	39,00 ± 10,25	5	55,37 ± 7,01	4	40,16 ± 6,61	3
Std. bicarbonate	19,82 ± 1,35	10	22,50 ± 1,60	3	18,02 ± 2,40	5	16,05 ± 1,43	4	15,73 ± 1,29	3

Mean ± 1 Sem NA = Number of animals No = Number of samples processed

TABLE 55 SERIAL MEAN LEVELS - GROUP 1(b) (Heterotopic allograft plus cholecystojejunocholecystostomy)

TEST	DAY											
	OP	NA 14 No	7	NA 14 No	14	NA 12 No	21	NA 10 No	28	NA 8 No
CSF Glutamine	10,65 ± 1,12	12	40,52 ± 5,33	13	52,95 ± 4,95	12	66,73 ± 6,53	9	66,06 ± 7,60	8
Venous NH_4	227,22 ± 28,81	9	291,81 ± 12,60	11	312,70 ± 19,84	10	390,10 ± 16,16	10	429,12 ± 54,97	8
Alkaline Phos.	9,60 ± 1,05	14	5,27 ± 0,45	14	6,80 ± 0,88	12	7,56 ± 1,07	9	6,08 ± 1,07	8
S.G.O.T.	34,23 ± 4,96	13	35,00 ± 6,04	14	37,91 ± 10,30	12	56,50 ± 10,75	10	35,62 ± 6,50	8
Cholesterol	109,07 ± 6,13	14	65,18 ± 6,05	11	57,50 ± 4,93	12	63,00 ± 8,10	9	61,57 ± 5,97	7
Total protein	6,18 ± 0,24	10	7,18 ± 0,41	9	6,96 ± 0,17	12	6,80 ± 0,28	8	7,26 ± 0,54	5
Albumin	1,85 ± 0,13	10	1,91 ± 0,12	9	1,70 ± 0,16	12	1,58 ± 0,19	8	1,51 ± 0,21	4
Globulin	4,33 ± 0,19	10	5,27 ± 0,42	9	5,26 ± 0,20	12	5,21 ± 0,17	8	5,22 ± 0,17	4
Haemoglobin	10,93 ± 0,37	11	10,77 ± 0,63	12	10,49 ± 0,55	10	10,69 ± 0,63	10	11,02 ± 0,90	8
Leucocytes x 10^3	15,04 ± 1,26	12	30,45 ± 3,11	12	25,26 ± 1,27	12	23,66 ± 2,48	10	20,56 ± 2,23	8
Platelets x 10^3	434,91 ± 44,50	12	391,23 ± 32,89	13	411,50 ± 39,35	10	387,10 ± 67,52	10	265,25 ± 27,01	8
Neutrophils %	46,46 ± 4,73	12	56,50 ± 4,56	12	61,66 ± 2,49	12	57,90 ± 4,64	10	52,75 ± 4,87	8
Lymphocytes %	41,91 ± 5,10	12	31,08 ± 3,57	12	26,16 ± 2,38	12	28,80 ± 3,53	10	31,50 ± 2,89	8
Eosinophils %	2,66 ± 0,93	12	2,25 ± 0,80	12	1,66 ± 0,89	12	1,80 ± 0,55	10	1,50 ± 1,05	8
Basophils %	1,16 ± 0,27	12	0,16 ± 0,11	12	0,16 ± 0,11	12	0,60 ± 0,22	10	0,62 ± 0,26	8
Monocytes %	8,75 ± 1,12	12	9,91 ± 1,55	12	10,33 ± 1,72	12	10,20 ± 1,16	10	13,62 ± 2,97	8
pH	–		–		–		–		–	
pCO_2	–		–		–		–		–	
Std. bicarbonate	–		–		–		–		–	

Mean ± 1 Sem NA = Number of animals No = Number of samples processed
– = No or insufficient data

TABLE 56 SERIAL MEAN VALUES - GROUP 2(a) (End-to-side portacaval shunt)

TEST	DAY											
	OP	NA 5 No	7	NA 5 No	14	NA 5 No	21	NA 5 No	28	NA 4 No
CSF Glutamine	9,88 ± 0,72	5	10,26 ± 0,86	5	7,78 ± 0,46	5	9,42 ± 1,05	5	9,80 ± 0,68	4
Venous NH_4	197,50 ± 17,21	4	227,25 ± 11,27	4	168,00 ± 16,56	5	220,75 ± 53,46	4	249,00 ± 9,29	4
Alkaline Phos.	5,18 ± 0,96	5	4,16 ± 0,49	5	6,82 ± 0,82	5	6,52 ± 0,98	5	6,37 ± 1,28	4
S.G.O.T.	30,00 ± 4,18	5	39,00 ± 4,30	5	33,00 ± 3,39	5	58,00 ± 17,21	5	47,50 ± 3,22	4
Cholesterol	107,40 ± 8,12	5	105,40 ± 10,12	5	103,40 ± 7,78	5	97,40 ± 9,20	5	102,5 ± 10,12	4
Total protein	5,32 ± 0,17	4	6,16 ± 0,47	5	6,46 ± 0,30	5	6,50 ± 0,18	5	7,37 ± 0,62	4
Albumin	1,70 ± 0,19	4	1,72 ± 0,22	4	1,86 ± 0,18	5	2,04 ± 0,18	5	2,20 ± 0,40	4
Globulin	3,62 ± 0,13	4	4,50 ± 0,54	4	4,60 ± 0,16	5	4,46 ± 0,05	5	5,17 ± 0,37	4
Haemoglobin	11,00 ± 0,52	5	9,96 ± 0,52	5	10,00 ± 0,51	5	10,44 ± 0,63	5	11,22 ± 0,69	4
Leucocytes x 10^3	18,80 ± 1,49	5	27,04 ± 4,14	5	22,48 ± 2,36	5	22,76 ± 2,60	5	16,02 ± 2,89	4
Platelets x 10^3	600,00 ± 81,77	5	610,00 ± 111,13	5	404,16 ± 66,90	5	410,00 ± 36,74	5	346,25 ± 43,51	4
Neutrophils %	57,00 ± 7,10	4	52,80 ± 3,12	5	32,40 ± 3,72	5	44,00 ± 6,01	4	27,75 ± 9,85	4
Lymphocytes %	34,75 ± 6,34	4	29,00 ± 4,35	5	35,60 ± 3,20	5	31,75 ± 4,26	4	42,00 ± 4,30	4
Eosinophils %	1,75 ± 1,03	4	2,40 ± 0,92	5	3,40 ± 2,71	5	2,00 ± 1,68	4	3,00 ± 1,58	4
Basophils %	1,00 ± 0,70	4	0,20 ± 0,20	5	0,60 ± 0,40	5	0,25 ± 0,25	4	0,25 ± 0,25	4
Monocytes %	5,75 ± 0,85	4	15,60 ± 1,93	5	28,00 ± 3,39	5	19,50 ± 4,80	4	27,00 ± 6,59	4
pH	7,39 ± 0,03	5	-		7,15 ± 0,04	5	-		7,27 ± 0,03	4
pCO_2	33,40 ± 2,77	5	-		51,00 ± 3,98	5	-		47,50 ± 4,40	4
Std. bicarbonate	20,56 ± 0,85	5	-		15,96 ± 2,03	5	-		19,57 ± 1,56	4

Mean ± 1 Sem NA = Number of animals No = Number of samples processed
- = No or insufficient data

TABLE 57 SERIAL MEAN VALUES - GROUP 2(b) (Sham laparotomy)

TEST	DAY															
	OP	NA 22	No	7	NA 22	No	14	NA 17	No	21	NA 14	No	28	NA 12	No
CSF Glutamine	11,00 ± 0,56	20		20,73 ± 1,93	22		24,08 ± 2,26	17		22,43 ± 2,56	14		23,39 ± 3,29	11	
Venous NH_4	199,30 ± 9,08	20		241,55 ± 21,60	18		259,12 ± 19,26	16		273,58 ± 31,59	12		252,36 ± 21,23	11	
Alkaline Phos.	6,11 ± 0,51	21		7,78 ± 0,82	22		7,15 ± 0,74	17		6,20 ± 1,04	14		5,38 ± 1,10	11	
S.G.O.T.	39,54 ± 3,08	22		88,63 ± 26,57	22		95,41 ± 20,76	17		98,21 ± 21,50	14		65,00 ± 14,53	11	
Cholesterol	89,82 ± 5,84	17		81,52 ± 5,98	19		78,41 ± 8,20	17		60,58 ± 11,08	12		47,80 ± 13,38	10	
Total protein	6,31 ± 0,14	16		6,55 ± 0,26	19		6,39 ± 0,19	17		6,29 ± 0,25	13		6,85 ± 0,25	9	
Albumin	1,80 ± 0,06	16		1,71 ± 0,09	19		1,80 ± 0,08	17		1,61 ± 0,09	13		1,70 ± 0,13	9	
Globulin	4,50 ± 0,13	16		4,84 ± 0,19	19		4,58 ± 0,14	17		4,67 ± 0,19	13		5,15 ± 0,20	9	
Haemoglobin	11,61 ± 0,31	21		10,42 ± 0,48	22		10,24 ± 0,63	17		8,94 ± 0,66	14		8,81 ± 0,75	11	
Leucocytes x 10^3	18,99 ± 1,19	21		31,70 ± 2,63	21		29,81 ± 3,34	17		28,25 ± 2,76	14		32,65 ± 3,92	10	
Platelets x 10^3	395,04 ± 32,67	21		533,42 ± 77,06	21		390,23 ± 67,45	17		383,07 ± 79,10	14		352,00 ± 61,86	11	
Neutrophils %	46,28 ± 3,29	21		54,68 ± 2,56	22		48,64 ± 4,02	17		44,14 ± 4,48	14		52,18 ± 6,98	11	
Lymphocytes %	37,23 ± 2,42	21		27,40 ± 2,13	22		35,58 ± 4,18	17		32,42 ± 3,66	14		32,54 ± 4,96	11	
Eosinophils %	5,42 ± 1,37	21		3,09 ± 0,58	22		3,47 ± 0,73	17		3,14 ± 0,98	14		2,36 ± 1,12	11	
Basophils %	0,57 ± 0,17	21		0,81 ± 0,21	22		1,52 ± 0,39	17		1,21 ± 0,53	14		0,81 ± 0,32	11	
Monocytes %	10,47 ± 1,18	21		13,95 ± 1,78	22		10,88 ± 1,66	17		19,07 ± 2,68	14		13,00 ± 2,73	11	
pH	7,28 ± 0,02	19		7,29 ± 0,02	9		7,23 ± 0,04	11		7,16 ± 0,03	10		7,22 ± 0,02	9	
pCO_2	47,26 ± 3,01	19		49,27 ± 2,19	9		39,95 ± 4,54	11		49,45 ± 4,21	10		44,38 ± 2,75	9	
Std. bicarbonate	20,25 ± 1,05	19		21,83 ± 1,38	9		17,72 ± 1,39	11		16,19 ± 1,00	10		17,07 ± 1,13	9	

Mean ± 1 Sem NA = Number of animals No = Number of samples processed

TABLE 58 SERIAL MEAN VALUES - GROUP 1(a+b) (Combined transplant group)

TEST	DAYS			
	0-7	0-14	0-21	0-28
CSF Glutamine	<0,025	<0,01	<0,01	<0,01
Venous NH_4	<0,01	<0,02	<0,005	<0,001
Alkaline Phos.	-	<0,01	-	-
S.G.O.T.*	<0,005	-	-	-
Cholesterol	-	-	-	-
Total Protein	<0,025	-	-	<0,05
Albumin	-	-	-	-
Globulin	<0,05	-	-	<0,005
Haemoglobin	-	-	<0,05	-
Leucocytes	<0,005	-	<0,01	<0,02
Platelets	n	n	n	n
Neutrophils	-	-	-	-
Lymphocytes	-	-	-	-
Eosinophils	n	n	n	n
Basophils	n	n	n	n
Monocytes	-	<0,05	-	-
pH	-	-	-	-
pCO_2	-	-	-	-
Standard Bicarb.	-	-	-	-

p< = significance of difference in mean levels
\- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation.

TABLE 59 THE SIGNIFICANCE OF CHANGES IN MEAN LEVELS IN GROUP 1(a)

TEST	DAYS			
	0-7	0-14	0-21	0-28
CSF Glutamine	<0,001	<0,001	<0,005	<0,02
Venous NH_4	-	-	-	-
Alkaline Phos.	-	-	-	-
S.G.O.T.*	<0,02	<0,025	<0,001	<0,005
Cholesterol	-	-	<0,025	<0,005
Total Protein	-	-	-	-
Albumin	<0,05	-	<0,005	-
Globulin	-	-	-	-
Haemoglobin	<0,05	<0,02	<0,005	<0,001
Leucocytes	<0,02	<0,02	-	<0,01
Platelets	n	n	n	n
Neutrophils	-	-	-	-
Lymphocytes	<0,025	-	-	-
Eosinophils	n	n	n	n
Basophils	n	n	n	n
Monocytes	<0,02	<0,05	<0,005	<0,02
pH	-	-	<0,05	-
pCO_2	-	-	-	-
Standard Bicarb.	-	-	-	-

p< = significance of difference in mean levels
\- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation.

TABLE 60 : THE SIGNIFICANCE OF CHANGES IN MEAN LEVELS IN GROUP 1(b)

TEST	DAYS						
	0-7	0-14	0-21	0-28	7-14	14-21	21-28
CSF Glutamine	<0,001	<0,001	<0,001	<0,001	-	-	-
Venous NH_4	<0,05	<0,02	<0,001	<0,005	-	<0,01	-
Alkaline Phos.	<0,001	<0,05	-	<0,05	-	-	-
S.G.O.T.*	-	-	<0,05	-	-	-	-
Cholesterol	<0,001	<0,001	<0,001	<0,001	-	-	-
Total Protein	<0,05	<0,02	-	<0,05	-	-	-
Albumin	-	-	-	-	-	-	-
Globulin	<0,05	<0,005	<0,005	<0,02	-	-	-
Haemoglobin	-	-	-	-	-	-	-
Leucocytes	<0,001	<0,001	<0,005	<0,025	-	-	-
Platelets	n	n	n	n	n	n	n
Neutrophils	-	<0,02	-	-	-	-	-
Lymphocytes	-	<0,01	<0,05	-	-	-	-
Eosinophils	n	n	n	n	n	n	n
Basophils	n	n	n	n	n	n	n
Monocytes	-	-	-	-	-	-	-
pH	0	0	0	0	0	0	0
pCO_2	0	0	0	0	0	0	0
Std. Bicarb.	0	0	0	0	0	0	0

p < = significance of difference in mean levels
\- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation

TABLE 61 THE SIGNIFICANCE OF CHANGES IN MEAN LEVELS IN GROUP 2(a)

TEST	DAYS						
	0-7	0-14	0-21	0-28	7-14	14-21	21-28
CSF Glutamine	-	<0,05	-	-	<0,025	-	-
Venous NH_4	-	-	-		<0,02	-	-
Alkaline Phos.	-	-	-	-	<0,02	-	-
S.G.O.T.*	-	-	-	<0,05	-	-	-
Cholesterol	-	-	-	-	-	-	-
Total Protein	-	<0,02	<0,005	<0,02	-	-	-
Albumin	-	-	-	-	-	-	-
Globulin	-	<0,005	<0,001	<0,01	-	-	-
Haemoglobin	-	-	-	-	-	-	-
Leucocytes	-	-	-	-	-	-	-
Platelets	n	n	n	n	n	n	n
Neutrophils	-	<0,01	-	<0,05	<0,005	-	-
Lymphocytes	-	-	-	-	-	-	-
Eosinophils	n	n	n	n	n	n	n
Basophils	n	n	n	n	n	n	n
Monocytes	<0,005	<0,001	<0,05	<0,025	<0,01	-	-
pH	0	<0,001	0	<0,025	0	0	0
pCO_2	0	<0,005	0	<0,02	0	0	0
Std. Bicarb.	0	-	0	-	0	0	0

p< = significance of difference in mean levels
\- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation

TABLE 62 THE SIGNIFICANCE OF CHANGES IN MEAN LEVELS IN GROUP 2(b)

TEST	DAYS			
	0-7	0-14	0-21	0-28
CSF Glutamine	<0,001	<0,001	<0,001	<0,001
Venous NH_4	-	<0,01	<0,02	<0,02
Alkaline Phos.	-	-	-	-
S.G.O.T.*	<0,001	<0,01	<0,001	-
Cholesterol	-	-	<0,02	<0,005
Total Protein	-	-	-	<0,05
Albumin	-	-	-	-
Globulin	-	-	-	<0,01
Haemoglobin	<0,05	<0,05	<0,001	<0,001
Leucocytes	<0,001	<0,005	<0,005	<0,001
Platelets	n	n	n	n
Neutrophils	<0,05	-	-	-
Lymphocytes	<0,005	-	-	-
Eosinophils	n	n	n	n
Basophils	n	n	n	n
Monocytes	-	-	<0,005	-
pH	-	-	<0,02	-
pCO_2	-	-	-	-
Standard Bicarb.	-	-	<0,02	<0,001

p < = significance of difference in mean levels
\- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation.

TABLE 63 THE SIGNIFICANCE OF CHANGES IN MEAN LEVELS IN GROUP 1(a+b)

TEST	DATE OF COMPARISON (DAYS)				
	OP	7	14	21	28
CSF Glutamine	-	-	-	-	-
Venous NH_4	-	-	-	< 0,01	< 0,02
Alkaline Phos.	-	-	-	-	-
S.G.O.T.*	-	-	-	-	< 0,025
Cholesterol	-	-	< 0,005	-	-
Total Protein	-	< 0,005	-	< 0,02	-
Albumin	-	< 0,025	-	< 0,01	-
Globulin	-	< 0,02	-	-	-
Haemoglobin	-	-	< 0,02	-	-
Leucocytes	-	-	-	-	-
Platelets	n	n	n	n	n
Neutrophils	-	-	-	-	-
Lymphocytes	-	-	-	-	-
Eosinophils	n	n	n	n	n
Basophils	n	n	n	n	n
Monocytes	-	-	< 0,005	-	< 0,05
pH	-	-	-	-	-
pCO_2	-	-	-	-	-
Standard Bicarb.	-	-	-	-	-

p< = significance of difference in mean levels
\- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation

TABLE 64 THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN GROUPS 1(a) and 1(b)

TEST	DATE OF COMPARISON (DAYS)				
	OP	7	14	21	28
CSF Glutamine	-	<0,005	<0,005	<0,001	<0,001
Venous NH_4	-	-	-	-	-
Alkaline Phos.	<0,005	-	-	-	-
S.G.O.T.*	-	<0,001	<0,05	-	-
Cholesterol	-	<0,05	<0,001	-	-
Total Protein	-	-	-	-	-
Albumin	-	-	-	-	-
Globulin	-	-	-	-	-
Haemoglobin	-	-	<0,05	-	-
Leucocytes	-	-	-	-	<0,05
Platelets	n	n	n	n	n
Neutrophils	-	-	<0,05	-	-
Lymphocytes	-	-	<0,02	-	-
Eosinophils	n	n	n	n	n
Basophils	n	n	n	n	n
Monocytes	-	-	<0,05	-	-
pH	0	0	0	0	0
pCO_2	0	0	0	0	0
Standard Bicarb.	0	0	0	0	0

p< = significance of difference in mean levels
\- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation

TABLE 65 THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN GROUPS 1(a) and 2(a)

TEST	DATE OF COMPARISON (DAYS)				
	OP	7	14	21	28
CSF Glutamine	-	<0,05	<0,01	<0,02	<0,02
Venous NH_4	-	-	<0,01	-	-
Alkaline Phos.	-	<0,02	-	-	-
S.G.O.T.*	<0,05	<0,005	-	-	-
Cholesterol	-	-	-	-	-
Total Protein	<0,02	-	-	-	-
Albumin	-	-	-	-	-
Globulin	<0,01	-	-	<0,025	-
Haemoglobin	-	-	<0,005	-	-
Leucocytes	-	-	-	-	<0,05
Platelets	n	n	n	n	n
Neutrophils	-	-	<0,02	-	-
Lymphocytes	-	-	-	-	-
Eosinophils	n	n	n	n	n
Basophils	n	n	n	n	n
Monocytes	-	-	<0,001	-	<0,02
pH	-	0	-	0	-
pCO_2	-	0	-	0	-
Standard Bicarb.	-	0	-	0	-

p< = significance of difference in mean levels
\- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation

TABLE 66 . THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN GROUPS 1(a) and 2(b)

TEST	DATE OF COMPARISON (DAYS)				
	OP	7	14	21	28
CSF Glutamine	-	<0,01	<0,001	<0,001	<0,05
Venous NH_4	-	<0,02	<0,02	<0,001	<0,01
Alkaline Phos.	<0,05	<0,025	-	-	-
S.G.O.T.*	-	<0,01	<0,02	<0,025	<0,02
Cholesterol	-	-	-	-	<0,025
Total Protein	-	<0,02	<0,05	<0,025	-
Albumin	-	<0,02	-	-	-
Globulin	-	-	<0,02	<0,02	-
Haemoglobin	-	-	-	<0,05	<0,02
Leucocytes	<0,02	-	-	-	<0,025
Platelets	n	n	n	n	n
Neutrophils	-	-	<0,05	<0,05	-
Lymphocytes	-	-	-	-	-
Eosinophils	n	n	n	n	n
Basophils	n	n	n	n	n
Monocytes	-	-	-	<0,005	-
pH	0	0	0	0	0
pCO_2	0	0	0	0	0
Standard Bicarb.	0	0	0	0	0

p< = significance of difference in mean levels
\- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation

TABLE 67 : THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN GROUPS 1(b) and 2(a)

TEST	DATE OF COMPARISON (DAYS)				
	OP	7	14	21	28
CSF Glutamine	-	<0,02	<0,005	<0,01	-
Venous NH_4	-	-	<0,05	-	-
Alkaline Phos.	-	<0,05	-	-	-
S.G.O.T.*	-	-	-	-	-
Cholesterol	-	<0,02	<0,02	<0,025	<0,005
Total Protein	<0,005	-	-	-	-
Albumin	-	-	-	<0,005	-
Globulin	<0,01	-	-	-	-
Haemoglobin	-	-	-	-	<0,02
Leucocytes	-	-	-	-	<0,025
Platelets	n	n	n	n	n
Neutrophils	-	-	-	-	-
Lymphocytes	-	-	-	-	-
Eosinophils	n	n	n	n	n
Basophils	n	n	n	n	n
Monocytes	-	-	<0,005	-	-
pH	<0,05	0	-	0	-
pCO_2	<0,02	0	-	0	-
Standard Bicarb.	-	0	-	0	-

p< = significance of difference in mean levels
\- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation

TABLE 68 : THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN GROUPS 1(b) and 2(b)

TEST	DATE OF COMPARISON (DAYS)				
	OP	7	14	21	28
CSF Glutamine	-	<0,005	<0,001	<0,001	<0,001
Venous NH_4	-	<0,01	<0,001	<0,001	<0,05
Alkaline Phos.	<0,025	-	-	-	-
S.G.O.T.*	-	-	-	-	-
Cholesterol	-	<0,005	<0,001	<0,02	<0,005
Total Protein	<0,05	-	-	-	-
Albumin	-	-	-	-	-
Globulin	<0,05	-	-	<0,005	-
Haemoglobin	-	-	-	-	-
Leucocytes	-	-	-	-	-
Platelets	n	n	n	n	n
Neutrophils	-	-	<0,001	-	<0,02
Lymphocytes	-	-	<0,05	-	<0,05
Eosinophils	n	n	n	n	n
Basophils	n	n	n	n	n
Monocytes	-	<0,05	<0,001	<0,01	<0,05
pH	0	0	0	0	0
pCO_2	0	0	0	0	0
Standard Bicarb.	0	0	0	0	0

p< = significance of difference in mean levels
\- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation

TABLE 69 : THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN GROUPS 2(a) and 2(b)

TEST	DATE OF COMPARISON (DAYS)				
	OP	7	14	21	28
CSF Glutamine	-	<0,001	<0,001	<0,001	<0,001
Venous NH_4	-	-	-	<0,005	<0,01
Alkaline Phos.	<0,005	<0,05	-	-	-
S.G.O.T.*	-	<0,001	<0,02	-	-
Cholesterol	<0,05	-	-	-	-
Total Protein	-	-	<0,05	-	-
Albumin	-	-	-	-	-
Globulin	-	-	<0,01	-	-
Haemoglobin	-	-	-	-	-
Leucocytes	<0,05	-	-	-	<0,02
Platelets	n	n	n	n	n
Neutrophils	-	-	<0,02	<0,05	-
Lymphocytes	-	-	-	-	-
Eosinophils	n	n	n	n	n
Basophils	n	n	n	n	n
Monocytes	-	-	-	<0,02	-
pH	0	0	0	0	0
pCO_2	0	0	0	0	0
Standard Bicarb.	0	0	0	0	0

p< = significance of difference in mean levels
\- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation

TABLE 70 : THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN GROUPS 1(a+b) and 2(a)

TEST	DATE OF COMPARISON (DAYS)				
	OP	7	14	21	28
CSF Glutamine	-	<0,02	<0,001	<0,01	<0,05
Venous NH_4	-	-	<0,02	-	-
Alkaline Phos.	-	<0,05	-	-	-
S.G.O.T.*	-	-	-	-	-
Cholesterol	-	-	-	-	<0,025
Total Protein	<0,005	-	-	-	-
Albumin	-	-	-	<0,05	-
Globulin	<0,005	-	-	-	-
Haemoglobin	-	-	-	-	-
Leucocytes	-	-	-	-	<0,025
Platelets	n	n	n	n	n
Neutrophils	-	-	<0,025	-	-
Lymphocytes	-	-	-	-	-
Eosinophils	n	n	n	n	n
Basophils	n	n	n	n	n
Monocytes	-	-	<0,001	-	<0,025
pH	-	0	-	0	-
pCO_2	<0,05	0	-	0	-
Standard Bicarb.	-	0	-	0	-

p< = significance of difference in mean levels
\- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation

TABLE 71 : THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN GROUPS 1(a+b) and 2(b)

APPENDIX 6

STATISTICAL ANALYSIS OF THE CHANGES IN MEAN BIOCHEMICAL AND HAEMATOLOGICAL VALUES RELATED TO THE HISTOLOGICAL QUALITY OF THE DONOR LIVERS - RELEVANT TO PART III

MEAN VALUES RELATED TO THE HISTOLOGICAL QUALITY AT WEEKLY INTERVALS - THE "TIME RELATED STUDY".

(refer to page 241 in the text for methods used)

MEAN VALUES RELATED TO THE HISTOLOGICAL QUALITY, IRRESPECTIVE OF THE POSTOPERATIVE PERIOD - THE "TIME UNRELATED STUDY".

(Refer to page 248 in the text for methods used)

TEST	PRE-OPERATIVE NORMAL	POST-OP DAY	GRADE							
			I	No.	II	No.	III	No.	IV	No.
GLUTAMINE	11,00 ± 2,47 (20)	7	14,56 ± 4,28	6	17,50 ± 6,40	6	21,58 ± 4,95	5	33,85 ± 8,27	4
		14	16,76 ± 1,92	5	25,57 ± 9,99	4	23,30 ± 2,20	2	29,46 ± 9,29	6
		21	16,04 ± 3,93	5	22,10 ± 8,02	3	NIL	-	31,26 ± 6,39	5
		28	13,70 ± 2,01	3	21,6	1	28,20 ± 13,01	4	27,26 ± 5,24	3
VENOUS NH_4	199,30 ± 39,61 (20)	7	259,80 ±101,59	5	241,40 ± 65,15	5	273,00 ± 99,07	3	184,50 ± 72,57	4
		14	229,40 ± 46,71	5	233,33 ± 20,98	3	263,00 ± 52,00	2	296,50 ± 96,91	6
		21	206,75 ± 62,39	4	261,33 ± 76,18	3	NIL	-	360,00 ±110,55	4
		28	278,00 ±104,90	4	185	1	269,00 ± 22,02	4	261,33 ± 66,43	3
ALKALINE PHOS.	6,11 ± 2,29 (21)	7	7,25 ± 2,64	6	7,80 ± 0,85	6	4,70 ± 1,69	5	10,92 ± 5,66	4
		14	7,90 ± 4,14	5	6,92 ± 2,05	4	8,60 ± 0,00	2	6,20 ± 2,44	6
		21	9,38 ± 3,83	5	4,30 ± 1,99	3	NIL	-	4,56 ± 2,62	5
		28	9,46 ± 1,61	3	3,8	1	4,12 ± 2,94	4	3,50 ± 2,78	3
S G O T	39,54 ± 14,13 (22)	7	72,50 ± 5,59	6	60,00 ± 18,70	6	48,00 ± 18,05	5	216,25 ±245,44	4
		14	118,00 ± 79,34	5	161,75 ± 89,41	4	35,00 ± 0,00	2	52,50 ± 50,55	6
		21	162,00 ± 97,75	5	70,00 ± 4,08	3	NIL	-	52,00 ± 26,94	5
		28	100,00 ± 43,20	3	70	1	62,50 ± 51,29	4	31,66 ± 4,71	3
CHOLESTEROL	89,82 ± 23,38 (17)	7	92,20 ± 12,20	5	96,83 ± 30,93	6	79,00 ± 20,43	4	60,25 ± 14,70	4
		14	65,20 ± 37,61	5	101,50 ± 29,94	4	$\binom{31}{100}$	2	78,33 ± 17,85	6
		21	67,20 ± 28,20	5	52,00 ± 41,40	3	NIL	-	38,66 ± 24,56	3
		28	69,66 ± 43,92	3	21	1	27,66 ± 21,48	3	55,66 ± 42,59	3
TOTAL PROTEIN	6,31 ± 0,54 (16)	7	6,34 ± 0,63	5	6,66 ± 1,08	6	6,37 ± 0,66	4	6,82 ± 1,71	4
		14	6,38 ± 0,69	5	6,27 ± 1,05	4	6,00 ± 0,30	2	6,61 ± 0,70	6
		21	5,86 ± 0,93	5	6,50 ± 0,98	3	NIL	-	6,52 ± 0,49	4
		28	7,10 ± 0,65	3	5,6	1	6,96 ± 0,44	3	6,90 ± 0,60	2
ALBUMIN	1,80 ± 0,25 (16)	7	1,80 ± 0,08	5	1,70 ± 0,46	6	1,70 ± 0,31	4	1,62 ± 0,57	4
		14	1,70 ± 0,32	5	1,77 ± 0,23	4	1,65 ± 0,45	2	1,96 ± 0,34	6
		21	1,42 ± 0,23	5	1,63 ± 0,24	3	NIL	-	1,77 ± 0,35	4
		28	1,83 ± 0,20	3	1,5	1	1,53 ± 0,26	3	1,25 ± 0,05	2
GLOBULIN	4,50 ± 0,51 (16)	7	4,54 ± 0,67	5	4,96 ± 0,66	6	4,67 ± 0,46	4	5,20 ± 1,28	4
		14	4,68 ± 0,42	5	4,50 ± 0,96	4	4,35 ± 0,15	2	4,65 ± 0,45	6
		21	4,44 ± 0,75	5	4,86 ± 0,75	3	NIL	-	4,75 ± 0,40	4
		28	5,26 ± 0,44	3	4,10	1	5,43 ± 0,54	3	5,65 ± 0,55	2
HAEMOGLOBIN	11,61 ± 1,41 (21)	7	10,70 ± 2,14	6	10,81 ± 1,19	6	10,60 ± 0,98	5	8,42 ± 3,15	4
		14	9,88 ± 2,36	5	11,00 ± 2,12	4	8,85 ± 3,85	2	10,50 ± 2,14	6
		21	8,64 ± 2,46	5	8,66 ± 2,23	3	NIL	-	9,18 ± 2,59	5
		28	10,36 ± 2,14	3	7,7	1	7,30 ± 2,27	4	9,66 ± 1,69	3
LEUCOCYTES	18,99 ± 5,36 (21)	7	29,30 ± 3,92	6	26,45 ± 5,99	6	38,28 ± 19,92	5	35,30 ± 7,10	3
		14	44,06 ± 11,21	5	23,27 ± 6,30	4	31,50 ± 6,20	2	21,75 ± 9,99	6
		21	30,16 ± 10,79	5	31,56 ± 10,45	3	NIL	-	24,38 ± 8,41	5
		28	31,33 ± 12,25	3	NIL	-	33,20 ± 1,51	4	33,23 ± 17,51	3

TABLE 72 MEAN VALUES FOR THE FUNCTIONAL GRADES - TIME RELATED

(Mean ± 1 SD) (Continued on the following page)

TEST	PRE-OPERATIVE NORMAL	POST-OP DAY	GRADE I	No.	II	No.	III	No.	IV	No.
PLATELETS	395,04 ± 146,11 (21)	7	709,40 ± 371,13	5	598,00 ± 386,79	6	499,60 ± 294,83	5	323,50 ± 105,76	4
		14	496,60 ± 273,33	5	239,50 ± 57,08	4	350,50 ± 181,50	2	415,33 ± 324,20	6
		21	356,00 ± 344,02	5	383,33 ± 50,21	3	NIL	-	411,80 ± 326,10	5
		28	251,00 ± 163,30	3	82	1	490,25 ± 167,43	4	358,66 ± 127,52	3
NEUTROPHILS	46,28 ± 14,73 (21)	7	53,50 ± 13,46	6	47,00 ± 8,38	6	61,20 ± 5,74	5	60,00 ± 13,00	4
		14	49,60 ± 7,98	5	45,75 ± 13,31	4	61,50 ± 1,50	2	45,50 ± 22,17	6
		21	39,60 ± 12,17	5	47,33 ± 15,15	3	NIL	-	42,40 ± 17,93	5
		28	41,66 ± 29,93	3	25	1	66,50 ± 13,27	4	52,66 ± 5,43	3
LYMPHOCYTES	37,23 ± 10,85 (21)	7	25,16 ± 8,91	6	35,33 ± 10,81	6	25,60 ± 5,85	5	23,50 ± 6,87	4
		14	32,20 ± 9,98	5	38,25 ± 10,03	4	32,50 ± 0,50	2	37,66 ± 24,97	6
		21	39,40 ± 15,94	5	29,33 ± 10,07	3	NIL	-	30,00 ± 8,74	5
		28	35,00 ± 21,18	3	57	1	26,50 ± 9,34	4	30,00 ± 8,83	3
EOSINOPHILS	5,42 ± 6,13 (21)	7	4,83 ± 3,43	6	1,16 ± 0,89	6	3,00 ± 2,44	5	3,75 ± 1,47	4
		14	2,40 ± 1,95	5	5,00 ± 2,54	4	(0) (1)	2	4,33 ± 3,29	6
		21	2,80 ± 2,13	5	6,00 ± 5,88	3	NIL	-	1,80 ± 1,72	5
		28	4,33 ± 6,12	3	1	1	2,00 ± 1,22	4	1,33 ± 0,94	3
BASOPHILS	0,57 ± 0,79 (21)	7	0,66 ± 1,49	6	0,83 ± 0,68	6	1,00 ± 0,89	5	0,75 ± 0,43	4
		14	0,80 ± 0,74	5	2,00 ± 1,22	4	(0) (1)	2	2,16 ± 2,03	6
		21	1,40 ± 1,85	5	2,33 ± 2,62	3	NIL	-	0,60 ± 1,20	5
		28	0,75 ± 1,29	3	0	1	1,00 ± 0,70	4	0,66 ± 0,94	3
MONOCYTES	10,47 ± 5,31 (21)	7	15,66 ± 12,11	6	15,50 ± 4,89	6	9,20 ± 1,46	5	12,00 ± 5,78	4
		14	15,00 ± 5,17	5	9,25 ± 6,60	4	5,50 ± 2,50	2	10,33 ± 6,74	6
		21	16,80 ± 6,49	5	15,00 ± 4,54	3	NIL	-	25,20 ± 12,05	5
		28	18,00 ± 5,88	3	17	1	6,50 ± 2,17	4	15,33 ± 11,89	3
pH	7,28 ± 0,12 (19)	7	7,31 ± 0,02	3	7,37 ± 0,02	3	(7,25) (7,24)	2	7,11	1
		14	7,22 ± 0,18	4	7,30 ± 0,02	4	7,10	1	(7,32) (7,03)	2
		21	(7,07) (6,95)	2	7,22 ± 0,09	3	NIL	-	7,16 ± 0,08	4
		28	7,25 ± 0,06	3	7,17	1	7,24 ± 0,06	3	(7,18) (7,17)	2
pCO_2	47,26 ± 12,80 (19)	7	46,50 ± 9,51	3	49,00 ± 2,16	3	(51) (51)	2	55	1
		14	36,25 ± 22,09	4	43,12 ± 7,24	4	41	1	(43) (38)	2
		21	(64) (63)	2	42,50 ± 12,57	3	NIL	-	48,0 ± 12,01	4
		28	42,00 ± 8,98	3	36,50	1	47,66 ± 6,59	3	(52) (42)	2
STD. BICARBONATE	20,25 ± 4,49 (19)	7	21,83 ± 2,77	3	25,83 ± 1,54	3	(19) (19,5)	2	15	1
		14	17,02 ± 4,89	4	20,37 ± 1,98	4	12	1	(21) (12,4)	2
		21	(18) (13,2)	2	17,00 ± 3,55	3	NIL	-	14,67 ± 1,23	4
		28	17,13 ± 0,44	3	(13,20)	1	19,16 ± 4,28	3	(17,3) (14,25)	2

TABLE 72 MEAN VALUES FOR THE FUNCTIONAL GRADES - TIME RELATED

(Mean ± 1 SD) (Continued from the previous page)

TEST	PRE-OPERATIVE NORMAL	POST-OP DAY	GRADE I	No.	II	No.	III	No.	IV	No.
GLUTAMINE	11,00 ± 2,47 (20)	7	17,26 ± 4,35	3	15,76 ± 6,84	6	18,63 ± 4,90	6	28,44 ± 10,33	5
		14	16,30 ± 1,88	4	24,52 ± 10,44	4	22,80	1	27,11 ± 8,85	7
		21	14,40	1	18,87 ± 6,78	7	NIL	-	31,26 ± 6,39	5
		28	16,30	1	20,23 ± 11,10	3	28,40 ± 12,10	3	23,77 ± 7,56	4
VENOUS NH_4	199,30 ± 39,61 (20)	7	200,66 ± 44,64	3	279,00 ±101,37	4	249,00 ± 66,50	4	230,60 ±111,98	5
		14	249,00 ± 28,41	4	198,00 ± 35,56	3	257	1	300,71 ± 88,29	7
		21	141	1	245,00 ± 69,30	6	NIL	-	360,00 ±110,55	4
		28	381	1	197,33 ± 31,67	3	254,33 ± 49,84	3	260,00 ± 57,58	4
ALKALINE PHOS.	6,11 ± 2,29 (21)	7	5,60 ± 0,80	3	8,05 ± 2,16	6	6,91 ± 2,00	6	6,04 ± 2,11	5
		14	7,37 ± 4,48	4	7,15 ± 2,31	4	9,1	1	6,31 ± 2,11	7
		21	11,7	1	6,87 ± 4,00	7	NIL	-	4,56 ± 2,62	5
		28	7,7	1	7,50 ± 4,15	3	4,86 ± 3,03	3	3,60 ± 2,41	4
S G O T	39,54 ± 14,13 (22)	7	73,33 ± 6,23	3	64,16 ± 12,38	6	54,16 ± 18,57	6	66,00 ± 27,09	5
		14	138,75 ± 75,61	4	132,50 ±105,50	4	152	1	31,42 ± 5,15	7
		21	185	1	119,28 ± 92,52	7	NIL	-	52,00 ± 26,94	5
		28	40	1	93,33 ± 52,49	3	85,00 ± 48,13	3	35,00 ± 7,07	4
CHOLESTEROL	89,82 ± 23,38 (17)	7	87,00 ± 13,58	3	99,20 ± 22,40	5	75,60 ± 27,88	5	66,00 ± 21,32	5
		14	60,25 ± 40,57	4	91,75 ± 27,26	4	124	1	79,42 ± 22,37	7
		21	64	1	61,14 ± 36,93	7	NIL	-	38,66 ± 24,56	3
		28	128	1	31,66 ± 19,60	3	16,00 ± 5,00	2	55,75 ± 36,88	4
TOTAL PROTEIN	6,31 ± 0,54 (16)	7	6,50 ± 0,71	3	6,84 ± 0,92	5	5,90 ± 0,68	5	7,00 ± 1,51	5
		14	6,37 ± 0,78	4	6,25 ± 1,05	4	6,5	1	6,57 ± 0,65	7
		21	4,9	1	6,27 ± 0,96	7	NIL	-	6,52 ± 0,49	4
		28	7,9	1	6,80 ± 0,35	3	6,00 ± 0,40	2	7,13 ± 0,59	3
ALBUMIN	1,80 ± 0,25 (16)	7	1,80 ± 0,08	3	1,84 ± 0,41	5	1,48 ± 0,27	5	1,78 ± 0,54	5
		14	1,72 ± 0,36	4	1,65 ± 0,15	4	2,11	1	1,95 ± 0,38	7
		21	1,1	1	1,55 ± 0,22	7	NIL	-	1,77 ± 0,35	4
		28	2,1	1	1,76 ± 0,12	3	1,45 ± 0,05	2	1,66 ± 0,59	3
GLOBULIN	4,50 ± 0,51 (16)	7	4,70 ± 0,72	3	5,00 ± 0,70	5	4,42 ± 0,46	5	5,22 ± 1,14	5
		14	4,65 ± 0,47	4	4,60 ± 0,96	4	4,4	1	4,61 ± 0,43	7
		21	3,8	1	4,71 ± 0,77	7	NIL	-	4,75 ± 0,40	4
		28	5,8	1	5,03 ± 0,24	3	4,55 ± 0,45	2	5,46 ± 0,51	3
HAEMOGLOBIN	11,61 ± 1,41 (21)	7	9,93 ± 2,74	3	11,50 ± 0,76	6	10,35 ± 0,89	6	10,04 ± 2,29	5
		14	9,65 ± 2,38	4	10,77 ± 1,96	4	12,8	1	10,65 ± 2,48	7
		21	6,9	1	8,90 ± 2,44	7	NIL	-	9,18 ± 2,59	5
		28	13,2	1	8,50 ± 1,00	3	6,33 ± 1,72	3	9,82 ± 1,49	4
LEUCOCYTES	18,99 ± 5,36 (21)	7	30,43 ± 3,95	3	28,03 ± 3,01	6	35,03 ± 17,02	6	32,36 ± 13,44	5
		14	41,62 ± 11,29	4	32,05 ± 13,79	4	18,7	1	22,44 ± 9,62	7
		21	35,00	1	30,07 ± 11,29	7	NIL	-	24,38 ± 8,41	5
		28	16,7	1	37,36 ± 6,81	3	32,45 ± 1,65	2	33,20 ± 15,16	4

TABLE 73 MEAN VALUES FOR THE GRADES OF REJECTION - TIME RELATED

(Mean ± 1 SD) (Continued on the following page)

TEST	PRE-OPERATIVE NORMAL	POST-OP DAY	GRADE							
			I	No.	II	No.	III	No.	IV	No.
PLATELETS	395,04 ± 146,11 (21)	7	590,00 ± 385,15	3	650,40 ± 309,79	5	570,00 ± 425,13	6	402,00 ± 220,20	5
		14	470,75 ± 300,07	4	336,75 ± 161,51	4	211	1	424,00 ± 308,95	7
		21	1000	1	275,71 ± 142,28	7	NIL	-	411,80 ± 326,10	5
		28	253,00	1	341,66 ± 208,49	3	256,00 ± 123,16	3	456,50 ± 202,26	4
NEUTROPHILS	46,28 ± 14,73 (21)	7	50,00 ± 15,55	3	51,50 ± 10,22	6	55,00 ± 10,11	6	61,00 ± 12,19	5
		14	48,00 ± 8,18	4	44,00 ± 11,22	4	63,00	1	48,14 ± 21,49	7
		21	38,00	1	43,14 ± 14,73	7	NIL	-	42,40 ± 17,93	5
		28	21,00	1	53,66 ± 26,23	3	52,00 ± 22,90	3	59,00 ± 11,93	4
LYMPHOCYTES	37,23 ± 10,85 (21)	7	24,33 ± 10,33	3	32,00 ± 8,22	6	29,00 ± 11,35	6	24,80 ± 7,60	5
		14	32,75 ± 11,09	4	40,50 ± 6,18	4	21,00	1	38,57 ± 22,39	7
		21	46,00	1	34,14 ± 15,31	7	NIL	-	30,00 ± 8,74	5
		28	43,00	1	31,33 ± 20,41	3	38,00 ± 15,93	3	26,75 ± 9,49	4
EOSINOPHILS	5,42 ± 6,13 (21)	7	3,33 ± 4,02	3	3,50 ± 3,14	6	2,16 ± 2,26	6	3,60 ± 1,35	5
		14	2,50 ± 2,17	4	4,00 ± 2,73	4	6,00	1	3,85 ± 3,27	7
		21	6,00	1	3,71 ± 4,49	7	NIL	-	1,80 ± 1,72	5
		28	13,00	1	1,00 ± 1,41	3	2,00 ± 0,81	3	1,00 ± 1,00	4
BASOPHILS	0,57 ± 0,79 (21)	7	1,33 ± 1,88	3	0,33 ± 0,47	6	0,66 ± 0,74	6	1,20 ± 0,74	5
		14	1,00 ± 0,70	4	1,25 ± 1,29	4	3,00	1	2,00 ± 1,92	7
		21	1,00	1	1,85 ± 2,35	7	NIL	-	0,60 ± 1,20	5
		28	3,00	1	0,33 ± 0,47	3	0,33 ± 0,47	3	1,00 ± 1,00	4
MONOCYTES	10,47 ± 5,31 (21)	7	21,00 ± 14,35	3	12,50 ± 5,56	6	13,00 ± 5,00	6	9,40 ± 4,22	5
		14	15,75 ± 5,53	4	10,50 ± 6,53	4	7,00	1	7,57 ± 5,15	7
		21	9,00	1	17,14 ± 5,61	7	NIL	-	25,20 ± 12,05	5
		28	20,00	1	13,66 ± 7,40	3	11,00 ± 4,32	3	12,25 ± 11,60	4
pH	7,28 ± 0,12 (19)	7	7,32	1	7,32 ± 0,03	3	7,33 ± 0,06	3	7,18 ± 0,06	2
		14	7,12 ± 0,08	3	7,36 ± 0,09	4	7,30	1	7,15 ± 0,12	3
		21	6,95	1	7,18 ± 0,10	4	NIL	-	7,16 ± 0,08	4
		28	7,25	1	7,21 ± 0,08	3	7,22 ± 0,05	2	7,21 ± 0,05	3
pCO_2	47,26 ± 12,80 (19)	7	33,50	1	52,00 ± 2,82	3	49,33 ± 2,35	3	53,00 ± 2,00	2
		14	42,33 ± 22,42	3	35,12 ± 11,59	4	50,00	1	40,66 ± 2,05	3
		21	63,00	1	47,87 ± 14,32	4	NIL	-	48,00 ± 12,01	4
		28	42,00	1	42,33 ± 8,99	3	39,75 ± 3,25	2	50,33 ± 6,23	3
STD. BICARBONATE	20,25 ± 4,49 (19)	7	18,00	1	24,00 ± 0,70	3	24,16 ± 3,51	3	17,00 ± 2,00	2
		14	14,20 ± 0,28	3	21,37 ± 3,02	4	21,50	1	15,13 ± 4,15	3
		21	13,20	1	17,25 ± 3,11	4	NIL	-	14,67 ± 1,23	4
		28	17,40	1	16,00 ± 1,47	3	16,10 ± 2,90	2	18,68 ± 4,29	3

TABLE 73 MEAN VALUES FOR THE GRADES OF REJECTION - TIME RELATED

(Mean ± 1 SD) (Continued from the previous page)

TEST	GROUP																	
	N		NA 22 No	I		LB 19 No	II		LB 14 No	III		LB 11 No	IV		LB 18 No
Glutamine	11,00 ±	0,56	20	15,39 ±	0,84	19	21,08 ±	2,34	14	24,30 ±	2,87	11	30,57 ±	1,95	18
Venous NH_4	199,30 ±	9,08	20	235,52 ±	20,38	17	239,66 ±	18,35	12	269,00 ±	22,64	9	278,88 ±	27,40	17
Alkaline Phos.	6,11 ±	0,51	21	8,33 ±	0,81	19	6,51 ±	0,59	14	5,20 ±	0,84	11	6,34 ±	1,06	18
S.G.O.T.	39,54 ±	3,08	22	112,36 ±	17,73	19	91,92 ±	18,40	14	50,90 ±	10,97	11	85,27 ±	33,78	18
Cholesterol	89,82 ±	5,84	17	71,22 ±	7,76	18	83,14 ±	11,41	14	58,88 ±	11,82	9	62,12 ±	7,47	16
Total protein	6,31 ±	0,14	16	6,34 ±	0,20	18	6,44 ±	0,29	14	6,48 ±	0,22	9	6,68 ±	0,26	16
Albumin	1,80 ±	0,06	16	1,67 ±	0,06	18	1,69 ±	0,09	14	1,63 ±	0,12	9	1,81 ±	0,12	16
Globulin	4,50 ±	0,13	16	4,67 ±	0,16	18	4,75 ±	0,22	14	4,85 ±	0,21	9	4,86 ±	0,19	16
Haemoglobin	11,61 ±	0,31	21	9,88 ±	0,57	19	10,18 ±	0,57	14	9,08 ±	0,85	11	9,53 ±	0,62	18
Leucocytes x 10^3	18,99 ±	1,19	21	33,73 ±	2,69	19	26,65 ±	2,29	13	35,20 ±	4,43	11	26,94 ±	3,06	17
Platelets x 10^3	395,04 ±	32,67	21	475,72 ±	85,41	18	412,71 ±	86,32	14	469,09 ±	76,70	11	384,50 ±	64,73	18
Neutrophils %	46.28 ±	3,29	21	46,94 ±	4,00	19	45,14 ±	3,52	14	63,18 ±	2,92	11	49,05 ±	4,47	18
Lymphocytes %	37,23 ±	2,42	21	32,31 ±	3,49	19	36,42 ±	3,31	14	27,18 ±	2,31	11	31,11 ±	4,06	18
Eosinophils %	5,42 ±	1,37	21	3,57 ±	0,84	19	3,28 ±	1,05	14	2,18 ±	0,64	11	3,00 ±	0,62	18
Basophils %	0,57 ±	0,17	21	0,94 ±	0,34	19	1,42 ±	0,45	14	0,90 ±	0,25	11	1,16 ±	0,38	18
Monocytes %	10,47 ±	1,18	21	16,15 ±	1,98	19	13,71 ±	1,64	14	7,54 ±	0,79	11	15,66 ±	2,70	18
pH	7,28 ±	0,02	19	7,21 ±	0,04	12	7,28 ±	0,08	11	7,21 ±	0,03	6	7,16 ±	0,03	9
pCO_2	47,26 ±	3,01	19	44,79 ±	5,14	12	43,95 ±	2,76	11	47,66 ±	2,56	6	46,88 ±	3,31	9
Std. bicarbonate	20,25 ±	1,05	19	18,01 ±	1,20	12	20,29 ±	1,46	11	18,00 ±	1,81	6	15,40 ±	0,86	9

Mean ± 1 Sem LB = Number of liver biopsies analysed NA = Number of animals No = Number of samples processed

TABLE 74 MEAN VALUES FOR THE FUNCTIONAL GRADES - TIME UNRELATED

TEST	GROUP											
	N	NA 22 No	I	LB 9 No	II	LB 20 No	III	LB 10 No	IV	LB 21 No
Glutamine	11,00 ± 0,56	20	16,41 ± 1,03	9	19,27 ± 2,05	20	21,98 ± 2,93	10	27,78 ± 1,97	21
Venous NH_4	199,30 ± 9,08	20	235,55 ± 24,63	9	235,75 ± 19,73	16	252,00 ± 21,22	8	286,65 ± 24,08	20
Alkaline Phos.	6,11 ± 0,51	21	7,30 ± 1,23	9	7,37 ± 0,75	20	6,52 ± 0,86	10	5,31 ± 0,56	21
S.G.O.T.	39,54 ± 3,08	22	111,11 ± 23,81	9	101,50 ± 18,37	20	73,20 ± 14,07	10	45,23 ± 5,30	21
Cholesterol	89,82 ± 5,84	17	77,11 ± 12,54	9	72,94 ± 8,90	19	66,75 ± 15,09	8	64,47 ± 7,03	19
Total protein	6,31 ± 0,14	16	6,42 ± 0,34	9	6,50 ± 0,22	19	6,00 ± 0,23	8	6,76 ± 0,22	19
Albumin	1,80 ± 0,06	16	1,72 ± 0,12	9	1,68 ± 0,06	19	1,55 ± 0,11	8	1,82 ± 0,11	19
Globulin	4,50 ± 0,13	16	4,70 ± 0,25	9	4,81 ± 0,18	19	4,45 ± 0,16	8	4,93 ± 0,18	19
Haemoglobin	11,61 ± 0,31	21	9,71 ± 0,95	9	9,99 ± 0,49	20	9,39 ± 0,80	10	10,00 ± 0,53	21
Leucocytes x 10^3	18,99 ± 1,19	21	34,38 ± 3,95	9	30,95 ± 2,31	20	32,67 ± 5,23	9	27,31 ± 2,80	21
Platelets x 10^3	395,04 ± 32,67	21	545,11 ± 125,03	9	397,52 ± 61,76	19	439,90 ± 124,06	10	422,04 ± 61,97	21
Neutrophils %	46,28 ± 3,29	21	44,55 ± 4,89	9	47,40 ± 3,66	20	54,90 ± 5,03	10	51,90 ± 4,17	21
Lymphocytes %	37,23 ± 2,42	21	32,55 ± 4,25	9	34,35 ± 3,10	20	30,90 ± 4,48	10	31,00 ± 3,52	21
Eosinophils %	5,42 ± 1,37	21	4,87 ± 1,58	9	3,30 ± 0,82	20	2,50 ± 0,71	10	2,76 ± 0,56	21
Basophils %	0,57 ± 0,17	21	1,33 ± 0,47	9	1,05 ± 0,38	20	0,80 ± 0,32	10	1,28 ± 0,33	21
Monocytes %	10,47 ± 1,18	21	17,22 ± 3,47	9	13,90 ± 1,51	20	11,80 ± 1,63	10	13,09 ± 2,46	21
pH	7,28 ± 0,02	19	7,14 ± 0,05	6	7,27 ± 0,03	14	7,29 ± 0,03	6	7,17 ± 0,02	12
pCO_2	47,26 ± 3,01	19	44,25 ± 8,14	6	43,92 ± 3,47	14	46,25 ± 2,34	6	47,58 ± 2,66	12
Std. bicarbonate	20,25 ± 1,05	19	15,20 ± 0,81	6	19,60 ± 1,08	14	21,03 ± 2,10	6	16,17 ± 1,07	12

Mean ± 1 Sem LB = Number of liver biopsies analysed NA = Number of animals No = Number of samples processed

TABLE 75 MEAN VALUES FOR THE GRADES OF REJECTION - TIME UNRELATED

TEST	HISTOLOGICAL GRADE							
	N-I	N-II	N-III	N-IV	I-II	II-III	III-IV	I-IV
Glutamine	<0,001	<0,001	<0,001	<0,001	<0,02	-	-	<0,001
Venous NH_4	-	<0,05	<0,005	<0,01	-	-	-	-
Alkaline Phos.	<0,02	-	-	-	-	-	-	-
S.G.O.T.*	<0,001	<0,001	-	-	-	<0,02	-	<0,02
Cholesterol	-	-	<0,02	<0,005	-	-	-	-
Total Protein	-	-	-	-	-	-	-	-
Albumin	-	-	-	-	-	-	-	-
Globulin	-	-	-	-	-	-	-	-
Haemoglobin	<0,01	<0,025	<0,005	<0,005	-	-	-	-
Leucocytes	<0,001	<0,005	<0,001	<0,02	-	-	-	-
Platelets	n	n	n	n	n	n	n	n
Neutrophils	-	-	<0,001	-	-	<0,001	<0,05	-
Lymphocytes	-	-	<0,02	-	-	<0,05	-	-
Eosinophils	n	n	n	n	n	n	n	n
Basophils	n	n	n	n	n	n	n	n
Monocytes	<0,02	-	-	-	-	<0,005	<0,05	-
pH	-	-	-	<0,02	-	-	-	-
pCO_2	-	-	-	-	-	-	-	-
Std. Bicarb.	-	-	-	<0,01	-	-	-	-

p< = significance of difference in mean levels
\- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation.

TABLE 76 : THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN THE GRADES OF FUNCTION - TIME UNRELATED

TEST	HISTOLOGICAL GRADE							
	N-I	N-II	N-III	N-IV	I-II	II-III	III-IV	I-IV
Glutamine	<0,001	<0,001	<0,001	<0,001	-	-	-	<0,001
Venous NH_4	-	-	<0,01	<0,005	-	-	-	-
Alkaline Phos.	-	-	-	-	-	-	-	-
S.G.O.T.*	<0,001	<0,001	<0,005	-	-	-	<0,025	<0,001
Cholesterol	-	-	-	<0,01	-	-	-	-
Total Protein	-	-	-	-	-	-	-	-
Albumin	-	-	<0,05	-	-	-	-	-
Globulin	-	-	-	-	-	-	-	-
Haemoglobin	<0,02	<0,01	<0,005	<0,02	-	-	-	-
Leucocytes	<0,001	<0,001	<0,005	<0,01	-	-	-	-
Platelets	n	n	n	n	n	n	n	n
Neutrophils	-	-	-	-	-	-	-	-
Lymphocytes	-	-	-	-	-	-	-	-
Eosinophils	n	n	n	n	n	n	n	n
Basophils	n	n	n	n	n	n	n	n
Monocytes	<0,05	-	-	-	-	-	-	-
pH	<0,025	-	-	<0,02	<0,05	-	<0,02	-
pCO_2	-	-	-	-	-	-	-	-
Std. Bicarb.	<0,02	-	-	<0,02	<0,02	-	<0,05	-

p< = significance of difference in mean levels
\- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation.

TABLE 77 : THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN THE GRADES OF REJECTION - TIME UNRELATED

TEST	GRADES COMPARED			
	I-I	II-II	III-III	IV-IV
CSF Glutamine	-	-	-	-
Venous NH_4	-	-	-	-
Alkaline Phos.	-	-	-	-
S.G.O.T.*	-	-	-	-
Cholesterol	-	-	-	-
Total Protein	-	-	-	-
Albumin	-	-	-	-
Globulin	-	-	-	-
Haemoglobin	-	-	-	-
Leucocytes	-	-	-	-
Platelets	n	n	n	n
Neutrophils	-	-	-	-
Lymphocytes	-	-	-	-
Eosinophils	n	n	n	n
Basophils	n	n	n	n
Monocytes	-	-	-	<0,025
pH	-	-	-	-
pCO_2	-	-	-	-
Standard Bicarb.	-	-	-	-

p = significance of difference in mean levels
\- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation.

TABLE 78 THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN THE TWO HISTOLOGICAL INDICES

BIBLIOGRAPHY

A.

1. Experience in the treatment of hepatic failure by intermittent liver hemoperfusions.
Abouna, G.M., Fisher, L.McA., Porter, K.A. and Andres, G.
Surg., Gynae. and Obstets. 137: 741, 1973.

2. Experimental and clinical heterotopic liver homotransplantation.
Absolon, K.B., Hagihara, P.F., Griffen Jr., W.O., and Lillehei, R.C.
Rev. Int. Hepat. 15: 1481, 1965.

3. Replantation of the liver in dogs.
Alican, F., and Hardy, J.D.
J. Surg. Res. 7: 368, 1967.

4. American College of Surgeons/National Institutes of Health (ACS/NIH) Organ Transplant Registry. Report February 1970.

5. Idem. Report January 1974.

6. Immunopathological studies of orthotopic human liver allografts.
Andres, G.A., Ansell, I.D., Halgrimson, C.G., Hsu, K.C., Porter, K.A., Starzl, T.E., Accinni, L., Calne, R.Y., Herbertson, B.M., Penn, I., Rendall, J.M. and Williams, R.
Lancet 1: 275, 1972.

7. Hepatic heterotransplantation.
Arena, A.J., Carter, J.H., Cooper, J.R., Margulies, J. and Welch, C.S.
Bull. Soc. Int. Chir. 25: 660, 1966.

8. Armitage, P.
in Statistical methods in medical research, ed.
Armitage, P., Blackwell Scientific Publications, Oxford and Edinburgh, 1971, p. 28.

9. Idem. p. 38

10. Idem. p. 85

11. Idem. p. 120

12. Idem. p. 141

13. Idem. p. 351

14. Idem. pp. 464-467

15. Orthotopic liver transplantation.
Aune, S.
Ann. Chir. et Gynae. Fenniae. 62: 175, 1973.

B.

16. Phenolphthalein monophosphate, a new substrate for alkaline phosphatase.
Babson, A.L.
Clinical Chemistry 11: 789, 1965.

17. Primary Carcinoma of the liver.
Bagshawe, A.F.
in Liver, eds. S.J. Saunders and J. Terblanche,
Pitman Medical, London, 1973, p. 299.

18. Hepatic resection.
Balasegaram, M.
in Liver, eds. S.J. Saunders and J. Terblanche,
Pitman Medical, London, 1973, p. 231.

19. Another look at rejection in pig liver homografts.
Battersby, C., Egerton, W.S., Balderson, G., Kerr, J.F.
and Burnett, W.
Surgery 76: 617, 1974.

20. Liver function in the pig. I. The effect of 30 minutes' normothermic ischaemia.
Battersby, C., Hickman, R., Saunders, S.J., and Terblanche, J.
Brit. J. Surg. 61: 27, 1974.

21. Function of Auxiliary liver allografts.
Beaudoin, J-G., Slapak, M., Phillips, M.J., Chandrasekaran, A.K., and MacLean, L.D.
Surg., Gynae. and Obstets. 130: 622, 1970.

22. Short term preservation of porcine livers.
Belzer, F.O., May, R., Berry, M.N., and Lee, J.C.
J. Surg. Res. 10: 55, 1970.

23. Heterotopic liver transplantation.
Bengmark, S., Börjesson, B., and Olsson, A.M.
Ann. Chir. et Gynae. Fenniae 62: 178, 1973.

24. Experimental auxiliary liver homotransplantation.
Bengoechea-Gonzalez, E., Awane, Y., and Reemtsma, K.
Arch. Surg. 94: 1, 1967.

25. The clinical features of primary carcinoma of the liver.
Berman, C.
Clinical Proceedings 3: 323, 1944.

26. The cerebral and peripheral uptake of ammonia in liver disease with an hypothesis for the mechanism of hepatic coma.
Bessman, S.P., and Bessman, A.N.
J. Clin. Investig. 34: 622, 1955.

27. The animal with an Eck fistula.
Bollman, J.L.
Physiol. Reviews 41: 607, 1961.

28. Anaesthesia for liver transplantation in the pig.
Bowes, J.B., Terblanche, J., Peacock, J.H., and Immelman, E.J.
Res. Vet. Sci. 10: 569, 1969.

29. Glutamine estimation in cerebrospinal fluid in liver disease. Its use as an assay of functional liver reserve before shunt operations.
Brandstaetter, S., and Barzilai, D.
Amer. J. Dig. Dis. 5: 945, 1960.

30. Hepatic Coma: present concepts of pathogenesis and therapy.
Breen, K.J., and Schenker, S.
in Progress in Liver Diseases, vol. IV, eds. H. Popper and F. Schaffner, Grune and Stratton, New York, 1972, p. 313.

31. Specific bacteriologic problems after orthotopic liver transplantation in dogs and pigs.
Brettschneider, L., Tong, J.L., Boose, D.S., Daloze, P.M., Smith, G.V., Huguet, C., Blanchard, H., Groth, C.G., and Starzl, T.E.
Arch. Surg. 97: 313, 1968.

C.

32. Observations on experimental and clinical liver transplantation.
Calne, R.Y.
Transplantation Proceedings IV: 773, 1972.

33. Allografting in the pig.
Calne, R.Y.
in Immunological Aspects of Transplantation Surgery, ed. R. Calne, MTP Publishing Co., 1973, p. 296.

34. Immunosuppressive effects of the orthotopically transplanted porcine liver.
Calne, R.Y., White, H.J.O., Binns, R.M., Herbertson, B.M., Millard, P.R., Pena, J., Salaman, J.R., Samuel, J.R., and Davis, D.R.
Transplantation Proceedings i: 321, 1969.

35. Prolonged survival of liver transplants in the pig.
Calne, R.Y., White, H.J.O., Yoffa, D.E., Binns, R.M., Maginn, R.R., Herbertson, R.M., Millard, P.R., Molina, V.P., and Davis D.R.
Brit. Med. J. 4: 645, 1967.

36. Observations of orthotopic liver transplantation in the pig.
Calne, R.Y., White, H.J.O., Yoffa, D.E., Maginn, R.R., Binns, R.M., Samuel, J.R., and Molina, V.P.
Brit. Med. J. 2: 478, 1967.

37. The differential diagnosis of neonatal hepatitis and biliary atresia.
Campbell, D.P., Poley, J.R., Alaupovic, P., and Smith, E.I.
J. Pediatric Surg. 9: 699, 1974.

38. Hepatic portoenterostomy - is it indicated in the treatment of biliary atresia?
Campbell, D.P., Poley, J.R., Bhatia, M., and Smith, E.I.
J. Pediatric Surg. 9: 329, 1974.

39. The inter-liver competition and portal blood in regeneration of auxiliary liver transplants.
Chandler, J.G., Lee, S., Krubel, R., Rosen, H., Nakaji, N.T., and Orloff, M.J.
Surg. Forum 22: 341, 1971.

40. Modified reagents for determination of urea and ammonia.
Chaney, A.L. and Marbach, E.P.
Clinical Chemistry 8: 131, 1962.

41. Liver regeneration following portacaval transposition in dogs.
Child, C.G., Barr, D., Holswade, G.R., and Harrison, C.S.
Ann. Surg. 138: 600, 1953.

42. Pathology of orthotopic liver transplantation in pigs and dogs. Autopsy and biopsy studies.
Collan, Y., Sivula, A., and Lempinen, M.
Ann. Chir. et Gynae. Fenniae 62: 204, 1973.

43. Auxiliary partial liver transplantation in the dog.
Corry, R.J., Chavéz-Peón, F., Miyakuni, T., and Malt, R.A.
Arch. Surg. 98: 799, 1969.

44. Supply and demand in kidney and liver transplantation: a statistical survey.
Couch, N.P.
Transplantation 4: 587, 1966.

45. Problems encountered with an electromagnetic blood flowmeter.
Crosier, J.H., Terblanche, J. and Immelman, E.J.
S. Afr. J. Surg. 11: 106, 1973.

46. A comparative study of the effects of portacaval shunting and sham laparotomies on young pigs.
Crosier, J., Immelman, E., Hickman, R., Webber, B., Uys, C.J. and Terblanche, J.
S. Afr. J. Surg. 12: 79, 1974.

47. Cerebrospinal fluid glutamine as an index of rejection in heterotopic liver transplantation in pigs: a controlled study.
Crosier, J.H., Immelman, E.J., Hickman, R., Uys, C.J. and Terblanche, J.
S. Afr. J. Surg. 13: 2, 1975.

48. A comparative study of two methods of biliary drainage in pigs subjected to auxiliary heterotopic hepatic transplantation.
Crosier, J., Immelman, E., Hickman, R., Uys, C.J., van den Ende, J. and Terblanche, J.
S. Afr. J. Surg. 13: 1, 1975.

49. A comparative study of two methods of biliary drainage in auxiliary hepatic transplantation in the pig.
Crosier, J., Immelman, E.J., Hickman, R., Uys, C.J., van den Ende, J. and Terblanche, J.
Paper presented at the Congress of the European Surgical Research Society, Paris, April, 1975.

D.

50. Auxiliary homotransplantation of the canine liver with the use of a "reverse" Welch technique.
Daloze, P.M., Huguet, C., Porter, K.A., and Starzl, T.E.
Surgery, 64: 934, 1968.

51. Blood flow in auxiliary canine liver homografts.
Daloze, P.M., Huguet, C., Groth, C.G., and Stoll, F.
J. Surg. Res. 9: 10, 1969.

52. Biostatistics - a foundation for analysis in the health sciences, ed. Daniel, W.W., John Wiley and Sons, Inc., New York, p. 127.

53. Experimental liver transplantation; - Basic observations in the pig.
Dent, D.M.
Ch. M. Thesis Univ. of Cape Town, 1972.

54. Gastric ulceration complicating experimental liver transplantation in the pig.
Dent, D.M., Uys, C.J., Hickman, R., Saunders, S.J., and Terblanche, J.
J. Surg. Res. 11: 289, 1971.

55. The natural history of liver allo- and autotransplantation in the pig.
Dent, D.M., Hickman, R., Uys, C.J., Saunders, S., and Terblanche, J.
Brit. J. Surg. 58: 407, 1971.

56. The application of ion-exchange column chromatography in the diagnosis of disorders in amino acid metabolism.
De Wet, P.M.
Paper read at the Congress of Medical Technologists of S.A., Johannesburg, September, 1974.

57. Documenta Geigy, Scientific Tables, Sixth edition, ed. K. Diem, pp 32-33.

58. Orthotopic liver transplantation for Wilson's Disease.
DuBois, R.S., Rodgerson, D.O., Martineau, G., Shroter, G., Giles, G., Lilly, J., Halgrimson, C.G., Starzl, T.E., Sternlieb, I. and Scheinberg, I.H.
Lancet, 1: 505, 1971.

59. Assessment of the leucocyte migration test for predicting renal allograft rejection.
Dunningham, T.H., and Castro, J.E.
Transplantation, 21: 270, 1976.

E.

60. Application of the leucocyte migration test to the diagnosis of rejection after liver transplantation.
Eddleston, A.L.W.F., Smith, M.G.M., Mitchell, C.G., and Williams, R.
Transplantation, 12: 11, 1971.

61. Treatment of liver failure.
Editorial-Brit. Med. J. 20 October 1973.

62. Isolated liver perfusion for reducing blood ammonia.
Eiseman, B., Knipe, P., McColl, H.A., and Orloff, M.J.
Arch. Surg. 83: 356, 1961.

63. Effect of external pressure on oxygen utilization by the liver.
Eiseman, B., Kawamura, T., Tate, I., Velasquez, A.
Ann. Surg. 171: 211, 1970.

64. Liver morphology.
Elias, H.
Biol. Rev. 30: 263, 1955.

65. The "Normal" liver of the pig; is it an example of purely portal (and therefore subclinical) cirrhosis?
Elias, H., Bond, E. and Lazarowitz, A.
Am. J. Vet. Res. Jan., 1954, p. 60.

F.

66. Radioisotope scanning in auxiliary liver transplantation.
Faris, T.D., Dickhaus, A.J., Marchioro, T.L. and Starzl, T.E.
Surg., Gynae. and Obstets. 123: 1261, 1966.

67. Allogeneic hepatic transplantation using cadaveric donors with clinical application.
Fonkalsrud, E.W., Joseph, W.L., Tocornal, J.A., Ono, H., Shafey, O.A., and Longmire, W.P.
J. Pediatric Surg. 3: 300, 1968.

68. Orthotopic and heterotopic liver homografts in man.
Fortner, J.G., Beattie, Jr., E.J., Shiu, M.H., Kawano, N., and Howland, W.S.
Ann. Surg. 172: 23, 1970.

69. Clinical liver heterotopic (auxiliary) transplantation.
Fortner, J.G., Kinne, D.W., Shiu, M.H., Howland, W.S., Kim, D.K., Castro, E.B., Yeh, S.D.J., Benua, R.S., and Krumins, S.
Surgery 74: 739, 1973.

70. Heterotopic (Auxiliary) liver allotransplantation for biliary atresia.
Fortner, J.G., Shiu, M.H., Kinne, D.W., Kim, D.K., Mabogunje, O., and Yeh, S.D.J.
Gastroenterology, 66: 693 , 1974.

71. Infections in recipients of liver homografts.
Fulginiti, V.A., Scribner, R., Groth, C.G., Putnam, C.W., Brettschneider, L., Gilbert, S., Porter, K.A., and Starzl, T.E.
New Engl. J. of Med. 279: 619, 1968.

G.

72. Orthotopic transplantation of the porcine liver.
Garnier, H., Clot, J.P., and Chomette, G.
Surg., Gynae. and Obstets. 130: 105, 1970.

73. Glutamine estimation in cerebrospinal fluid in cases of liver cirrhosis and hepatic coma.
Gilon, E., Szeinberg, A., Tauman, G. and Bodonyi, E.
J. Lab. Clin. Med. 53: 714, 1959.

74. Some concepts on ammonia, porta-systemic shunts, and encephalopathy.
Gips, C.H., Hindfelt, B., Holmin, T., and Kardel, T.
Scand. J. Gastroenterology, 8: 385, 1973.

75. Heterotopic liver transplantation after liver damage.
Gliedman, M.L., Pangan, J., Minkowitz, S., Popowitz, L., and Karlson, K.E.
Trans. Amer. Soc. Artif., Int. Organs XI: 205, 1965.

76. Homotransplantation of the canine liver.
Goodrich, Jr., E.O., Welch, H.F., Nelson, J.A., Beecher, T.S., and Welch, C.S.
Surgery, 39: 244, 1956.

77. Abdominopelvic cavity.
in Grant's Method of Anatomy, eds. J.C. Boileau Grant and J.V. Basmajian, E. & S. Livingstone Limited.
Edinburgh and London, 1965, pp. 231-234.

78. Metabolic effects of hepatic replacement in Wilson's Disease.
Groth, C.G., Dubois, R.S., Corman, J., Gustafsson, A., Iwatsuki, S., Rodgerson, D.O., Halgrimson, C.G., and Starzl, T.E.
Transplantation Proceedings, V: 829, 1973.

79. An analysis of 62 cases of primary carcinoma of the liver based on 24 400 necropsies at Bellevue Hospital.
Gustafson, E.G.
Ann. Intern. Med. 11: 889, 1937.

H.

80. Experimental studies on homologous heterotopic liver transplantation.
Hagihara, P., and Absolon, K.B.
Surg. Gynae. and Obstets. 119: 1297, 1964.

81. Auxiliary liver transplantation: effect of host portacaval shunt. Experimental and clinical observations.
Halgrimson, C.G., Marchioro, T.L., Faris, T.D., Porter, K.A., Peters, G.N. and Starzl, T.E.
Arch. Surg. 93: 107, 1966.

82. The lymphocytes of the blood.
in Histology, ed. A.W. Ham, Sixth edition, 1969.
J.B. Lippincott Company, Philadelphia. pp 273-288.

83. Blood Ammonia - Accuracy and precision.
Di Giorgio, J. (quote page 526)
in Clinical Chemistry: Principles and Technics,
eds. Henry, R.J., Cannon, D.C., and Winkelman, J.W.
Harper and Row publishers, second edition, 1974.
chapter 17, p. 518.

84. The pathology of liver transplants.
Herbertson, B.M., Millard, P.R., and Ansell, I.D.
in Clinical Organ Transplantation, ed. R.Y. Calne,
1971, p. 421.

85. Advantages of Auxiliary liver homotransplantation in rats.
Hess, F., Jerusalem, C., and van der Heyde, M.N.
Arch. Surg. 104: 76, 1972.

86. Current concepts relating to metabolic changes after portacaval shunts, and alcohol and the pancreas.
(1) Portacaval shunts.
Hickman, R., Crosier, J.H., Meyers, O.L., Engelbrecht, G.H.C., Keraan, M., Immelman, E.J. and Terblanche, J.
S. Afr. J. Surg. 12: 201, 1974.

87. Transhepatic metabolism after end-to-side portacaval shunt in the young pig.
Hickman, R., Crosier, J.H., Saunders, S.J., and Terblanche, J.
Surgery, 76: 601, 1974.

88. Problems with an electromagnetic blood flowmeter.
Hickman, R., Crosier, J.H., Smith, P., Immelman, E.J., and Terblanche, J.
S. Afr. Med. J. 49: 261, 1975.

89. The use of domestic pigs in medical research in South Africa.
2. Physiological data.
Hickman, R., Saunders, S.J., and Terblanche, J.
J. S. Afr. vet. med. Ass. 41: 105, 1970.

90. Liver function in the pig. Total hepatic and portal flow values in vivo.
Hickman, R., Saunders, S.J., and Terblanche, J.
S. Afr. J. Lab. Clin. Med. 48: 1197, 1974.

91 Anorexia and weight loss in the portacaval-shunted pig.
Hickman, R. and Terblanche, J.
Surgery, 80: 569, 1976.

92. Biochemical values in normal anaesthetized South African pigs.
Hickman, R., Terblanche, J., Simson, E., Dent, D., and Saunders, S.J.
S. Afr. Med. J. 44: 531, 1970.

93. Cerebrospinal fluid glutamine as a measure of hepatic encephalopathy.
Hourani, B.T., Hamlin, E.M., and Reynolds, T.B.
Arch. Intern. Med. 127: 1033, 1971.

94. Pathology of liver transplantation in the pig.
Hunt, A.C.
in Colston Papers, vol. 19, The Liver, ed. A.E. Read., Butterworths, London, 1967, p. 337.

95. Liver transplantation in the pig.
Hunt, P., Cocks, J., de Boer, W., Dennis, P., Gordon, E., Jablonski, P., Sali, A., Tannenberg, A., Yoffa, D., and Watts, J. McK.
Med. J. Aust. 2: 261, 1970.

96. Platelet changes following clinical and experimental hepatic homotransplantation.
Hutchison, D.E., Genton, E., Porter, K.A., Daloze, P.M., Huguet, C., Brettschneider, L., Groth, C.G., and Starzl, T.E.
Arch. Surg. 97: 27, 1968.

I.

97. The avoidance of donor atrophy in auxiliary liver allo-transplantation in the pig.
Immelman, E.J., van Leenhof, J.W., Terblanche, J., Saunders, S.J., and Uys, C.J.
Europ. Soc. Exp. Surg. 7th Congress, 1972, p. 288.

98. Recognition and prevention of intra-operative Warm-ischaemia in liver transplantation.
Immelman, E.J., Peacock, J.H., Hobbs, K.E.F., Mitra, S.K., Hunt, A.C., and Bowes, J.B.
Brit. Med. J. 2: 220, 1968.

99. Heterotopic liver allotransplantation in the pig.
Immelman, E.J., van Leenhoff, J.W., Saunders, S.J., Uys, C.J., and Terblanche, J.
in Liver, eds. S.J. Saunders and J. Terblanche, Pitman Medical, London, 1973, p. 210.

J.

100. Heterotopic liver transplantation.
II. Unfavorable outflow conditions as a possible cause for late graft failure.
Jerusalem, C., van der Heyde, M.N., Schmidt, W.J. and Tjebbes, F.A.
Europ. Surg. Res. 4: 186, 1972.

101. The jaundiced newborn baby.
in Clinical Paediatric Surgery, ed. Peter G. Jones, Wright, Bristol, 1970, pp 106-110.

K.

102. The determination of urea, ammonia and urease.
Kaplan, A.
Methods of Biochemical Analysis, 17: 311, 1969.

103. A note on the spectrophotometric assay of glutamic oxalacetic transaminase in human blood serum.
Karmen, A.
J. Clin. Investig. 34: 131, 1955.

104. Occlusion of the hepatic artery in man.
Kim, D.K., Kinne, D.W., and Fortner, J.G.
Surg., Gynae, and Obstets. 136: 966, 1973.

105. Heterotopic Auxiliary liver transplantation in rats. Use of the infrahepatic vena cava as the efferent vessel.
Kort, W.J., Wolff, E.D., and Eastham, W.N.
Transplantation, 12: 415, 1971.

106. Congenital biliary atresia. II. Analysis of the therapeutic problem.
Krovetz, L.J.
Surgery, 47: 468, 1960.

107. Temporary auxiliary life-sustaining liver transplantation following acute hepatic necrosis.
Kuster, G.G.R., and Woods, J.E.
Surg. Forum 22: 337, 1971.

108. Auxiliary liver transplantation in the dog as temporary support in acute fulminating hepatic necrosis.
Kuster, G.G.R., and Woods, J.E.
Ann. Surg. 176: 732, 1972.

L.

109. Liver transplantation.
Leading article.
Lancet, ii: 29, 1974.

L.

110. Primary carcinoma of the liver.

Lemmer, K.E.

Arch. Surg. 61: 599, 1950.

111. Orthotopic liver transplantation in the pig.

Lempinen, M., Salmenkivi, K., and Sivula, A.

Acta. Chir. Scand. 137: 265, 1971.

112. Long-term survival after orthotopic liver transplantation in pigs.

Lempinen, M., Sivula, A., and Collan, Y.

Ann. Chir. et Gynae. Fenniae 62: 219, 1973.

113. Auxiliary liver transplantation in acute liver failure.

Lilly, J.R., Anderson, K.D., Hill, J.L., Rosser, S.B., and Randolph, J.G.

J. Pediatric Surg. 7: 492, 1972.

114. Primary cancer of the liver.

Lin, T-Y.

Scand. J. Gastroenterology 6: 223, 1970.

115. New Technique of hepatic lobectomy and results of hepatic lobectomy for primary carcinoma of the liver.

Lin, T-Y.

in Liver, eds. S.J. Saunders and J. Terblanche, Pitman Medical, London, 1973, p. 230.

116. Personal communication - Cape Town, May, 1974

Longmire, W.P., Jr.

117. Obstructive jaundice in early infancy.

Louw, J.H., and McKenzie, D.

S. Afr. Med. J. 35: 657, 1961.

118. The regulation of ammonia metabolism in mammalian tissues.
Lund, P., Brosnan, J.T., and Eggleston, L.V.
in Essays in Cell Metabolism, eds. Bartley, Kornberg and Quayle, Wiley - Interscience, London, 1970, p. 167.

M.

119. The effect of host liver damage, portacaval shunt, and various immunosuppressive regimes on canine auxiliary liver transplantation.
Macpherson, S.G., Miller, S.E.P., Calman, K.C., McSween, R.N.M., Buxton, B.F., and Bell, P.R.F.
Transplantation, 14: 175, 1972.

120. Hepatic sepsis after liver transplantation in dogs and pigs.
McSween, R.N.M.
Arch. Path. 88: 166, 1969.

121. Abdominal Operations.
Editor R. Maingot, Fifth edition, Appleton-Century-Crofts, New York, 1969.
Neoplasms of the liver - Clinical course p. 1021.

122. Idem:- Hepatic artery injury. pp 805-806

123. Idem:- Roux-Y method. pp 461-463.

124. A life supporting heterotopic autotransplant of the canine liver without portal blood.
Maki, T., Blackburn, G.L., Slapak, M., and McDermott, W.V. Jr.
Surg., Gynae. and Obstets. 136: 951, 1973.

125. The specific influence of nonhepatic splanchnic venous blood flow on the liver.
Marchioro, T.L., Porter, K.A., Brown, B.I., Faris, T.D., Herrmann, T.J., Sudweeks, A., and Starzl, T.E.
Surg. Forum 16: 280, 1965.

126. Physiologic requirements for auxiliary liver homotransplantation.
Marchioro, T.L., Porter, K.A., Dickinson, T.C., Faris, T.D., and Starzl, T.E.
Surg., Gynae. and Obstets. 121: 17, 1965.

127. The effect of partial portacaval transposition on the canine liver.
Marchioro, T.L., Porter, K.A., Brown, B.I., Otte, J-B., and Starzl, T.E.
Surgery 61: 723, 1967.

128. Delayed biliary duct obstruction after orthotopic liver transplantation.
Martineau, G., Porter, K.A., Corman, J., Launois, B., Schroter, G.T., Palmer, W., Putnam, C.W., Groth, C.G., Halgrimson, C.G., Penn, I., and Starzl, T.E.
Surgery 72: 604, 1972.

129. Homotransplantation of the canine liver: a new technic.
Mehrez, I.O., Nabseth, D.C., Kekis, B.P., Apostolou, K., Gottlieb, L.S., and Deterling, R.A.
Ann. Surg. 159: 416, 1964.

130. The nature of the rejection response in pig liver homotransplants.
Mieny, C.J.
S. Afr. Med. J. 42: 670, 1968.

131. Homotransplantation of the liver in pigs.
Mieny, C.J., Moore, A.R., Homatas, J., and Eiseman, B.
S. Afr. J. Surg. 5: 109, 1967.

132. Partial heterotopic liver homograft in dogs utilizing portal arterialization.
Mito, M., Ackroyd, F.W., Covelli, V.H., Eyskens, E., Katayama, I., and McDermott, Jr., W.V.
Ann. Surg. 165: 20, 1967.

133. Studies on the outflow tracts of the liver - III, qualitative observations in normal man and in patients with hepatic cirrhosis.
Moreno, A.H., Burchell, A.R., Rousselot, L.M. and Burke, J.H.
Ann. Surg. 170: 63, 1969.

134. Auxiliary liver transplantation in dogs with arterialization of the portal vein.
Mouzas, G.L., van Heeckeren, D. and Matano, I.
Internat. Surg. 47: 220, 1967.

135. Liver transplantation in man.
The significance, patterns, and control of infection.
Murray-Lyon, I.M., Evans, D.B., Foster, W.D., Holden, R.J., Rake, M.O., Stern, H., Calne, R.Y., and Williams, R.
Brit. J. Surg. 57: 280, 1970.

136. Cholestatic Phenomenon in hepatic allograft rejection in the primate.
Myburgh, J.A., Abrahams, C., Mendelsohn, D., Mieny, C.J., and Bersohn, I.
Transplantation Proceedings III: 501, 1971.

N.

137. Auxiliary homotransplantation of the porcine liver into the retroperitoneal space.
Novak, V., Hill, L., Rothwell-Jackson, R., Velasquez, A., Wright, R., and Eiseman, B.
Surgery, 67: 678, 1970.

138. Studies to prolong function of experimental auxiliary liver grafts.
Novak, V., Hill, L., Velasquez, A., Douglas, M., and Eiseman, B.
J. Surg. Res. 10: 123, 1970.

O.

139. Primary Carcinoma of the liver.
Ong., G.B. and Chan, P.K.W.
Surg., Gynae. and Obstets. 143: 31, 1976.

140. Current progress and problems in portal hypertension.
Orloff, M.J.
in Liver, eds. S.J. Saunders and J. Terblanche, Pitman Medical, London, 1973, p. 273.

141. Site of origin of the hepatotrophic portal blood factor responsible for liver regeneration.
Orloff, M.J., Lee, S., Charters, A.C., Chandler, J.G., Sgro, J.C., Grambort, D.E. and Broelsch, C.E.
Gastroenterology 66: 755, 1974.
Abstract Annual Meeting of Amer. Gastroent. Assoc. et al.

P.

142. A rapid, accurate method for the determination of total cholesterol in serum.
Pearson, S., Stern, S. and McGavack, T.H.
Analytical Chemistry, 25: 813, 1953.

143. Pathology of liver transplantation.
Porter, K.A.
Transplantation Reviews 2: 129, 1969.

144. Pathology of the auxiliary homograft.
Porter, K.A.
in Experiences in Hepatic transplantation, eds. T.E. Starzl and C.W. Putnam, 1969, p. 516.

145. Pathology of the orthotopic homograft and heterograft.
Porter, K.A.
in Experiences in Hepatic transplantation, eds.
T.E. Starzl and C.W. Putnam, 1969, p. 422.

146. The role of portal blood in regeneration and function of completely revascularized partial hepatic autografts.
Price, J.B., Voorhees, A.B., and Britton, R.C.
Surgery, 62: 195, 1967.

R.

147. Influence of the source of portal vein blood on dog auxiliary hepatic allograft function in recipients with hepatic insufficiency.
Ranson, J.H.C., Garcia-Moran, M., Becker, F. and Localio, S.A.
Surg. Forum. 22: 345, 1971.

148. Portal inflow and auxiliary hepatic allograft function.
Ranson, J.H.C., Garcia-Moran, M., Becker, F.F. and Localio, S.A.
Surg., Gynae. and Obstets. 135: 769, 1972.

149. Progress report. Acute hepatic necrosis and fulminant hepatic failure.
Rueff, B. and Benhamou, J.P.
Gut. 14: 805, 1973.

150. The future of constructive surgery.
Russell, P.S.
Transplantation Proceedings VI: 147, 1974.

S.

151. Liver pathology.
Sandritter, W. and Wartman, W.B.
in Colour Atlas and Textbook of Tissue and Cellular Pathology, eds. W. Sandritter and W.B. Wartman, 4th edition, 1973, p 117.

152. Saunders, S.J.: - In discussion on liver transplantation.
in Liver, eds. S.J. Saunders and J. Terblanche,
Pitman Medical, London, 1973, p. 220.

153. Acute hepatic failure.
in Liver, eds. S.J. Saunders and J. Terblanche,
Pitman Medical, London, 1973, pp. 113-137.

154. The treatment of acute liver failure.
Saunders, S.J., Hickman, R., McDonald, R. and Terblanche, J.
in Progress in Liver Diseases, vol. IV, eds. H. Popper and F. Schaffner, Grune and Stratton, New York, 1972, p. 333.

155. Hepatic competition in auxiliary allotransplantation.
Sawada, M., Price, J.B. (Jr.), Takeshige, K., and Voorhees, A.B. (Jr.)
Surg. Forum 22: 344, 1971.

156. Heterotopic auxiliary liver transplantation.
Part 1. A physiological concept of functional competition.
Schalm, L.
Arch. Chir. Neerl. 18: 283, 1966.

157. Liver biopsy interpretation.
Scheuer, P.
Bailliere, Tindall and Cassell, London, 1968.

158. Congenital biliary atresia.
in Principles of Surgery, ed. Schwartz, S.I.,
McGraw-Hill Book Company, New York, 1969, p. 1081.

159. Simplified technic for human auxiliary liver transplantation.
Sheil, A.G.R., Rogers, J., May, J., Storey, B., Kuruvila, J.T., George, C., Berry, F.R. and Stewart, J.H.
Amer. J. Surg. 117: 359, 1969.

160. Auxiliary canine liver transplantation from cadaver donors.
Sheil, A.G.R., Rogers, J.H., Halliday, J.P., Storey, B.G., Kelly, G.E. and Mason, R.
Arch. Surg. 100: 290, 1970.

161. Diseases of the Liver and Biliary System.
ed. Sheila Sherlock, Fifth edition, Blackwell Scientific Publications, Oxford, 1975.
Hepatic transplantation, pp. 792-799.

162. Idem:- Portal venous system and portal hypertension. p. 197.

163. Idem:- Carcinoma of the bile ducts. p. 662.

164. Rejection of the homotransplanted dog liver in the absence of hepatic insufficiency.
Sicular, A., Paronetto, F., Kark, A.E., Dreiling, D.A., Burrows, L. and Popper, H.
Proc. Soc. Exp. Biol. and Med. 112: 760, 1963.

165. Methods and results of partial liver autotransplantation.
Sigel, B. and Acevedo, F.J.
Surg., Gynae. and Obstets. 114: 731, 1962.

166. Effect of portosystemic shunting and decreased blood flow on partial heterotopic liver autotransplants.
Sigel, B., Baldia, L.B. and Dunn, M.R.
Surg. Forum 16: 288, 1965.

167. A new acid-base nomogram. An improved method for the calculation of the relevant blood acid-base data.
Siggaard-Andersen, O. and Engel, K.
Scand. J. Clin. & Lab. Investig. 12: 177, 1960.

168. Studies of hepatic function in dogs with Eck fistula or portacaval transposition.
Silen, W., Mawdsley, D.L., Weirich, W.L. and Harper, H.A.
Arch. Surg. 74: 964, 1957.

169. Anatomy of the domestic animals.
Sisson, S. and Grossman, J.D.
Third Edition, W.B. Saunders. pp. 490-499 and pp. 756-757.

170. Causes of death in the early postoperative period in orthotopic liver transplantations in the dog.
Sivula, A., Lempinen, M., Paloheimo, M. and Turunen, M.T.
Ann. Chir. et Gynae. Fenniae 62: 199, 1973.

171. Auxiliary liver homotransplantation.
A new technique and an evaluation of current techniques.
Slapak, M., Beaudoin, J.G., Lee, H.M., and Hume, D.M.
Arch. Surg. 100: 31, 1970.

172. Long-term preservation of the canine liver using auxiliary autotransplantation as a functional test.
Slapak, M. and Maki, T.
Transplantation Proceedings VI: 295, 1974.

173. Twenty-four hour liver preservation by the use of continuous pulsatile perfusion and hyperbaric oxygen.
Slapak, M., Wigmore, R.A. and MacLean, L.D.
Transplantation 5: 1154, 1967.

174. Further studies with a sine wave electromagnetic flowmeter.
Smith, P., Crosier, J.H., Hickman, R. and Terblanche, J.
S. Afr. J. Surg. 12: 80, 1974.

175. The metabolic response of the liver to portacaval shunt.
Smith, G.W. and Mouzas, G.L.
Surgery, 68: 341, 1970.

176. Primary carcinoma of the liver.
Spatt, S.D. and Grayzel, D.M.
Amer. J. Med. 5: 570, 1948.

177. Studies of the blood ammonia in liver disease. Its diagnostic, prognostic, and therapeutic significance.
Stahl, J.
Ann. Int. Med. 58: 1, 1963.

178. Experience in Hepatic Transplantation.
Eds. T.E. Starzl with the assistance of C.W. Putnam, W.B. Saunders Company, Philadelphia, 1969.
Congenital biliary atresia, pp. 8-9.

179. Idem:- Chapter 16. Infectious complications, excluding partial hepatic gangrene. p. 329.

180. Idem:- Chapter 14. Early liver rejection in patients without hepatic gangrene. p. 277.

181. Idem:- Chapter 5. Donor hepatectomy and liver preservation. p. 41.

182. Idem:- Chapter 15. Acute rejection and hepatic gangrene. p. 308.

183. Personal communication.
Starzl, T.E. Cape Town, August, 1973.

184. Personal experience with orthotopic liver transplantation.
Starzl, T.E., Corman, J., Groth, C.G., Halgrimson, C.G., Penn, I., Putnam, C.W., Schroter, G. and Gustafsson, A.
Transplantation Proceedings IV: 759, 1972.

185. The origin, hormonal nature, and action of hepatotrophic substances in portal venous blood.
Starzl, T.E., Francavilla, A., Halgrimson, C.G., Francavilla, F.R., Porter, K.A., Brown, T.H. and Putnam, C.W.
Surg., Gynae, and Obstets. 137: 179, 1973.

186. Orthotopic homotransplantation of the human liver.
Starzl, T.E., Groth, C.G., Brettschneider, L., Penn, I., Fulginiti, V.A., Moon, J.B., Blanchard, H., Martin, A.J., and Porter, K.A.
Ann. Surg. 168: 392, 1968.

187. Progress in and deterrents to orthotopic liver transplantation, with special reference to survival, resistance to hyperacute rejection, and biliary duct reconstruction.
Starzl, T.E., Ishikawa, M., Putnam, C.W., Porter, K.A., Picache, R., Husberg, B.S., Halgrimson, C.G. and Schroter, G.
Transplantation Proceedings VI: 129, 1974.

188. Studies on the rejection of the transplanted homologous dog liver.
Starzl, T.E, Kaupp, H.A. Jr., Brock, D.R. and Linman, J.W.
Surg., Gynae. and Obstets. 112: 135, 1961.

189. Avenues of future research in homotransplantation of the liver with particular reference to hepatic supportive procedures, antilymphocyte serum, and tissue typing.
Starzl, T.E., Marchioro, T.L., Faris, T.D., McCardle, R.J. and Iwaski, Y.
Amer. J. Surg. 112: 391, 1966.

190. Factors determining short- and long-term survival after orthotopic liver homotransplantation in the dog.
Starzl, T.E., Marchioro, T.L., Porter, K.A., Taylor, P.D., Faris, T.D., Herrmann, T.J., Hlad, C.J., and Waddell, W.R.
Surgery, 58: 131, 1965.

191. Immunosuppression after experimental and clinical homotransplantation of the liver.
Starzl, T.E., Marchioro, T.L., Rowlands, D.T. Jr., Kirkpatrick, C.H., Wilson, W.E.C., Rifkind,D. and Waddell, W.R.
Ann. Surg. 160: 411, 1964.

192. Orthotopic liver transplantation in ninety-three patients.
Starzl, T.E., Porter, K.A., Putnam, C.W., Schroter, G.P.J., Halgrimson, C.G., Weil, R., Hoelscher, M. and Reid, H.A.S.
Surg., Gynae. and Obstets. 142: 487, 1976.

193. Autopsy findings in a long-surviving liver recipient.
Starzl, T.E., Porter, K.A., Schroter, G., Corman, J., Groth, C.G., and Sharp, H.L.
New Engl. J. Med. 289: 82, 1973.

194. Cerebrospinal fluid glutamine in the diagnosis of hepatic coma.
Steigman, E., Kafzemi, F. and Dubin, A., et al.
Amer. J. Gastroent. 40: 378, 1962.

195. Orthotopic autotransplantation and allotransplantation of the liver: functional and structural patterns in the dog.
Stuart, F.P., Torres, E., Hester, W.J., Dammin, G.J. and Moore, F.D.
Ann. Surg. 165: 325, 1967.

T.

196. The strategy of liver transplantation.
Terblanche, J. and Riddell, A.G.
in Colston Papers, vol. 19, The Liver, ed. A.E. Read., Butterworths, London, 1967, p. 321.

197. The technique of orthotopic liver homotransplantation in the pig.
Terblanche, J., Peacock, J.H., Bowes, J., and Hobbs, K.E.F.
J. Surg. Res. 8: 151, 1968.

198. Orthotopic liver homotransplantation: an experimental study in the unmodified pig.
Terblanche, J., Peacock, J.H., Hobbs, K.E.F., Hunt, A.C., Bowes, J., Tierris, E.J., Palmer, D.B. and Blecher, T.E.
S. Afr. Med. J. 42: 486, 1968.

199. Progress in liver transplantation and the treatment of bile duct carcinoma.
Terblanche, J.
S. Afr. Med. J. 45: 1149, 1971.

200. U tube drainage in the palliative therapy of carcinoma of the main hepatic duct junction.
Terblanche, J. and Louw, J.H.
Surg. Clinics of North Amer. 53: 1245, 1973.

201. Homotransplantation of the canine liver. Survival and histology with and without azathioprine.
Thomford, N.R., Shorter, R.G. and Hallenbeck, G.A.
Arch. Surg. 90: 527, 1965.

202. Homotransplantation of an auxiliary dog liver into the pelvis - effect of portacaval shunt in the prevention of liver atrophy.
Tretbar, L.L., Beven, E.G. and Hermann, R.E.
Surg. Forum 16: 219, 1965.

203. The effects of portacaval shunt and portal flow occlusion on canine auxiliary liver homotransplants.
Tretbar, L.L., Beven, E.G. and Hermann, R.E.
Surgery 61: 733, 1967.

U.

204. Experimental auxiliary liver allotransplantation with special reference to biliary duct obstruction and hepatic arterial blood restriction of host liver.
Uchida, H., Motsay, G.J., Ruiz, O. and Lillehei, R.C.
Amer. J. Surg. 119: 138, 1970.

205. The pathology of liver transplantation in the pig.
Uys, C.J., Dent, D.M., Immelman, E.J., Saunders, S.J. and Terblanche, J.
in Liver, eds. S.J. Saunders and J. Terblanche,
Pitman Medical, London, 1973, p. 213.

V.

206. Heterotopic auxiliary liver transplantation. Part II.
A technique for heterotopic partial liver homotransplantation in dogs.
Van der Heyde, M.N.
Arch. Chir. Neerl. 18: 285, 1966.

207. Experimental thoracic implantation of partial liver homografts in the dog.
Van der Heyde, M.N., Bilski, R., Jerusalem, C. and Reinking, J.W.
European Soc. for Experimental Surg. 6th Congress, 1971, p. 283.

208. Auxiliary liver-graft without portal blood. Experimental autotransplantation of left liver lobes.
Van der Heyde, M.N. and Schalm, L.
Brit. J. Surg. 55: 116, 1968.

209. Survival of hepatectomized dogs with partial liver transplants.
Van der Heyde, M.N., Cooper, J.R., Carter, J.H. and Welch, C.S.
Surgery 59: 1079, 1966.

210. Functional requirements for the auxiliary livergraft.
Van der Heyde, M.N., Jerusalem, C., Jap, P.H.K., Hess, F. and Lens, J.
in Liver, eds. S.J. Saunders and J. Terblanche,
Pitman Medical, London, 1973, p. 211.

211. The influence of haemodynamics on the micro-structure of the heterotopic liver graft.
Van der Heyde, M.N., Jerusalem, C., Schmidt, W.J.H., Bilsky, R., Reinking, J.W., Tjebbes, F. and Jap, P.
Europ. Soc. Exp. Surg. 5th Congress, 1970, p. 152.

212. Heterotopic liver transplantation. 1. Postoperative course and histological feature of the canine auxiliary homograft.
van der Heyde, M.N., Jerusalem, C., Schmidt, W.J. and Tjebbes, F.A.
Europ. Surg. Res. 3: 454, 1971.

213. Heterotopic auxiliary liver transplantation. Part III. Functional competition - cause of atrophy of the auxiliary liver transplant.
Van der Heyde, M.N., Vink, M., Stol, H., Dicke, H.W. and Schalm, S.W.
Arch. Chir. Neerl. 18: 293, 1966.

W.

214. The gallbladder as a conduit between the liver and intestine.
Waddell, W.R. and Grover, F.L.
Surgery, 74: 524, 1973.

215. Bile secretion following liver transplantation in man.
Waldram, R., Kemp, A., Williams, R. and Calne, R.Y.
Gut. 14: 819, 1973.

216. A note on transplantation of the whole liver in dogs.
Welch, C.S.
Transplantation Bulletin 2: 54, 1955.

217. Auxiliary liver transplantation: A successful model without portal blood or portacaval shunt.
Wexler, M.J., Farkouh, E.F., Farrer, P.A., Slapak, M., and MacLean, L.D.
Ann. Surg. 175: 357, 1972.

218. Regeneration and maintenance of integrity of canine liver. The role of arterial, portal and systemic venous blood.
Wexler, M., Slapak, M., Mizumoto, R., Latzina, A., Giles, G., Soto, E. and McDermott, W.V. Jr.
Arch. Surg. 101: 267, 1970.

219. A method for the determination of glutamine in cerebrospinal fluid and the results in hepatic coma.
Whitehead, T.P. and Whittaker, S.R.F.
J. Clin. Path. 8: 81, 1955.

220. The glutamate and glutamine content of rat brain after portacaval anastomosis.
Williams, A.H., Kyu, M.H., Fenton, J.C.B. and Cavanagh, J.B.
J. Neuro Chem. 19: 1073, 1972.

221. Current position of liver transplantation.
Williams, R.
in Liver, eds. S.J. Saunders and J. Terblanche,
Pitman Medical, London, 1973, p. 198.

222. Liver transplantation in man - III, studies of liver function, histology, and immunosuppressive therapy.
Williams, R., Calne, R.Y., Ansell, I.D., Ashby, B.S., Cullum, P.A., Dawson, J.L., Eddleston, A.L.W.F., Evans, D.B., Flute, P.T., Herbertson, P.M., Joysey, V., McGregor, A.M.C., Millard, P.R., Murray-Lyon, I.M., Pena, J.R., Rake, M.O. and Sells, R.A.
Brit. Med. J. 3: 12, 1969.

223. Liver transplantation: A clinical and immunological appraisal.
Williams, R. and Smith, M.G.M.
in Progress in Liver Diseases, vol. IV, eds. H. Popper and F. Schaffner, Grune and Stratton, New York, 1972, p. 433.

224. Liver transplantation in man: the frequency of rejection, biliary tract complications, and recurrence of malignancy based on an analysis of 26 cases.
Williams, R., Smith, M., Shilkin, K.B., Herbertson, B., Joysey, V., and Calne, R.Y.
Gastroenterology 64: 1026, 1973.

225. Disseminated intravascular coagulation in fulminant hepatic failure.
Williams, R.
in Liver, eds. S.J. Saunders and J. Terblanche,
Pitman Medical, London, 1973, p. 126.

226. Williams, R.:- In discussion on liver transplantation.
in Liver, eds. S.J. Saunders and J. Terblanche,
Pitman Medical, London, 1973, p. 220.

227. Studies in serum proteins - γ - a rapid procedure for the estimation of total protein, true albumin, total globulin, α globulin, β globulin and γ globulin in 1,0 ml of serum.
Wolfson, W.Q., Cohn, C., Calvary, E. and Ichiba, F.
Amer. J. Clin. Path. 18: 723, 1948.

Z.

228. Studies on heterotopic auxiliary liver transplantation in rats.
Zelder, O., Thermann, M., Bode, C. and Fischer, M.
Europ. Soc. Exp. Surg. 7th Congress, 1972, p. 373.

229. Hepatic Failure.
Zimmerman, H.J.
in The Liver, Eds. E.A. Gall and F.K. Mostofi,
The Williams & Wilkins Company, Baltimore, 1973, p. 384.

STATISTICAL ADDENDUM

STATISTICAL ADDENDUM

The statistical method used was criticised by one of the examiners on the grounds of:

"I have reservations about the presentation and the statistical analyses of the haematological and biochemical results (Part II, chapter 3, pages 196 - 235). The author performed a prodigious number of student t-tests on all possible combinations of groups and times after transplantation, and has tabulated p values for all of them. From these he draws conclusions which are not only confusing, but of questionable validity. For example, S.G.O.T. (p. 207 - 208): group 1(b) revealed a "significant, sustained evaluation" on days 7, 14, 21 and 28; in group 1(a) the mean level "was significantly elevated on day 7 but not thereafter"; however, "there were no significant differences in the serial mean levels between groups 1(a) and 1(b) except on day 28". Are these groups similar or different in this regard? His final conclusion on page 224 is that they are "comparable". I presume that by this he means they are not different. The author used log transformation of the data because the standard deviations were very large, and examination of the raw data in the appendix confirms the enormous range. The data are certainly not normally distributed and the validity of using parametric statistics here, even with log transformation, is questioned. Common sense and simple inspection of the raw data suggest that the use of medians would be a more realistic measure of the central tendency of the data, and a non-parametric test (e.g. Mann Whitney U test) more appropriate. Appropriate calculations indicate median values of 40, 75, 55, 55 and 35 for group 1(a) and 35, 50, 45, 100 and 120 for group 1(b) on days 0, 7, 14, 21 and 28 respectively. This conveys a different picture than does Fig. 58a on page 207 F, with a progressive and significant ($p<0.05$) deviation of the curves developing on day 21. What the relevance this is to the method of biliary drainage following auxiliary liver transplantation is another matter. Similar problems occur in the comparisons drawn with most of the other haematologic and biochemical criteria. Furthermore, at no stage does Dr. Crosier indicate the P value he regards as significant. Presumably this is the conventional 0,05. This is not necessarily realistic in all circumstances, particularly where a consistent trend occurs in serial comparisons. It would be preferable to give the P values rather than to dismiss any value greater than 0,05 as "not significant". The statement, underlined, on page 224 that "groups 1(a) and 1(b) are thus comparable biochemically and biochemically, suggesting that the type of biliary drainage did not influence the post-operative levels of these factors" may or may not be substantiated by re-examination of all the data.

"Inspection of the raw data, and the consistency of the trends graphically depicted in Figures 69 and 70, suggest that the main conclusion in Part III, namely that CSF glutamine levels are accurately related to the quality of the donor liver, will be substantiated by non-parametric statistical methods."

This examiner recommended "recalculation by simple non-parametric methods of the more relevant comparisons".

In view of the above the following was done:

(A) The Thesis and raw data were submitted to an independent professional statistician, Dr. D.J. van Sehalkwyk, for opinion about the statistical method used. He reported:

"Basically I am in agreement with the comments of the examiner although not necessarily with his recommendations."

"The method recommended by the examiner viz. the Mann-Whitney U test suffers from the disadvantage that the underlying assumption is that the populations have the same distributions but only differ in locality. With differing variances this is not the case."

(B) The author subsequently requested the assistance of Dr. van Schalkwyk in re-analysing the data. A series of analyses were devised by, and executed under the direction of Dr. van Schalkwyk, using the original raw data.

1. For Part II, Chapter 3, Section IV, the re-analyses were performed as follows:-

   The patterns of changes in the 19 biochemical and haematological factors in the 4 groups were analysed and compared at weekly intervals by means of pairwise comparisons of group means using Tukey's multiple comparison method based on the Studentized Range (231). Where necessary, transformations were done on the data to stabilise the within-group variances.

   The results of these analyses, in terms of probability levels, are summarised in Tables 79 - 89 on pages 414 - 424. It can be seen that the main trends and conclusions are almost identical to those obtained with the original analyses.

   In addition, Dr. van Schalkwyk replotted all the graphs using the original raw data but transformed where appropriate. The graphs were almost identical in all respects to those originally obtained.

2. For Part III, Chapters 2 and 3, the re-analyses were performed as follows:-

   (i) For each of the 19 biochemical and haematological variables, a one-way analysis of Variance was

done with the Functional Liver Index (and Degree of Rejection) as covariate and "days after operation" as treatment groups, to test:

(a) Whether a significant relationship exists between the variable measured and the Functional Liver Index (or Degree of Rejection), the "zero slope";

(b) Whether the relationship is constant over time, i.e. whether the regression slopes are equal.

The results are summarised in Table 90 on page 425.

(ii) The correlation between the Functional Liver Index (and Degree of Rejection) and 5 of the variables, was calculated for each of days 7, 14, 21 and 28, using group 2(b) animals as controls on each of these days. The results are summarised in Tables 91 - 94 on pages 426-428.

Once more, it can be seen that the main trends and conclusions are almost identical to those obtained with the original analyses.

After completion of these analyses, Dr. van Schalkwyk reported as follows:-

"As a result of these analyses Dr. Crosier's original conclusions are borne out, namely that

i) the two transplant groups did not differ;

ii) CSF glutamine is the only variable that showed clear and sustained differences between the transplant and non-transplant groups as well as changes from the pre-operative levels in all but the control groups; and

iii) the single best predictor of the Functional Index or degree of rejection is CSF glutamine."

(C) The author re-analysed representative samples of the data from Part II, Chapter 3, Section IV, and Part III, Chapter 2, using the Mann-Whitney U-Test (230), as suggested by the examiner.

The results of these non-parametric analyses are summarised in Tables 80, 82 - 84, 93 and 94 on pages 415, 417-419 and 427-428.

It is clear that the Mann-Whitney U-Tests produced trends and conclusions almost identical to those obtained with the t-test, in each of the representative samples.

DISCUSSION ON THE RE-ANALYSIS OF THE DATA

The more relevant data has been re-analysed by 2 different methods. The analyses carried out under the direction of a professional statistician and the Mann-Whitney U-Tests performed by the author, both provided results and conclusions which are almost identical to those the author achieved when using the t-test as described on pages 340 - 342. The t-test appears to have been sufficiently robust to detect the essential differences between and similarities in the various groups.

The two subsequent statistical analyses have merely confirmed, and in a few instances clarified the main trends that were obtained and do not alter any of the basic conclusions derived from the t-tests.

ACKNOWLEDGEMENTS

The re-analysis of the data proved to be a very considerable undertaking. The author wishes to express his appreciation to the Institutions, Departments and individuals who supported or played a part in the re-assessment:

IN THE INSTITUTE FOR BIOSTATISTICS OF THE SOUTH AFRICAN MEDICAL RESEARCH COUNCIL

Dr. D.J. van Schalkwyk , Ph.D. (London), for his independent assessment of the original statistical analysis, and for subsequently planning and directing a re-analysis of all the data.

Miss J. Cassidy, B.A. (Hons.), for carrying out the re-analysis.

IN THE UNIVERSITY OF CAPE TOWN

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Dr. A. Wolpowitz, Department of Cardiac Surgery, for providing the facilities for carrying out the Mann-Whitney U-Tests.

ADDITIONAL BIBLIOGRAPHY

230 The Mann-Whitney U Test.
in Nonparametric Statistics for the Behavioral Sciences,
ed. Sidney Siegel, International Student Edition,
McGraw-Hill Kogakusha, Ltd., Tokyo, pp. 116-127.

231 Statistical Methods, eds. Snedecor, G.W., and Cochran, W.G.,
Sixth Edition, The Iowa State University Press, Ames, Iowa, U.S.A.,
1967, pp. 271-275.

KEY TO TABLES IN THE ADDENDUM

1. The original tables have been used, as far as possible, to enable the reader to compare the results of the three analyses with ease.

(a) For Tables 79 - 89, and 93 - 94, letter Gothic typestyle (e.g. 0,001) has been used to illustrate the probability values obtained with the original t-tests, while Light Italic typestyle (e.g. *0,001*) has been used, in the same tables, for Dr. van Schalkwyk's results.

(b) For Tables 80, 82 - 84, and 93 - 94, Prestige Elite typestyle (e.g. 0,001) has been used for the Mann-Whitney U-Test results.

(c) - = Probability value greater than 0,05 for each type of analysis.

2. Where transformations have been used in the calculations, the variables have been identified as follows:

* = with Log transformation

† = with Square root transformation

3. Mult. Comparison = Multiple Comparison Test.

TEST	DATE OF COMPARISON (DAYS)				
	OP	7	14	21	28
CSF Glutamine	-	-	-	-	-
*	-	-	-	-	-
Venous NH_4	-	-	-	<0,01	<0,001
*	-	-	-	*<0,05*	-
Alkaline Phos.	-	-	-	-	-
*	-	-	-	-	-
S.G.O.T. *	-	-	-	-	<0,02
*	-	-	-	-	-
Cholesterol	-	-	<0,005	-	-
	-	-	*0,01*	-	-
Total Protein	-	<0,02	-	<0,01	-
	-	-	-	-	-
Albumin	-	-	-	-	-
	-	-	-	-	-
Globulin	-	<0,05	-	<0,02	<0,05
*	-	-	-	-	-
Haemoglobin	-	<0,05	<0,02	-	-
	-	-	-	-	-
Leucocytes	-	-	-	-	-
	-	-	-	-	-
Platelets	-	-	-	-	-
	-	-	-	-	-
Neutrophils	-	-	-	-	-
	-	-	-	-	-
Lymphocytes	-	-	-	-	-
	-	-	-	-	-
Eosinophils †		<0,01	-	-	-
	-	-	-	-	-
Basophils †	-	-	-	-	-
	-	-	-	-	*<0,05*
Monocytes	-	-	<0,005	-	<0,025
	-	-	-	-	-
pH	-	-	-	-	-
	-	-	-	-	-
pCO_2	-	-	-	-	-
*	-	-	-	-	-
Standard Bicarb.	-	-	-	-	-
*	-	-	-	-	-

p< = significance of difference in mean levels
\- = no significant difference in mean levels (p>0,05)
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation
† = with square root transformation

TABLE 79 : THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN GROUPS 1(a) and 1(b) - USING TWO METHODS OF STATISTICAL ANALYSIS.

(Refer to page 198F.)

TEST	DAYS 0-7	0-14	0-21	0-28	STATISTICAL ANALYSIS	TEST	DAYS 0-7	0-14	0-21	0-28
CSF Glutamine *	<0,025	<0,01	<0,01	<0,01	t-test	Platelets	n	n	n	n
	-	*<0,001*	*<0,01*	*<0,001*	*Mult. Comparison*		-	-	-	-
	<0,05	<0,001	<0,005	<0,001	Mann-Whitney		-	-	-	-
Venous NH_4 *	<0,01	<0,02	<0,005	<0,001	t-test	Neutrophils	-	-	-	-
	-	-	*<0,05*	-	*Mult. Comparison*		-	-	-	-
	<0,003	<0,005	<0,003	<0,002	Mann-Whitney		-	-	-	-
Alkaline Phos. *	-	<0,01	-	-	t-test	Lymphocytes	-	-	-	-
	-	-	-	-	*Mult. Comparison*		-	-	-	-
	-	-	-	-	Mann-Whitney		-	-	-	-
S.G.O.T. *	<0,005	-	-	-	t-test	Eosinophils †	n	n	n	n
*	*<0,05*	-	-	-	*Mult. Comparison*		-	-	-	-
	<0,03	-	-	-	Mann-Whitney		-	-	-	-
Cholesterol	-	-	-	-	t-test	Basophils †	n	n	n	n
	-	-	-	*<0.05*	*Mult. Comparison*		-	*<0,05*	-	-
	-	-	-	-	Mann-Whitney		-	<0,02	-	-
Total Protein	<0,025	-	-	<0,05	t-test	Monocytes	-	<0,05	-	-
	<0,05	-	-	-	*Mult. Comparison*		-	*<0,05*	-	-
	<0,03	-	-	-	Mann-Whitney		-	-	-	-
Albumin	-	-	-	-	t-test	pH	-	-	-	-
	-	-	-	-	*Mult. Comparison*		-	-	-	-
	-	-	-	-	Mann-Whitney		-	-	-	-
Globulin *	<0,05	-	-	<0,005	t-test	pCO_2 *	-	-	-	-
	-	-	-	-	*Mult. Comparison*		-	-	-	-
	<0,041	-	-	<0,009	Mann-Whitney		-	-	-	-
Haemoglobin	-	-	<0,05	-	t-test	Standard Bicarb.*	-	-	-	-
	-	-	-	-	*Mult. Comparison*		-	-	-	-
	-	-	-	-	Mann-Whitney		-	-	-	-
Leucocytes	<0,005	-	<0,01	<0,02	t-test	* = with log transformation				
	<0,01	-	*<0,05*	*<0,01*	*Mult. Comparison*	† = with sq. root transformation				
	<0,003	-	<0,02	<0,02	Mann-Whitney					

TABLE 80 : SIGNIFICANCE OF CHANGES IN GROUP 1(a) - USING THREE DIFFERENT METHODS OF STATISTICAL ANALYSIS.
(Refer to page 349).

TEST	DAYS			
	0-7	0-14	0-21	0-28
CSF Glutamine	<0,001	<0,001	<0,005	<0,02
*	*<0,001*	*<0,001*	*<0,01*	*<0,01*
Venous NH_4	-	-	-	-
*	-	-	-	-
Alkaline Phos.	-	-	-	-
*	-	-	-	-
S.G.O.T.	<0,02	<0,025	<0,001	<0,005
*	*<0,001*	*<0,01*	*<0,001*	*<0,05*
Cholesterol	-	-	<0,025	<0,005
	-	*<0,05*	*<0,05*	*<0,05*
Total Protein	-	-	-	-
	-	-	-	-
Albumin	<0,05	-	<0,005	-
	-	-	-	-
Globulin	-	-	-	-
*	-	-	-	-
Haemoglobin	<0,05	<0,02	<0,005	<0,001
	<0,05	*<0,01*	*<0,01*	-
Leucocytes	<0,02	<0,02	-	<0,01
	<0,01	*<0,001*	*<0,05*	*<0,05*
Platelets	n	n	n	n
	-	-	-	-
Neutrophils	-	-	-	-
	-	-	-	-
Lymphocytes	<0,025	-	-	-
	-	-	-	-
Eosinophils	n	n	n	n
†	-	-	-	-
Basophils	n	n	n	n
†	-	-	-	-
Monocytes	<0,02	<0,05	<0,005	<0,02
	<0,05	-	*<0,01*	-
pH	-	-	<0,05	-
	-	-	-	-
pCO_2	-	-	-	-
*	-	-	-	-
Standard Bicarb.	-	-	-	-
*	-	-	-	-

p< = significance of difference in mean levels
\- = no significant difference in mean levels
0 = no or insufficient data
n = statistical analysis not performed
† = with square root transformation
* = with log transformation

TABLE 81 : THE SIGNIFICANCE OF CHANGES IN MEAN LEVELS IN GROUP 1(b) - USING TWO METHODS OF STATISTICAL ANALYSIS.

(Refer to page 350)

TEST	DAYS				STATISTICAL ANALYSIS	TEST	DAYS			
	0-7	0-14	0-21	0-28			0-7	0-14	0-21	0-28
CSF Glutamine *	<0,001 *<0,001* <0,002	<0,001 *<0,001* <0,002	<0,001 *<0,001* <0,002	<0,001 *<0,001* <0,002	t-test *Mult. Comparison* Mann-Whitney	Platelets	n – –	n – –	n – –	n – <0,02
Venous NH_4 *	<0,05 *<0,05* <0,05	<0,02 *<0,05* <0,05	<0,001 *<0,01* <0,002	<0,005 *<0,01* <0,02	t-test *Mult. Comparison* Mann-Whitney	Neutrophils	– – –	<0,02 *<0,05* <0,02	– – –	– – –
Alkaline Phos. *	<0,001 *<0,001* –	<0,05 *<0,05* –	– – –	<0,05 – –	t-test *Mult. Comparison* Mann-Whitney	Lymphocytes	– – –	<0,01 *<0,05* <0,02	<0,05 – –	– – –
S.G.O.T. * *	– – –	– – –	<0,05 – –	– – –	t-test *Mult. Comparison* Mann-Whitney	Eosinophils †	n – –	n – –	n – –	n – –
Cholesterol	<0,001 *<0,001* <0,002	<0,001 *<0,001* <0,002	<0,001 *<0,001* <0,002	<0,001 *<0,01* <0,002	t-test *Mult. Comparison* Mann-Whitney	Basophils †	n *<0,05* <0,05	n *<0,05* <0,02	n – –	n – –
Total Protein	<0,05 *<0,01* –	<0,02 – <0,02	– – –	<0,05 – –	t-test *Mult. Comparison* Mann-Whitney	Monocytes	– – –	– – –	– – –	– – –
Albumin	– – –	– – –	– – –	– – –	t-test *Mult. Comparison* Mann-Whitney	pH	0 *0* 0	0 *0* 0	0 *0* 0	0 *0* 0
Globulin *	<0,05 *<0,01* –	<0,005 *<0,01* <0,02	<0,005 – <0,02	<0,02 – <0,05	t-test *Mult. Comparison* Mann-Whitney	pCO_2	0 *0* 0	0 *0* 0	0 *0* 0	0 *0* 0
Haemoglobin	– – –	– – –	– – –	– – –	t-test *Mult. Comparison* Mann-Whitney	Standard Bicarb.	0 *0* 0	0 *0* 0	0 *0* 0	0 *0* 0
Leucocytes	<0,001 *<0,01* <0,002	<0,001 *<0,01* <0,002	<0,005 *<0,05* <0,02	<0,025 – –	t-test *Mult. Comparison* Mann-Whitney	* = with log transformation † = with sq. root transformation				

TABLE 82 : SIGNIFICANCE OF CHANGES IN GROUP 2(a) - USING THREE DIFFERENT METHODS OF STATISTICAL ANALYSIS.

(Refer to page 351).

TEST	DAYS				STATISTICAL ANALYSIS
	0-7	0-14	0-21	0-28	
CSF Glutamine *	-	<0,05	-	-	t-test
	-	-	-	-	*Mult. Comparison*
	-	-	-	-	Mann-Whitney
Venous NH_4 *	-	-	-	-	t-test
	-	-	-	-	*Mult. Comparison*
	-	-	-	<0,03	Mann-Whitney
Alkaline Phos. *	-	-	-	-	t-test
	-	-	-	-	*Mult. Comparison*
	-	-	-	-	Mann-Whitney
*	-	-	-	<0,05	t-test
S.G.O.T. *	-	-	-	-	*Mult. Comparison*
	-	-	-	<0,05	Mann-Whitney
Cholesterol	-	-	-	-	t-test
	-	-	-	-	*Mult. Comparison*
	-	-	-	-	Mann-Whitney
Total Protein	-	<0,02	<0,005	<0,02	t-test
	-	*<0,05*	-	-	*Mult. Comparison*
	-	<0,02	<0,02	<0,03	Mann-Whitney
Albumin	-	-	-	-	t-test
	-	-	-	-	*Mult. Comparison*
	-	-	-	-	Mann-Whitney
Globulin *	-	<0,005	<0,001	<0,01	t-test
	-	*<0,01*	*<0,05*	*<0,01*	*Mult. Comparison*
	-	<0,02	<0,02	<0,03	Mann-Whitney
Haemoglobin	-	-	-	-	t-test
	-	-	-	-	*Mult. Comparison*
	-	-	-	-	Mann-Whitney
Leucocytes	-	-	-	-	t-test
	-	-	-	-	*Mult. Comparison*
	-	-	-	-	Mann-Whitney

TEST	DAYS			
	0-7	0-14	0-21	0-28
Platelets	n	n	n	n
	-	-	-	-
	-	-	-	-
Neutrophils	-	<0,01	-	<0,05
	-	-	-	*<0,05*
	-	<0,02	-	-
Lymphocytes	-	-	-	-
	-	-	-	-
	-	-	-	-
Eosinophils †	n	n	n	n
	-	-	-	-
	-	-	-	-
Basophils †	n	n	n	n
	-	-	-	-
	-	-	-	-
Monocytes	<0,005	<0,001	<0,05	<0,025
	<0,05	*<0,001*	*<0,05*	*<0,001*
	<0,02	<0,02	-	<0,03
pH	0	<0,001	0	<0,025
	0	-	*0*	-
	0	<0,01	0	<0,05
pCO_2 *	0	<0,005	0	<0,02
	0	-	*0*	-
	0	<0,05	0	-
Standard Bicarb. *	0	-	0	-
	0	-	*0*	-
	0	<0,05	0	-

* = with log transformation
† = With sq. root transformation

TABLE 83 : SIGNIFICANCE OF CHANGES IN GROUP 2(b) - USING THREE DIFFERENT METHODS OF STATISTICAL ANALYSIS.

(Refer to page 352).

TEST	DAYS					STATISTICAL ANALYSIS	TEST	DAYS				
	OP	7	14	21	28			OP	7	14	21	28
CSF Glutamine *	- - -	- - -	- - -	- - -	- - -	t-test *Mult. Comparison* Mann-Whitney	Platelets	n - -	n - -	n - -	n - -	n - -
Venous NH_4 *	- - -	- - <0,05	- - -	<0,01 *<0,05* <0,03	<0,02 - -	t-test *Mult. Comparison* Mann-Whitney	Neutrophils	- - -	- - -	- - -	- - -	- - -
Alkaline Phos.*	- - -	- - -	- - -	- - -	- - -	t-test *Mult. Comparison* Mann-Whitney	Lymphocytes	- - -	- - -	- - -	- - -	- - -
* S.G.O.T. *	- - -	- - -	- - -	- - -	<0,025 - -	t-test *Mult. Comparison* Mann-Whitney	Eosinophils †	n - -	n - -	n - -	n - -	n - -
Cholesterol	- - -	- - -	<0,005 *<0,01* <0,02	- - -	- - -	t-test *Mult. Comparison* Mann-Whitney	Basophils †	n - -	n - -	n - <0,05	n - -	n *<0,05* <0,03
Total Protein	- - -	<0,005 - <0,02	- - -	<0,02 - -	- - -	t-test *Mult. Comparison* Mann-Whitney	Monocytes	- - -	- - -	<0,005 - <0,02	- - -	<0,05 - <0,05
Albumin	- - -	<0,025 - <0,02	- - -	<0,01 - <0,03	- - -	t-test *Mult. Comparison* Mann-Whitney	pH	- - -	- - -	- - -	- - -	- - -
Globulin *	- - -	<0,02 - <0,05	- - -	- - -	- - -	t-test *Mult. Comparison* Mann-Whitney	pCO_2 *	- - -	- - -	- - -	- - -	- - -
Haemoglobin	- - -	- - -	<0,02 - <0,02	- - -	- - -	t-test *Mult. Comparison* Mann-Whitney	Standard Bicarb*	- - -	- - -	- - -	- - -	- - -
Leucocytes	- - -	- - -	- - -	- - -	- - -	t-test *Mult. Comparison* Mann-Whitney	* = with log transformation † = with sq. root transformation					

TABLE 84 : THE SIGNIFICANCE OF DIFFERENCES BETWEEN GROUPS 1(a) and 1(b) - USING THREE DIFFERENT METHODS OF STATISTICAL ANALYSIS. (refer to page 354).

TEST	DATE OF COMPARISON (DAYS)				
	OP	7	14	21	28
CSF Glutamine	-	<0,005	<0,005	<0,001	<0,001
*	-	*<0,01*	*<0,01*	*<0,001*	*<0,001*
Venous NH_4	-	-	-	-	-
*	-	-	-	-	-
Alkaline Phos.	<0,005	-	-	-	-
*	-	-	-	-	-
S.G.O.T. *	-	<0,001	<0,05	-	-
*	-	*<0,05*	-	-	-
Cholesterol	-	<0,05	<0,001	-	-
	-	-	*<0,01*	-	-
Total Protein	-	-	-	-	-
	-	-	-	-	-
Albumin	-	-	-	-	-
	-	-	-	-	-
Globulin	-	-	-	-	-
*	-	-	-	-	-
Haemoglobin	-	-	<0,05	-	-
	-	-	-	-	-
Leucocytes	-	-	-	-	<0,05
	-	-	-	-	-
Platelets	n	n	n	n	n
	-	-	-	-	-
Neutrophils	-	-	<0,05	-	-
	-	-	-	-	-
Lymphocytes	-	-	<0,02	-	-
	-	-	-	-	-
Eosinophils	n	n	n	n	n
†	-	-	-	-	-
Basophils	n	n	n	n	n
†	-	-	*<0,01*	-	-
Monocytes	-	-	<0,05	-	-
	-	-	-	-	-
pH	0	0	0	0	0
	0	*0*	*0*	*0*	-
pCO_2	0	0	0	0	0
*	*0*	*0*	*0*	*0*	-
Standard Bicarb.	0	0	0	0	0
*	*0*	*0*	*0*	*0*	-

p< = significance of difference in mean levels
\- = no significant difference in mean levels (p>0,05)
0 = no or insufficient data
n = statistical analysis not performed
† = with square root transformation
* = with log transformation

TABLE 85 : THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN GROUPS 1(a) and 2(a) - USING TWO METHODS OF STATISTICAL ANALYSIS.

(Refer to page 355)

TEST	DATE OF COMPARISON (DAYS)				
	OP	7	14	21	28
CSF Glutamine	-	<0,05	<0,01	<0,02	<0,02
*	-	-	*<0,001*	*<0,01*	*<0,05*
Venous NH_4	-	-	<0,01	-	-
*	-	-	*<0,01*	-	-
Alkaline Phos.	-	<0,02	-	-	-
*	-	-	-	-	-
S.G.O.T. *	<0,05	<0,005	-	-	-
*	-	-	-	-	-
Cholesterol	-	-	-	-	-
	-	-	-	-	-
Total Protein	<0,02	-	-	-	-
	-	-	-	-	-
Albumin	-	-	-	-	-
	-	-	-	-	-
Globulin	<0,01	-	-	<0,025	-
*	-	-	-	-	-
Haemoglobin	-	-	<0,005	-	-
	-	-	-	-	-
Leucocytes	-	-	-	-	<0,05
	-	-	-	-	-
Platelets	n	n	n	n	n
	-	-	-	-	-
Neutrophils	-	-	<0,02	-	-
	-	-	-	-	-
Lymphocytes	-	-	-	-	-
	-	-	-	-	-
Eosinophils	n	n	n	n	n
†	-	-	-	-	-
Basophils	n	n	n	n	n
†	-	-	-	-	-
Monocytes	-	-	<0,001	-	<0,02
	-	-	*<0,001*	-	*<0,05*
pH	-	0	-	0	-
	-	*0*	-	*0*	-
pCO_2	-	0	-	0	-
*	-	*0*	-	*0*	-
Standard Bicarb.	-	0	-	0	-
*	-	*0*	-	*0*	-

p< = significance of difference in mean levels
\- = no significant difference in mean levels (p>0,05)
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation
† = with square root transformation

TABLE 86 : THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN GROUPS 1(a) and 2(b) - USING TWO METHODS OF STATISTICAL ANALYSIS.

(Refer to page 356)

TEST	DATE OF COMPARISON (DAYS)				
	OP	7	14	21	28
CSF Glutamine	-	<0,01	<0,001	<0,001	<0,05
*	-	*<0,01*	*<0,001*	*<0,001*	*<0,01*
Venous NH_4	-	<0,02	<0,02	<0,001	<0,01
*	-	*<0,05*	-	*<0,01*	*<0,01*
Alkaline Phos.	<0,05	<0,025	-	-	-
*	-	*<0,05*	-	-	-
S.G.O.T. *	-	<0,01	<0,02	<0,025	<0,02
*	-	*<0,001*	-	-	-
Cholesterol	-	-	-	-	<0,025
	-	-	-	-	-
Total Protein	-	<0,02	<0,05	<0,025	-
	-	-	-	-	-
Albumin	-	<0,02	-	-	-
	-	-	-	-	-
Globulin	-	-	<0,02	<0,02	-
*	-	-	-	*<0,05*	-
Haemoglobin	-	-	-	<0,05	<0,02
	-	-	-	-	-
Leucocytes	<0,02	-	-	-	<0,025
	-	-	-	-	-
Platelets	n	n	n	n	n
	-	-	-	-	-
Neutrophils	-	-	<0,05	<0,05	-
	-	-	-	-	-
Lymphocytes	-	-	-	-	-
	-	-	-	-	-
Eosinophils	n	n	n	n	n
†	-	-	-	-	-
Basophils	n	n	n	n	n
†	-	-	-	-	-
Monocytes	-	-	-	<0,005	-
	-	-	-	-	-
pH	0	0	0	0	0
	0	*0*	*0*	*0*	-
pCO_2	0	0	0	0	0
*	*0*	*0*	*0*	*0*	-
Standard Bicarb.	0	0	0	0	0
*	*0*	*0*	*0*	*0*	-

p< = significance of difference in mean levels
- = no significant difference in mean levels (p>0,05)
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation
† = with square root transformation

TABLE 87 : THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN GROUPS 1(b) and 2(a) - USING TWO METHODS OF STATISTICAL ANALYSIS.

(Refer to page 357).

TEST	DATE OF COMPARISON (DAYS)				
	OP	7	14	21	28
CSF Glutamine	-	<0,02	<0,005	<0,01	-
*	-	*<0,05*	*<0,001*	*<0,05*	-
Venous NH_4	-	-	<0,05	-	-
*	-	-	-	-	-
Alkaline Phos.	-	<0,05	-	-	-
*	-	-	-	-	-
S.G.O.T. *	-	-	-	-	-
*	-	-	-	-	-
Cholesterol	-	<0,02	<0,02	<0,025	<0,005
	-	-	*<0,05*	-	*<0,05*
Total Protein	<0,005	-	-	-	-
	-	-	-	-	-
Albumin	-	-	-	<0,005	-
	-	-	-	-	-
Globulin	<0,01	-	-	-	-
*	-	-	-	-	-
Haemoglobin	-	-	-	-	<0,02
	-	-	-	-	-
Leucocytes	-	-	-	-	<0,025
	-	-	-	-	-
Platelets	n	n	n	n	n
	-	-	-	-	-
Neutrophils	-	-	-	-	-
	-	-	-	-	-
Lymphocytes	-	-	-	-	-
	-	-	-	-	-
Eosinophils	n	n	n	n	n
†	-	-	-	-	-
Basophils	n	n	n	n	n
†	-	-	-	-	-
Monocytes	-	-	<0,005	-	-
	-	-	*<0,01*	-	-
pH	<0,05	0	-	0	-
	-	*0*	-	*0*	-
pCO_2	<0,02	0	-	0	-
*	-	*0*	-	*0*	-
Standard Bicarb.	-	0	-	0	-
*	-	*0*	-	*0*	-

p< = significance of difference in mean levels
\- = no significant difference in mean levels (p>0,05)
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation
† = with square root transformation

TABLE 88 : THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN GROUPS 1(b) and 2(b) - USING TWO METHODS OF STATISTICAL ANALYSIS.

(Refer to page 358)

TEST	DATE OF COMPARISON (DAYS)				
	OP	7	14	21	28
CSF Glutamine	-	<0,005	<0,001	<0,001	<0,001
*	*<0,05*	*<0,001*	*<0,001*	*<0,001*	*<0,001*
Venous NH_4	-	<0,01	<0,001	<0,001	<0,05
*	-	-	*<0,01*	*<0,05*	*<0,01*
Alkaline Phos.	<0,025	-	-	-	-
*	-	-	-	-	-
S.G.O.T. *	-	-	-	-	-
*	-	-	-	-	-
Cholesterol	-	<0,005	<0,001	<0,02	<0,005
	-	-	*<0,05*	-	-
Total Protein	<0,05	-	-	-	-
	-	-	-	-	-
Albumin	-	-	-	-	-
	-	-	-	-	-
Globulin	<0,05	-	-	<0,005	-
*	-	-	-	-	-
Haemoglobin	-	-	-	-	-
	-	-	-	-	-
Leucocytes	-	-	-	-	-
	-	-	-	-	-
Platelets	n	n	n	n	n
	-	-	-	-	-
Neutrophils	-	-	<0,001	-	<0,02
	-	-	*<0,01*	-	-
Lymphocytes	-	-	<0,05	-	<0,05
	-	-	-	-	-
Eosinophils	n	n	n	n	n
†	-	-	-	-	-
Basophils	n	n	n	n	n
†	-	-	-	-	-
Monocytes	-	<0,05	<0,001	<0,01	<0,05
	-	-	*<0,01*	-	-
pH	0	0	0	0	0
	0	*0*	*0*	*0*	-
pCO_2	0	0	0	0	0
*	*0*	*0*	*0*	*0*	-
Standard Bicarb.	0	0	0	0	0
*	*0*	*0*	*0*	*0*	-

p< = significance of difference in mean levels
\- = no significant difference in mean levels (p>0,05)
0 = no or insufficient data
n = statistical analysis not performed
* = with log transformation
† = with square root transformation

TABLE 89 : THE SIGNIFICANCE OF DIFFERENCES IN MEAN LEVELS BETWEEN GROUPS 2(a) and 2(b) - USING TWO METHODS OF STATISTICAL ANALYSIS.

(Refer to page 359).

VARIABLE	PROBABILITY LEVELS			
	FUNCTIONAL INDEX		DEGREE OF REJECTION	
	ZERO SLOPE	EQUALITY OF SLOPE	ZERO SLOPE	EQUALITY OF SLOPE
CSF Glutamine	$0,4x10^{-19}$	0,9221	$0,2x10^{-14}$	0,4769
Venous NH_4	0,0460	0,0217	0,0288	0,0734
Alkaline Phos.	0,0709	0,0018	0,1494	0,0368
S.G.O.T.	0,6589	0,1306	0,5087	0,6225
Cholesterol	0,0002	0,3666	0,0002	0,1893
Total Protein	0,6074	0,7097	0,7703	0,6468
Albumin	0,4051	0,4848	0,3038	0,2344
Globulin	0,2777	0,7065	0,3747	0,8083
Haemoglobin	0,2261	0,6475	0,6308	0,3093
Leucocytes	0,3367	0,1083	0,3287	0,1187
Platelets	0,4421	0,3638	0,5467	0,7797
Neutrophils	0,0208	0,2761	0,0127	0,2025
Lymphocytes	0,3602	0,6554	0,4660	0,6421
Eosinophils	0,6691	0,8237	0,7057	0,4279
Basophils	0,0659	0,6405	0,0590	0,6955
Monocytes	0,0084	0,0119	0,0015	0,0053
pH	0,6183	0,2865	0,9821	0,4091
pCO_2	0,7081	0,7703	0,6440	0,6449
Standard Bicarb.	0,3510	0,5754	0,7432	0,7654

TABLE 90 : SUMMARY OF ONE-WAY ANALYSIS OF VARIANCE

VARIABLE	DAY 7			DAY 14			DAY 21			DAY 28		
	No	Corr	p	No	Corr	p	No	Corr	p	No	Corr	p
CSF Glutamine	26	0,804	<0,001	22	0,817	<0,001	18	0,843	<0,001	14	0,845	<0,001
Alkaline Phos	26	0,368	-	22	0,104	-	18	0,448	-	14	0,651	<0,05
S.G.O.T.	26	0,374	-	22	0,078	-	18	0,225	-	14	0,497	-
Cholesterol	24	0,474	<0,05	22	0,194	-	16	0,574	<0,05	14	0,521	-
Leucocytes	25	0,295	-	22	0,224	-	18	0,003	-	13	0,487	-

TABLE 91:: CORRELATION BETWEEN THE FUNCTIONAL LIVER INDEX AND THE VARIABLES

VARIABLE	DAY 7			DAY 14			DAY 21			DAY 28		
	No	Corr	p	No	Corr	p	No	Corr	p	No	Corr	p
CSF Glutamine	25	0,670	<0,001	21	0,806	<0,001	18	0,851	<0,001	14	0,707	<0,01
Alkaline Phos	25	0,311	-	21	0,074	-	18	0,362	-	14	0,489	-
S.G.O.T.	25	0,251	-	21	0,177	-	18	0,099	-	14	0,201	-
Cholesterol	23	0,506	<0,05	21	0,120	-	16	0,581	<0,05	14	0,561	<0,05
Leucocytes	25	0,200	-	21	0,206	-	18	0,031	-	13	0,557	<0,05

TABLE 92 : CORRELATION BETWEEN THE DEGREE OF REJECTION AND THE VARIABLES

Note: Group 2(b) animals used as controls on each of the days.

POST OP DAY	FUNCTIONAL GRADES COMPARED										TEST	CORRELATION
	N-I	N-II	N-III	N-IV	I-II	I-III	I-IV	II-III	II-IV	III-IV		"N"-GRADES I-IV
7	<0,02 -	<0,01 <0,02	<0,001 <0,002	<0,001 <0,002	- -	<0,05 <0,03	<0,005 <0,005	- -	<0,01 <0,02	<0,05 -	t MW	*<0,001*
14	<0,001 <0,002	<0,005 <0,002	0 <0,02	<0,001 <0,002	- -	0 <0,05	<0,025 <0,002	0 -	- -	0 -	t MW	*<0,001*
21	<0,005 <0,02	<0,02 <0,02	0 <0,02	<0,001 <0,002	- -	0 0	<0,005 <0,002	0 0	- -	0 0	t MW	*<0,001*
28	- <0,02	0 0	<0,005 <0,002	<0,005 <0,002	0 -	- -	<0,02 <0,05	0 -	0 -	- -	t MW	*<0,001*

p< = significance
\- = no significant difference
0 = no or insufficient data

t = t-test
MW = Mann-Whitney U-Test
"N" = Group 2(b) levels on each day

TABLE 93 : THE SIGNIFICANCE OF SERIAL CHANGES IN MEAN CSF GLUTAMINE LEVELS - USING THREE DIFFERENT METHODS OF STATISTICAL ANALYSIS.

(Refer to page 243F.)

POST OP DAY	REJECTION GRADES COMPARED										TEST	CORRELATION
	N-I	N-II	N-III	N-IV	I-II	I-III	I-IV	II-III	II-IV	III-IV		"N"-GRADES I-IV
7	<0,005 <0,02	<0,05 -	<0,005 <0,002	<0,001 <0,002	- -	- -	- <0,036	- -	<0,05 <0,041	- -	t MW	*<0,001*
14	<0,001 <0,02	<0,01 <0,002	0 0	<0,001 <0,002	- -	0 0	<0,05 <0,003	0 0	- -	0 0	t MW	*<0,001*
21	0 0	<0,005 <0,001	0 0	<0,001 <0,002	0 0	0 0	0 0	0 0	<0,01 <0,009	0 0	t MW	*<0,001*
28	0 0	<0,05 -	<0,01 <0,02	<0,005 <0,002	0 0	0 0	0 0	- -	- -	- -	t MW	*<0,01*

p< = significance
\- = no significant difference
0 = no or insufficient data

t = t-test
MW = Mann-Whitney U-Test
"N" = Group 2(b) levels on each day

TABLE 94 : THE SIGNIFICANCE OF SERIAL CHANGES IN MEAN CSF GLUTAMINE LEVELS - USING THREE DIFFERENT METHODS OF STATISTICAL ANALYSIS.

(Refer to page 245F.)