ORTHOTOPIC NONAUXILIARY ALLOTRANSPLANTATION OF PART OF THE LIVER IN DOGS

Druk: Dissertatie Drukkerij Wibro, Helmond, Telefoon 04920-23981.

ORTHOTOPIC NONAUXILIARY ALLOTRANSPLANTATION OF PART OF THE LIVER IN DOGS

(Orthotope niet-auxiliaire allotransplantatie van een deel van de lever bij honden)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE ERASMUS UNIVERSITEIT ROTTERDAM, OP GEZAG VAN DE RECTOR MAGNIFICUS PROF. DR. M. W. VAN HOF EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN. DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP WOENSDAG 27 JUNI 1984 TE 14.00 UUR.

DOOR

NIKOLAAS MARIA AUGUST BAX

GEBOREN TE SCHERPENHEUVEL, BELGIË

BEGELEIDINGSCOMMISSIE:

Promotoren	:	Prof. Dr. J.C. Molenaar
		Prof. Dr. J. Jeekel
Overige leden	:	Prof. Dr. J.L. Terpstra (RU Leiden)
		Prof. Dr. R.O. van der Heul

To all sick children

C O N T E N T S

CHAPTER 1.	Rationale and aim of the study	11
CHAPTERS 2 - 5.	Review of the literature	15
CHAPTER 2.	The need for liver transplantation	17
2.1.	Treatment of terminal liver failure	17
2.2.	The need for pediatric liver transplantation in The Netherlands	20
CHAPTER 3.	Modes of liver transplantation	29
3.1.	Classification and nomenclature	29
3.2.	Discussion	30
3.2.1.	Vascular supply and biliary drainage of the graft	30
3.2.2.	The origin of the graft	30
3.2.3.	Heterotopic auxiliary transplantation	31
3.2.3.1.	The problem of graft atrophy	31
3.2.3.2.	Modifications of the venous anastomoses	32
3.2.3.3.	The concept of functional competition	34
3.2.3.4.	Intrinsic hepatic growth control factors	34
3.2.4.	Orthotopic nonauxiliary transplantation	35
3.3.	Comments	36
CHAPTER 4.	Problems associated with clinical orthotopic liver transplantation	45
4.1.	The donor liver	45
4.1.1.	Shortage of donor livers	45
4.1.2.	The discriminatory role of the size of the donor organ in recipient selection	45
4.1.3.	Preservation of the donor liver	46
4.1.3.1.	The role of the temperature	46
4.1.3.2.	The role of the composition of the preservation fluid	48

4.2. The preoperative condition of the recipient 50
4.3. Anastomotic problems 51
4.4. Rejection 53

CHAPTER 5. Results of orthotopic liver transplantation in children 63

CHAPTERS 6 - 11. The experiments 67

CHAPTER 6. The experimental design 69

6.1. The experimental animal: the beagle 69
6.2. Mitigation of rejection 70
6.3. The design of a control model 70
6.4. The orthotopic nonauxiliary mode of partial liver transplantation 71
6.5. The age of the animals at operation and the size of the partial grafts 71
6.6. The duration of liver ischemia 72
6.7. The duration of the period under study 72

CHAPTER 7. The surgical techniques and over-all results 75

CHAPTER 8. Peroperative complications 97

CHAPTER 9. Vascular and biliary complications 123

9.1. Vascular complications 123
9.1.1. Methods of diagnosis 123
9.1.2. Results 137
9.1.3. Discussion 137
9.2. Biliary complications 138
9.2.1. Methods of diagnosis 138
9.2.2. Results 139
9.2.3. Discussion 142

CHAPTER 10. Liver function 145

10.1. Clinical assessment 145
10.2. Biochemical assessment 145
10.2.1. Methods 145
10.2.2. Results 147
10.3. Discussion 160
10.4. Conclusions 163

CHAPTER 11. Pathology of the liver 165

11.1. Methods 165
11.1.1. Sampling of liver specimens 165
11.1.2. Processing of liver specimens 166
11.2. Results 166
11.2.1. Methods of sampling 166
11.2.2. Microscopical findings 167
11.3. Discussion 177
11.4. Conclusions 181

CHAPTER 12. General discussion and conclusions 185

SUMMARY 191

SAMENVATTING 194

ACKNOWLEDGMENTS 197

CURRICULUM VITAE 201

CHAPTER 1. RATIONALE AND AIM OF THE STUDY

At present, end-stage hepatic disease can only be cured by orthotopic liver transplantation. As a pediatric surgeon the writer of this thesis was interested in the problems that hamper the development of pediatric orthotopic liver transplantation and particularly in the problem of the shortage of appropriately sized donor livers.

Matching of the size of the donor liver with the size of the recipient liver has been a general principle in clinical orthotopic liver transplantation,[1-6] and insurmountable problems have been encountered when livers that were too large were transplanted.[7,8] Matching for size is a special problem in pediatric orthotopic liver transplantation. The weight of the liver of the full-term infant is doubled at 2 years and tripled at 3 years; at 5 years it has increased 6 times, and the liver of an adult, weighing approximately 1500 gr, is 12 to 13 times as large as that of the newborn.[9] Because of these age-related differences in the size of the liver, age-matched donor livers are required in pediatric liver transplantation. Pediatric donor livers, however, constitute only a small part of the total donor pool.[10] Moreover, adult candidates for liver transplantation compete for these livers,[4,11] which aggravates the problem of the shortage of donor livers in pediatric liver transplantation. This competition will increase further when heterotopic auxiliary transplantation becomes a truly therapeutic procedure. The group of pediatric liver transplantation candidates younger than 6 years of age is the most severely affected by the

shortage of age-related donor livers.[6]

The use of livers from anencephalic newborns in these children is theoretically attractive,[2,4] and there is one report of a case in which such a liver was used in an adult recipient.[7] However, this potentially large pool of donor livers - the incidence of anencephaly being 1 in 1,000 births[12] - is likely to decrease as a result of increasing prenatal diagnosis of the condition and the associated increasing abortion rate of these fetuses. Moreover, it is not certain whether successful transplantation of such livers is possible.

An alternative solution for the problem of the discriminatory role of the size of the donor liver in recipient selection for orthotopic liver transplantation, would be the use of only part of a donor liver. The left lateral segment of an adult human liver, which accounts for about 25% of the total liver weight,[9] could be used for this purpose. Survival after removal of all but that segment of the liver has proven to be possible in patients requiring hepatic tumor surgery.[13]

The experimental study reported here was performed to assess in dogs the feasibility of replacing the native liver by part of a donor liver.

REFERENCES

1. Starzl TE, Putnam CW: Experience in Hepatic Transplantation. Philadelphia, Saunders, 1969, pp 133-134
2. Calne RY: Surgical aspects of clinical liver transplantation in 14 cases. Br J Surg 56:729-736, 1969
3. Fortner JG, Howland WS, Beattie EJ: The donor in human liver transplantation. Surg Gynecol Obstet 130:988-994, 1970

4. Marshal VC: Selection of donors for liver transplantation, in Calne RY (ed): Clinical Organ Transplantation. Oxford, Blackwell, 1971, pp 347-349
5. Weil III R, Putnam CW, Porter KA, et al: Transplantation in children. Surg Clin North Am 56:467-476, 1976
6. Starzl TE, Iwatsuki S, Van Thiel DH, et al: Evolution of liver transplantation. Hepatology 2:614-636, 1982
7. Starzl TE, Putnam CW: Experience in Hepatic Transplantation. Philadelphia, Saunders, 1969, pp 502-504
8. Starzl TE, Koep LJ, Halgrimson CG, et al: Fifteen years of clinical liver transplantation. Gastroenterology 77:375-388, 1979
9. Vaughan VC, MacKay RJ, Behrman RE: Liver and bile ducts, in Vaughan VC, MacKay RJ, Behrman RE, et al (eds): Nelson's Textbook of Pediatrics. Philadelphia, Saunders, 1979, pp 1109-1110
10. Fox PS, Failla JP, Kauffman HM, et al: The cadaver donor. Logistics of supply and demand in an urban population. JAMA 222:162-167, 1972
11. Calne RY: Transplantation of the liver. Ann Surg 188:129-138, 1978
12. Warkany JW: Congenital Malformations. Chicago, Year Book Medical Publishers, 1971, pp 189-190
13. Starzl TE, Bell RH, Beart AW, et al: Hepatic trisegmentectomy and other liver resections. Surg Gynecol Obstet 141:429-437, 1975

CHAPTERS 2 - 5. REVIEW OF THE LITERATURE ON LIVER TRANSPLANTATION

CHAPTER 2. THE NEED FOR LIVER TRANSPLANTATION

2.1. Treatment of terminal liver failure

The liver has so many different functions, for example synthesis, storage, homeostasis, and detoxification, that it is highly unlikely that an effective artificial liver will become available during the next few decades.[1] Even the results of short-term support of the acutely failing liver provided by various methods such as exchange transfusion, plasmaphoresis, total body washout, cross circulation, extracorporeal liver perfusion, exchange resin or activated charcoal hemoperfusion, and hemodialysis, have been disappointing.[2-4]

Although liver transplantation is not generally accepted as a form of short-term support in cases of acute liver failure, because of the usually uncertain prognosis, the poor surgical risk, the possibility of transmission of the original disease to the graft, and the possible interference with the regeneration of the native liver tissue,[4-6] this method has been the most successful in prolonging survival or allowing recovery from experimentally induced acute liver failure of various origins.[7-11]

In chronic end-stage hepatic disease, the only chance of cure and long-term survival is provided by liver transplantation.

REFERENCES

1. Hamburger J: The future of transplantation. Transplant Proc 13:10-12, 1981
2. Sherlock S: Diseases of the Liver and Biliary System. Oxford, Blackwell, 1975, pp 107-121
3. Berk PD, Martin JF, Scharschmidt BF, et al: Current status of artificial hepatic support systems, in Popper H, Schaffner F (eds): Progress in Liver Disease. New York, Grune and Straton, 1976, pp 398-417
4. Saunders SJ, Seggie J, Kirsch RE, et al: Acute liver failure, in Wright R, Alberti KGMM, Karran S, et al (eds): Liver and Biliary Disease. London, Saunders, 1979, pp 569-584
5. Calne RY: Liver transplantation, in Wright R, Alberti KGMM, Karran S, et al (eds): Liver and Biliary Disease. London, Saunders, 1979, pp 1180-1190
6. Starzl TE, Putnam CW: Experience in Hepatic Transplantation. Philadelphia, Saunders, 1969, pp 11-12
7. Chandrasakeram AK, Beadoin JG, Slapak M: The effect of auxiliary liver transplantation on posthepatectomy coma in the dog. Arch Surg 103:728-734, 1971
8. Kuster GGR, Woods JE: Auxiliary liver transplantation in the dog as temporary support in acute fulminating hepatic necrosis. Ann Surg 176:732-735, 1971
9. Diaz A, Ricco JB, Franco D, et al: Temporary liver transplantation in acute liver failure. Arch Surg 112:74-78, 1977
10. Sutherland DER, Numata M, Matas AJ, et al: Hepatocellular transplantation in acute liver failure. Surgery 82:124-132, 1977

11. Terpstra OT, Reuvers CB, Kooy PPM, et al: Auxiliary transplantation of a partial liver graft in the dog and the pig. Neth J Surg 35:188-191, 1983

2.2. The need for pediatric liver transplantation in The Netherlands

THE INCIDENCE AND FATALITY OF BILIARY ATRESIA IN THE NETHERLANDS
-An Indicator for the Need for Pediatric Liver Transplantation-

N.M.A. Bax and J.C. Molenaar.
From the Department of Pediatric Surgery, University Children's Hospital, Rotterdam, The Netherlands.
Published in J Jpn Soc Pediatr Surg 8:1023-1027, 1982.
Reproduced by permission.

Summary

Two hundred of the 310 children who died in The Netherlands over a 10-year period due to a liver or biliary-tract condition could have been potential candidates for liver transplantation. Seventy-five percent of these candidates had biliary atresia, the incidence of which is estimated to be 0.93 per 10,000 live births in this country. At the current annual birth rate of 180,000, 16 to 17 new cases may be expected each year and 13 to 14 of these patients will die if the results of treatment remain unchanged. Even if the high Japanese standard of treatment can be reached, a considerable number of patients with biliary atresia will progress to end-stage hepatic disease. The results of liver transplantation achieved in these patients have been poor, which underlines the need for further experimental work.

Introduction

This retrospective study was undertaken to obtain a rough idea of the number of pediatric patients in The Netherlands annually who could be considered candidates for liver transplantation.

Results

Mortality due to liver and biliary-tract conditions in The Netherlands and potential candidacy for liver transplantation.

According to the Netherlands Central Bureau for Statistics, at least 310 children younger than 15 years of age died in The Netherlands between 1969 and 1978 as the result of a liver or biliary-tract condition (Table 1).

Table 1. Mortality due to liver and biliary-tract conditions in three pediatric age groups in The Netherlands in the period 1969 - 1978. (Netherlands Central Bureau for Statistics).

		*	<1 year n	1-3 years n	4-14 years n	total n	%
1	injury	(864)	0	10	22	32	10.32
2	primary tumors	(155.0; 155.1) (211.5; 230.5)	8	12	8	28	9.03
3	infectious hepatitis	(070)	3	5	3	11	3.53
4	acute and subacute necrosis	(570)	3	2	2	7	2.25
5	cirrhosis	(571)	18	13	17	48	15.48
6	congenital anomalies	(751.6)	99	46	10	155	50.00
7	others	(573; 576)	18	6	5	29	9.35
	total		149	94	67	310	100.00

* = international classification of diseases[14].

It is unlikely that the children whose death was due to an injury would have been potential candidates for liver transplantation, because in these patients bleeding, and less frequently biliary peritonitis, are the main causes of death rather than liver failure.[1-3]

Liver transplantation for primary hepatic malignancy not treatable by partial hepatectomy, has not been very successful. Calne reported a 60% recurrence rate in patients grafted for primary hepatoma, and Starzl found an 89% recurrence rate in patients who survived beyond 3 months.[4,5] The limited experience with liver transplantation for primary malignancy of the liver in children has not shown better results. All 3 children with a hepatoma who were transplanted by Starzl died, one after 143 days due to massive metastases and two after a little more than a year due to tumor recurrence.[6]

In spite of the high fatality rate associated with fulminant hepatic failure, this condition is generally not accepted as an indication for liver transplantation.[7,8] Auxiliary liver transplantation has, however, had the most success in prolonging survival or allowing recovery in experimentally induced acute liver failure.[9-11] Clinical experience with liver transplantation in acute liver failure has been too limited to provide a basis for definite conclusions, and further experimental work is badly needed. The major logistical problem posed by the need to find an appropriate cadaveric donor organ within the very brief time span in which such patients are legitimate candidates for liver transplantation is not likely to be solved in the foreseeable future.[12]

Chronic end-stage hepatic disease is considered to be the main indication for liver transplantation.[13] Roughly two-thirds of the children who died in The Netherlands in the period 1969-1978 due to a liver or biliary-tract condition had chronic end-stage hepatic disease and could have been potential candidates for liver transplantation.

Seventy-five percent of these patients had a congenital anomaly of the hepato-biliary system. Because most of the congenital anomalies listed under Nr. 751.6 of the International Classification of Diseases[14] are compatible with long-term survival, the children who died were in all probability suffering from biliary atresia. Obviously, the incidence and fatality rate of this condition in The Netherlands will to a great extent determine the yearly number of pediatric patients who might be considered candidates for liver transplantation in any given year.

The fatality rate of biliary atresia in the University Children's Hospital in Rotterdam, The Netherlands.

To obtain an idea of the fatality rate associated with biliary atresia in The Netherlands, the results of treatment in one leading center for pediatric surgery were analysed. Thirty- four children with biliary atresia were treated in the period 1970-1979 in the University Children's Hospital in Rotterdam. Cases of what is called hypoplasia of the intrahepatic biliary system were excluded.

Eleven patients underwent an exploratory laparotomy only, and all these patients died. Of the remaining 23 patients in whom a bilio-digestive anastomosis was established, six survived, the follow-up period ranging from 4 to 12 years, and 5 of these patients have remained free of jaundice. Therefore,the over-all fatality rate amounted to 82%.

The incidence of biliary atresia in The Netherlands.

On the assumption that the over-all results of treatment of biliary atresia were much the same throughout The Netherlands in the period 1970-1979 and did not differ substantially from the results obtained in

the period 1969-1978, the total number of new patients with biliary atresia in The Netherlands in the latter period can be estimated to have been 189; of these, 155 (82%) died and 34 (18%) survived.

In the same period, 2,012,600 children were born, and therefore the incidence of biliary atresia can be estimated to be 0.93 per 10,000 live births. At an actual annual birth rate of 180,000 in The Netherlands, 16 to 17 new cases per year may be expected and 13 to 14 of these children will die if the results of treatment are not improved.

Discussion

It has long been believed that biliary atresia is more common in Japan than in other countries and that this explains the greater attention given to the condition in that country.[15,16] This premise has never been substantiated [17] and the limited data available, including the present results, have not furnished any proof that the incidence is greater among the Japanese.[22-25]

Some improvement in the fatality rate associated with biliary atresia in The Netherlands can be expected now that Kasai's approach [26-29] has been generally accepted as the standard method of treatment. Moreover, it is slowly being recognized that the age at operation is an extremely important factor for the ultimate prognosis. Most important perhaps is the growing awareness that the Japanese treatment results are far better, and this is a challenge to improve the results elsewere. Sawaguchi reported a one-year survival rate of 48% and a five-year survival rate of 24% in a series of 117 patients treated at the National Children's Hospital in Tokyo in the period 1967-1973. Three other patients died 5, 7, and 10 years postoperatively.[21,30] Of the 55 patients treated by Kasai at the Tohoku University Hospital in Sendai in the

period 1971-1976, 89% had postoperative bile flow but only 49% were alive and free of jaundice in 1977.[18,29] Better results are obtained in the group of children operated on before the 60th day of life, especially in the more recent series, in terms of not only postoperative bile excretion but also the achievement of a jaundice-free state. In these children cholangitis is much less of a problem and the 5 year jaundice-free survival seems likely to improve.[19- 21,29]

In spite of major advances in the treatment of biliary atresia a considerable number of patients still die of end-stage hepatic disease, even in Japan. Theoretically, liver transplantation after an unsuccessful Kasai procedure remains an attractive approach. In patients with end-stage hepatic disease based on biliary atresia, Starzl favors removal of the native liver not only because orthotopic nonauxiliary grafting has been more successful than heterotopic auxiliary grafting but also in view of the high incidence of incidental malignancies present in the native liver.[6] Furthermore, in view of the high incidence of recurrent cholangitis after an unsuccessful Kasai procedure, the native liver should be considered an infected organ and should be removed.

However, the results of orthotopic liver transplantation in children with biliary atresia are poor. Of the 48 children transplanted by Starzl between March 1963 and January 1978, only 33% survived for one year and only 17% were alive at the time of publication. The bilio-digestive anastomosis in these children has been a major problem.[6] The absence of a normal extrahepatic biliary tract precludes a choledocho-choledochostomy and this may be one of the reasons why children transplanted for other reasons did distinctly better.

Further experimental work is badly needed in order to make liver transplantation in children with end-stage hepatic disease a more acceptable procedure.

REFERENCES

1. Schärli AF: Das Bauchtrauma, in Von Rehn J (ed): Unfall- Verletzungen bei Kindern. Berlin, Springer Verlag, 1971, pp 187-192
2. Touloukian RJ: Pediatric Trauma. New York, Wiley, 1978, pp 407-415
3. Welch KJ: Abdominal injuries, in Randolph JG, Ravitch MM, Welch KJ, et al (eds): The Injured Child. Chicago, Year Book Medical Publishers, 1979, pp 174-189
4. Calne RY: Transplant surgery: current status. Br J Surg 67:765-771, 1980
5. Starzl TE, Koep LJ, Halgrimson CG, et al: Fifteen years of clinical liver transplantation. Gastroenterology 77:375-388, 1979
6. Starzl TE, Koep LJ, Schröter GPJ, et al: Liver replacement for pediatric patients. Pediatrics 63:825-829, 1979
7. Saunders JC, Seggie J, Kirsch RE, et al: Acute liver failure, in Wright R, Alberti KGMM, Karran S, et al (eds): Liver and Biliary Disease. London, Saunders, 1979, pp 569-584
8. Calne RY: Liver transplantation, in Wright R, Alberti KGMM, Karran S, et al (eds): Liver and Biliary Disease. London, Saunders, 1979, pp 1180-1190
9. Sutherland DER, Numata M,Matas AJ, et al: Hepatocellular transplantation in acute liver failure. Surgery 82:124-132,1977
10. Kuster GGR, Woods JE: Auxiliary liver transplantation in the dog as temporary support in acute fulminating hepatic necrosis. Ann Surg 176:732-735, 1971

11. Diaz A, Ricco JB, Franco D, et al: Temporary liver transplantation in acute liver failure. Arch Surg 112:74-78, 1977
12. Putnam CW, Starzl TE: Transplantation of the liver. Surg Clin North Am 57:361-373, 1977
13. Terblanche J, Koep LJ, Starzl TE: Liver transplantation. Med Clin North Am 63:507-521, 1979
14. International Classification of Diseases. Manual of the International Statistical Classification of Diseases. Geneva, World Health Organization, 1:1967
15. Bill AH: Biliary atresia and choledochal cyst. Introduction. Prog Pediatr Surg 6:1-3, 1974
16. Bill AH: Biliary atresia and choledochal cyst. Editor's note. Prog Pediatr Surg 6:62, 1974
17. Hays DM, Kimura K: Biliary Atresia. The Japanese Experience. Cambridge, Harvard University Press, 1980, pp 27-31
18. Hays DM, Kimura K: Biliary Atresia. The Japanese Experience. Cambridge, Harvard University Press, 1980, pp 94
19. Hays DM, Kimura K: Biliary Atresia. The Japanese Experience. Cambridge, Harvard University Press, 1980, pp 106
20. Hays DM, Kimura K: Biliary Atresia. The Japanase Experience. Cambridge, Harvard University Press, 1980, pp 107-126
21. Hays DM, Kimura K: Biliary Atresia. The Japanese Experience. Cambridge, Harvard University Press, 1980, pp 148
22. Shim KT, Kasai M, Spence MA: Racial influence on the incidence of biliary atresia. Prog Pediatr Surg 6:53-62, 1974
23. Shim KT, Kasai M, Spence MA: Race and biliary atresia, in Kasai M, Shiraki K (eds): Cholestasis in Infancy. Tokyo, University of Tokyo Press, 1980, pp 5-10

24. Psacharopoulos HT, Mowat RP, Williams R: Epidemiological study of hepatitis syndrome in infancy in Southeast England: incidence and early course, in Kasai M, Shiraki K (eds):Cholestasis in Infancy.Tokyo, University of Tokyo Press, 1980, pp 11-17
25. Suzuki H, Katsushima N: Incidence of biliary atresia in Sendai, in Kasai M, Shiraki K (eds): Cholestasis in Infancy. Tokyo, University of Tokyo Press, 1980, pp 19-23
26. Kasai M, Suzuki S: A new operation for non-correctable biliary atresia: hepatic porto-enterostomy. Shujutru 13:733-739, 1959
27. Kasai M, Kimura S, Asakura Y, et al: Surgical treatment of biliary atresia. J Pediatr Surg 3:665-675, 1968
28. Kasai M: Treatment of biliary atresia with special reference to hepatic porto-enterostomy and its modifications. Prog Pediatr Surg 6:5-52, 1974
29. Kasai M; Hepatic porto-enterostomy and its modifications, in Kasai M, Shiraki K (eds): Cholestasis in Infancy. Tokyo, University of Tokyo Press, 1980, pp 337-344
30. Sawaguchi S, Akiyama H, Nakajo T: Long-term follow-up after radical operation for biliary atresia, in Kasai M, Shiraki K (eds): Cholestasis in Infancy. Tokyo, University of Tokyo Press, 1980, pp 371-379

CHAPTER 3. MODES OF LIVER TRANSPLANTATION

3.1. Classification and nomenclature

Livers or liver fragments have been transplanted in many different ways, which can be classified according to:

a. The vascularization of the graft

In free grafting no anastomoses are performed.[1] A special form of free grafting is hepatocellular transplantation, in which suspensions of liver cells are injected into the bloodstream, the peritoneal cavity, or a solid organ.[2-7]

In collaterally vascularized grafts, the autochthonous blood supply is severed after a collateral circulation from adjacent organs has been established.[8-11] Arterialization of hepatic segments to be autotransplanted has also been performed by direct vascular implantation.[12]

In classic liver transplantation, the graft is vascularized by direct vascular anastomoses.

b. The origin of the graft

In autotransplantation the graft is taken from and reintroduced into the same individual. An allo- or homograft is a graft taken from a different individual of the same species. A xenograft is a graft taken from a different species.

c. The functional destination of the graft

Depending on whether the native liver has been removed or left in situ, the graft will have to function in a nonauxiliary or an auxiliary way.

d. The anatomical destination of the graft

In orthotopic transplantation the graft is placed in an anatomical position which usually but not necessarily requires total removal of the native liver.[13-16] In heterotopic transplantation the graft is placed in a nonanatomical position.

3.2. Discussion

Only a few modes of liver transplantation have survived in experimental practice, and even fewer have reached the stage of clinical application.

3.2.1. Vascular supply and biliary drainage of the graft

Experience showed that a liver graft, even a small one, needs a direct anastomotic blood supply as well as biliary drainage, and this situation has hampered the use of small liver fragments for transplantation. In hepatocellular transplantation, no anastomoses have to be performed, but the biliary drainage system of the native liver must be intact. Promising results have been obtained experimentally with this mode of liver transplantation, but its value for the treatment of acute liver failure and hepatic enzyme defects in man remains to be proven.

3.2.2. The origin of the graft

Autotransplantation is of interest for the study of the fate of a graft in the absence of rejection. The procedure is, however, a difficult one, because of the relative shortness of the vascular pedicles. Moreover, extensive manipulation of the liver is hard to avoid and liver damage is likely to occur. It is therefore hardly surprising that the experimental results have been

poor.[17-22] This mode of transplantation is unsuitable for the study of techniques for long-term preservation of the liver, at least when the whole liver is to be autotransplanted, because the duration of the anhepatic phase and of the period during which the organ must be preserved is of course the same.[17] The use of xenografts has been abandoned because of the severe and uncontrolable immunological reactions.[23-25] For clinical liver transplantation only allografts are used at present.

3.2.3. Heterotopic auxiliary transplantation

This mode of liver transplantation is theoretically very attractive. Since the native liver does not have to be removed, the surgical procedure is much less hazardous and the graft can be removed if it fails or the native liver recovers. Considerable attention has been paid to this mode of liver transplantation, but the results have been much less satisfactory than those obtained with orthotopic liver transplantation, both experimentally and clinically.[27-30]

3.2.3.1. The problem of graft atrophy

According to the original technique developed by Welch and Goodrich, the donor suprahepatic caval vein was anastomosed end-to-end with the proximal part of the transected recipient infrarenal caval vein and the donor portal vein end-to-end with the inferior part of the transected recipient infrarenal caval vein. The donor infrahepatic caval vein was closed. The graft was also supplied with arterial blood and biliary drainage.[31,32]

After the introduction of immunosuppressive drugs early in the Sixties, some dogs in which Starzl et al. used this technique survived for at least a few weeks. At autopsy, however, the grafts showed marked atrophy and a greater degree of cellular invasion and hepatocyte loss

than was seen in orthotopic grafts.[33] This observation, confirmed by others,[34,35] gave a rise to an extensive discussion concerning the etiology of the atrophy, and most of the hypotheses put forward at that time, on such points as dependency of the graft on portal blood and functional competition between the native liver and the graft, are still topical.

3.2.3.2. Modifications of the venous anastomoses

The caval anastomosis

Sicular et al. introduced the end-to-side anastomosis between the donor suprahepatic and the recipient infrahepatic caval vein.[36] Bengoechea-Gonzalez et al. used the donor infrahepatic caval vein for this anastomosis.[37] In one series of experiments, Marchioro et al. interposed the donor retro-hepatic caval vein between the transected ends of the recipient infrarenal caval vein.[38] Transplanting part of the donor liver heterotopically, van der Heyde et al. resected most of the donor retrohepatic caval vein, leaving only a patch for the subsequent end-to-side anastomosis.[39] Although in the rat the donor infrahepatic caval vein has been considered superior to the donor suprahepatic caval vein for the end-to-side anastomosis,[40,41] the latter vessel seems preferable in larger animals.[42] At least in dogs, the hepatic veins enter the caval vein in a cranial direction at a narrow angle, and this situation makes the use of the donor infrahepatic caval vein for the end-to-side anastomosis with the recipient infrahepatic caval vein hemodynamically unfavorable. Le Compt et al. and Jerusalem et al. have underscored the importance of having a venous outflow of the donor liver as close to the heart as possible, and referred in this connection to the work of Moreno et al.[43-45]

The portal anastomosis: quantity versus quality of the venous supply.

Much experimental work on the portal vein anastomosis has been published. Hagihara et al. removed the spleen and established an end-to-end anastomosis between the donor portal and recipient splenic veins.[46] Mehrez et al. did the same, but also constricted the recipient portal vein to promote portal flow through the graft.[47] In an impressive series of experiments, Marchioro et al. showed that graft atrophy could be prevented by a complete (and not by an incomplete) diversion of the nonhepatic splanchnic venous blood flow through the graft.[38] Halgrimson et al. showed that atrophy of a graft provided with systemic venous blood through the portal vein, could be partially prevented by a portocaval shunt.[48] In split transposition experiments in which one part of the liver received all of the nonhepatic splanchnic venous blood and the other part received systemic venous blood, Marchioro et al. found atrophy of the part receiving systemic venous blood even though the flow to both parts had been equal.[49] In a classic Welch-Goodrich model, Daloze et al. studied the venous flow through the graft and through the native liver and found no difference.[50] In experiments in which the native liver received systemic venous blood and the graft nonhepatic splanchnic venous blood, atrophy was still more pronounced in the graft.[51]

The discussion on the relative importance of the flow and the quality of the venous blood continued, and liver transplantation experiments in which the donor portal vein was arterialized tended to support the flow theory.[52] Splanchnic flow diversion experiments in dogs led Starzl et al. to conclude that liver lobes receiving blood from the pancreatic, gastroduodenal, and splenic veins did better than the liver lobes receiving blood from the bowel, and that the hepatotrophic factors are pancreatic in origin.[53] However, Child et al. and later

Malt stated that direct perfusion of the liver with portal blood is not essential for hepatic regeneration.[54,55] Hess et al. concluded from transplantation experiments in rats that the survival of the graft is determined by the total liver flow in combination with the functional state of the liver rather than by the quality of venous blood.[56]

3.2.3.3. The concept of functional competition

It has long been recognized that ligation of one of the hepatic bile ducts causes atrophy of the corresponding liver lobe and hypertrophy of the other lobes.[57] The theory concerning functional competition was put forward by Schalm et al. in 1956,[58] and was extended to the field of auxiliary liver transplantation in 1966.[59] As early as 1965, Thomford et al. reported that removal of the native liver had a protective effect on the heterotopic liver.[34] Many experiments followed in which the native liver was handicapped by portal blood diversion and bile-duct ligation.[39,60-65] The results were, however, difficult to evaluate in view of the differences between the models, the unpredictable effect of rejection, and the inability to achieve consistent long-term survival.

3.2.3.4. Intrinsic hepatic growth control factors

The factors that regulate the size of a normal liver are still poorly understood. If there really is a growth inhibiting substance in nonregenerating liver tissue, as has been suggested, it is not surprising that the auxiliary liver cannot maintain its size because reparative regeneration of the damage in the graft due to ischemia and rejection is inhibited by the native liver. However, the presence of such an intrahepatic inhibitory substance remains controversial.[66]

In contrast there is increasing evidence that regenerating liver tissue contains a factor that augments

a regenerative response. It has been shown in dogs that intraportal injection of a cytosol extract from regenerating liver reverses the atrophy caused by a complete portocaval shunt[67] and promotes regeneration after partial hepatectomy.[66] The factor involved here was neither glucagon nor insulin.[66,67] No growth-promoting effect was seen when these extracts were injected into the systemic venous circulation or the peritoneal cavity.[66] Whether such extracts are effective when infused into the portal vein of nonregenerating livers, is still uncertain.[66]

The existence of a growth-inhibiting substance in normal nonregenerating liver tissue and of a growth-stimulating substance in regenerating liver tissue could explain why the normal native liver has to be handicapped to prevent atrophy of the auxiliary liver.

3.2.4. Orthotopic nonauxiliary transplantation

Orthotopic liver transplantation is the only mode for which large-scale clinical trials have been performed. Since the first efforts to replace a human liver in 1963,[68] more than 350 orthotopic transplantations have been carried out by the two leading teams in the world. The over-all results, although better than the results achieved with other modes of liver transplantation, have been poor until rather recently.[69] Smaller series of clinical orthotopic liver transplantations have been reported by other teams. Of particular interest in The Netherlands is the clinical liver transplantation program started by the Groningen University Hospital in 1979. By the end of 1983, Krom and his team had transplanted 32 patients.[70]

The results obtained in clinical orthotopic liver transplantation are presented in Chapter 5.

3.3. Comments

Three modes of liver transplantation continue to be of clinical interest. The hepatocellular mode, however, requires an intact biliary drainage system and is therefore unsuitable for patients with biliary atresia. The heterotopic auxiliary mode has not lost its theoretical attraction, but the results have been poor, both experimentally and clinically. Moreover, even if the problem of graft atrophy were solved, the logistics of a supply of appropriately sized donor organs would hamper clinical application. The experimental study reported in this thesis can therefore be seen as a first step toward the development of a heterotopic auxiliary model making use of partial livers. After this experimental study, liver transplantation research was continued in the same center and a heterotopic auxiliary model using partial livers was designed, and gave promising results.[71] The results of these experiments indicate that graft atrophy can be prevented and that long-term graft survival can be obtained by avoiding and controlling rejection.

At present, only the orthotopic nonauxiliary mode has proven to have unequivocal value in human medicine. Furthermore, as pointed out in section 2.2, it seems wise to remove the native liver in children with biliary atresia at the time of transplantation, because once a hepatic porto-enterostomy has been performed the native liver should be considered a potentially infected organ, and also because incidental carcinomas have been found in the native livers of patients with biliary atresia. In addition, removal of the native liver creates space for the insertion of the graft.

REFERENCES

1. Cameron GR, Oakley CL: Transplantation of the liver. J Pathol 38:17-28, 1934
2. Matas AJ, Sutherland DER, Steffes MW, et al: Hepatocellular transplantation for metabolic deficiencies: decrease of plasma bilirubin in Gunn rats. Science 192:892-894, 1976
3. Groth GC, Arborgh B, Bjorken C, et al: Correction of hyperbilirubinemia in glucuronyl transferase deficient rat by intraportal hepatocyte transplantation. Transplant Proc 9:313-316, 1977
4. Matas AJ, Desnick RJ, Najarian JS, et al: Clinical and experimental transplantation in enzymatic deficiency disease. Surg Gynecol Obstet 146:975-982, 1978
5. Sutherland DER, Numata M, Matas AJ, et al: Hepatocellular transplantation in acute liver failure. Surgery 82:124-132, 1977
6. Kusano M, Ebata H, Onishi T, et al; Transplantation of cryopreserved isolated hypatocytes into rat spleen. Transplant Proc 13:848-854, 1981
7. Makowka L, Rotstein LE, Falk RE, et al: Allogeneic and xenogeneic hepatocyte transplantation. Transplant Proc 13:855- 859, 1981
8. Seneviratne RD: Transplantation of a lobe of the liver in the rat. J Pathol 70:271-283, 1955
9. Benichoux R, Rauber G, Marchal C, et al: L'épiploo et la gastro-hépatoplastie dans la révascularisation du foie. Lyon Chir 58:94-104, 1962
10. Grisham JW, Leong GF, Hole BV: Heterotopic partial autotransplantation of rat liver: technic and demonstration of structure and function of the graft. Cancer Res 24:1475-1495, 1964

11. Sigel B, Acevedo FJ: Methods and results of partial liver autotransplantation. Surg Gynecol Obstet 114:731-740, 1962
12. Goldsmidt HS, Chen WF, Alday ES, et al: Autotransplantation of hepatic segments arterialized by direct vascular implantation. J Surg Res 17:411-416, 1975
13. Smith B, Antunes C, Degrefte GJM, et al: Partial orthotopic liver transplantation. Excerpta Medica International Congress Series 152:81, 1967
14. Smith B: Segmental liver transplantation from a living donor. J Pediatr Surg 4:126-132, 1969
15. Mizumoto R, Yokota T, Ohsawa J, et al: Orthotopic partial hepatic transplantation in dog. Jpn J Surg 4:121-129, 1974
16. Mizumoto R, Yokota T, Ohsawa J, et al: Survival of both donor and recipient in partial hepatic transplantation of dog. Jpn J Surg 4:130-136, 1974
17. Kestens PJ, McDermott WV: Perfusion and replacement of the canine liver. Surgery 50:196-206, 1961
18. Alican F, Hardy JD: Replantation of the liver in dogs. J Surg Res 7:368-375, 1967
19. Stuart FP, Torres E, Hester WJ, et al: Orthotopic autotransplantation and allotransplantation of the liver: functional and structural patterns in the dog. Ann Surg 165:325- 340, 1967
20. Aoki H, Wang TC, Matsumura H, et al: Partial liver transplantation with revascularization in dogs. Preliminary report on new method and its theoritical background. Keio J Med 16:205-221, 1967
21. Maki T, Blackburn GL, Slapak M, et al: A life supporting heterotopic autotransplant of the canine liver without portal blood. Surg Gynecol Obstet 136:951-957, 1973

22. Maki T, Blackburn GL, McDermott WV: Autotransplantation of canine liver preserved by asanguineous intermittent perfusion method. J Surg Res 16:604-610, 1974
23. Starzl TE, Putnam CW: Experience in Hepatic Transplantation. Philadelphia, Saunders, 1969, pp 408-421
24. Calne RY, Davis DR, Pena JR, et al: Hepatic allografts and xenografts in primates. Lancet 1:103-106, 1970
25. Wegmann A, Leger L, Monsallier F, et al: Histochimie d'une greffe hétérotopique d'un foie de babouin sur une patiente atteinte d'hépatite fulminante. Presse Med 78:403- 408, 1970
26. Terblanche J, Dent DM, Uys CJ, et al: Baboon into pig liver and kidney xenotransplantation. S Afr Med J 44:919-923, 1970
27. Calne RY: The present status of liver transplantation. Transplant Proc 9:209-216, 1977
28. Starzl TE, Putnam CW: Experience in Hepatic Transplantation. Philadelphia, Saunders, 1969, pp 475-515
29. Fortner JG,Kim DK, Shiu MH, et al: Heterotopic (auxiliary) liver transplantation in man. Transplant Proc 9:217-221, 1977
30. De Jonge MWC: Biliodigestieve Anastomose. Auxiliaire Levertransplantatie. Leiden. Doctoral Thesis, 1983, pp 70-71
31. Welch CS: A note on transplantation of the whole liver in dogs. Transplant Bull 2:54-55, 1955
32. Goodrich EO, Welch HF, Nelson JA, et al: Homotransplantation of the canine liver. Surgery 39:244-251, 1956

33. Starzl TE, Marchioro TL, Rowlands DT, et al: Immunosuppression after experimental and clinical homotransplantation of the liver. Ann Surg 160:411-439, 1964
34. Thomford NR, Shorter RG, Hallenbeck GA: Homotransplantation of the canine liver. Arch Surg 90:527-538, 1965
35. Tretbar LL, Beven EG, Hermann RE: Homotransplantation of an auxiliary dog liver into the pelvis; effect of portocaval shunt in the prevention of graft atrophy. Surg Forum 16:219-221, 1965
36. Sicular A, Dreiling DA, Paronetto F, Kark AE: Studies of rejection of the homotransplanted canine liver. Surg Forum 14:202-204, 1963
37. Bengoechea-Gonzalez E, Awane Y, Reemtsma K: Experimental auxiliary liver homotransplantation. Arch Surg 94:1-7, 1967
38. Marchioro TL, Porter KA, Dickinson TC, et al: Physiological requirements for auxiliary liver homotransplantation. Surg Gynecol Obstet 121:17-31, 1965
39. Van der Heyde MN, Cooper JR, Carter JH, et al: Survival of hepatectomized dogs with partial liver transplants. Surgery 59:1079-1082, 1966
40. Hess F, Jerusalem C, Van der Heyde MN: Advantages of auxiliary homografting in rats. Arch Surg 104:76-80, 1972
41. Wolff ED: Liver Transplantation in the Rat. Rotterdam. Doctoral Thesis, 1976
42. Immelman EJ, Van Leenhof JW, Terblanche J, et al: The avoidance of donor atrophy in auxiliary liver allotransplantation in the pig. 7th Congress European Society Experimental Surgery, 1972, p 288

43. Le Compte Y, De Riberolles C, Grange D, et al: Canine intrathoracic hepatic homograft. Arch Surg 109:809-811, 1974
44. Jerusalem C, Van der Heyde MN, Smidt WJ, et al: Heterotopic liver transplantation. II. Unfavorable outflow conditions as a possible cause for late graft failure. Eur Surg Res 4:186-197, 1972
45. Moreno AH, Burchell AR, Rousselot LM, et al: Studies on outflow tracts of the liver. III. Qualitative observations in normal man and in patients with cirrhosis. Ann Surg 170:63-73, 1969
46. Hagihara P, Absolon KB: Experimental studies on homologous heterotopic liver transplantation. Surg Gynecol Obstet 119:1297- 1304, 1964
47. Mehrez IO, Nabseth DC, Kekis BP, et al: Homotransplantation of the canine liver: a new technic. Arch Surg 159:416-423, 1964
48. Halgrimson CG, Marchioro TL, Faris TD, et al: Auxiliary liver transplantation: effect of host portocaval shunt. Arch Surg 93:107-118, 1966
49. Marchioro TL, Porter KA, Brown BI, et al: The specific influence of nonhepatic splanchnic venous blood flow on the liver. Surg Forum 16:280-282, 1965
50. Daloze PM, Huguet C, Groth CG, et al: Blood flow in auxiliary canine liver homografts. J Surg Res 9:10-12, 1979
51. Daloze PM, Huguet C, Porter KA, et al: Auxiliary homotransplantation of the canine liver with the use of a reverse Welch technique. Surgery 64:934-939, 1968
52. Mito M, Ackroyd FW, Covelli VH, et al: Partial heterotopic liver homograft in dogs utilizing portal arterialization. Ann Surg 165:20-32, 1967

53. Starzl TE, Francavilla A, Halgrimson CG, et al: The origin, hormonal nature, and action of hepatotrophic substances in portal venous blood. Surg Gynecol Obstet 137:179-199, 1973
54. Child III CG, Barr D, Holswade GR, et al: Liver regeneration following portocaval transposition in dogs. Ann Surg 138:600-608, 1953
55. Malt RA: Editorial comment. Arch Surg 109:811, 1974
56. Hess F, Willemen A, Jerusalem C: Survival of auxiliary rat liver grafts with decreased portal blood flow. Eur Surg Res 10:444-455, 1978
57. McMaster PD, Rous P: The biliary obstruction required to produce jaundice. J Exp Med 33:731-750, 1921
58. Schalm L, Bax HR, Mansens BJ: Atrophy of the liver after occlusion of bile ducts or portal vein and compensatory hypertrophy of the unoccluded portion and its clinical importance. Gastroenterology 31:131-155,1956
59. Schalm L: Heterotopic auxiliary liver transplantation. I. A physiological concept of functional competition. Arch Chir Neerl 18:283-284, 1966
60. Gliedman ML, Pangan J, Minkowitz S, et al: Heterotopic liver transplantation after liver damage. Trans Am Soc Artif Intern Organs 11:205-210, 1965
61. Slapak M, Beaudoin JG, Lee HM, et al: Auxiliary liver homotransplantation. A new technique and evaluation of current techniques. Arch Surg 100:31-41, 1970
62. Corry RJ, Chavèz-Peòn F, Miyakuni T, et al: Auxiliary partial liver transplantation in the dog. Arch Surg 98:799-802, 1969

63. Beaudoin JG, Slapak M, Phillips MJ, et al: Function of auxiliary liver allografts. Surg Gynecol Obstet 130:622-635, 1970
64. Sheil AGR, Rogers JH, Halliday JP, et al: Auxiliary canine liver transplantation from cadaver donors. Arch Surg 100:290-294, 1970
65. Ushida H, Motsay GJ, Ruiz O, et al: Experimental auxiliary liver allotransplantation with special reference to biliary duct obstruction of host liver. Am J Surg 119:138-146, 1970
66. Terblanche J, Porter KA, Starzl TE, et al: Stimulation of hepatic regeneration after partial hepatectomy by infusion of a cytosol extract from regenerating dog liver. Surg Gynecol Obstet 151:538-544, 1980
67. Starzl TE, Terblanche J, Porter KA, et al: Growth-stimulating factor in regenerating canine liver. Lancet 1:127-130, 1979
68. Starzl TE, Marchioro TL, Von Kaulla KN, et al: Homotransplantation of the liver in humans. Surg Gynecol Obstet 117:659-676, 1963
69. Starzl TE, Iwatsuki S, Van Thiel DH, et al: Evolution of liver transplantation. Hepatology 2:614-636, 1982
70. Krom RAF: Personal communication. December 1983
71. Terpstra OT, Reuvers CB, Kooy PPM, et al: Auxiliary transplantation of a partial liver graft in the dog and the pig. Neth J Surg 35:188-191, 1983

CHAPTER 4. PROBLEMS ASSOCIATED WITH CLINICAL ORTHOTOPIC LIVER TRANSPLANTATION

4.1. The donor liver

4.1.1. Shortage of donor livers

Potential cadaveric liver donors outnumber by far the eligible recipients, and theoretically there should be no shortage.[1-3] The actual procurement of donor livers has, however, been a major problem.[2,3] Moreover, as pointed out in Chapter 1, the size matching between donor and recipient is obligatory and this means a greater demand for small donor livers. Because of the prevailing shortage of appropriately sized donor livers, both of the leading transplantation teams in the world have transplanted across a positive cytotoxic crossmatch.[4-6] One of these teams has even transplanted despite blood-group incompatibility.[5,6] For the same reason and because of the time constraints on liver preservation, tissue matching has never had a fair trial.[4-6] The use of tissue-matched livers would, however, require an enormous donor pool.[7]

4.1.2. The discriminatory role of size of the donor organ in recipient selection

This problem forms the main subject of the present thesis, and has been discussed in Chapter 1. In 1981, Starzl and his team used only 30 liver grafts among 176 available organs. The inability to use the other 146

livers was usually due to a disparity in size between the donor and recipient, or incompatibility of the donor and recipient blood groups, or saturation of the Pittsburgh facilities for liver recipients.[6]

4.1.3. Preservation of the donor liver

Organs deprived of circulating blood undergo damage. The degree of the damage depends mainly on the duration of the deprivation and on the temperature to which the organ is exposed during this period. Cooling of the donor liver alone cannot entirely prevent damage, and this has evoked an ongoing discussion as to the optimal composition of the preservation fluids to be used.

4.1.3.1. The role of temperature

Normothermic ischemia

The study of the maximal tolerable normothermic ischemic time of the liver has been hampered by the fact that acute occlusion of the portal vein in many species is poorly tolerated hemodynamically and has profound effects on the bowel.[8] Raffuci and Wangensteen circumvented these problems by a concomitant occlusion of the superior mesenteric artery during the occlusion of the arterial and portal venous supply to the liver.[9] Based on these experiments it was long believed that 20 minutes was the maximal tolerable normothermic ischemic time of the liver. However, although congestion in the splanchnic venous system in these experiments was avoided by the occlusion of the superior mesenteric artery, normothermic ischemia of the bowel occurred concomitantly. In later experiments in dogs and pigs in which the portal venous system was decompressed during total hepatic inflow occlusion by means of a portocaval shunt or an external bypass, the maximal tolerable normothermic hepatic ischemic time appeared to be much longer than 20 minutes.[10-12] Sixty minutes of

normothermic hepatic ischemia in dogs and 120 minutes in pigs is considered to be safe.[13-15] In man, one hour of normothermic hepatic ischemia seems to be compatible with life.[16] Studies of this kind should, however, be evaluated critically, because the temperature of the liver drops considerably during vascular exclusion and even moderate cooling, i.e., 24 to 31°C, is known to have a protective effect with respect to ischemic damage.[17,18] Moreover to assess the maximal tolerable normothermic hepatic ischemic time, the inflow occlusion has to be complete because an intact collateral circulation has shown to allow a longer tolerable period of hepatic artery and portal vein occlusion.[19]

The maximal tolerable period of normothermic ischemia alone is certainly insufficient for liver transplantation purposes in view of the lengthy duration of the procedure. Moreover, in clinical liver transplantation a large part of this period may be consumed even before the donor operation has been started, because hemodynamic instability is usually associated with the status of donorship. This situation underlines the importance of early optimal care of possible donor candidates.[3] During the donor hepatectomy the organ may sustain further damage due to manipulation. Various drugs have been used to counteract the reactive vasoconstriction during removal of donor organs,[20] but the prevention by gentle manipulation is undoubtedly more important. In dogs, manipulation of the donor liver has been held to be at least partially responsible for the high incidence of liver outflow block during liver transplantation.[21-24] This phenomenon is ascribed to spasm of the small intraparenchymal hepatic veins, which have an extraordinarily well developed muscular coat, at least in dogs.[21]

The evaluation of the extent of normothermic ischemic damage in the donor liver seems to remain a major problem, as indicated by one of Starzl's statements:

"Even recently we transplanted hopelessly damaged organs taken from apparently good donors".[5]

Cold ischemia

Hypothermia remains the cornerstone of organ preservation.[20] The initial cooling of the donor liver has been performed by organ surface cooling,[22] total body surface cooling,[21] total body extracorporeal circulation cooling,[25] transportal vein infusion cooling,[21] or combinations of these methods. The ideal preservation temperature is still not known, but is believed to be between 0 and 10°C.[20] Since the acceptance of heart-beating donors in organ transplantation, the initial cooling has been achieved by transportal vein infusion of a solution at 4°C. Washing out of blood seems to be important, but the problem of immersional storage in a solution at 0°C versus continuous perfusional storage with a solution at 4°C has not been completely solved.[20] Immersional storage is most commonly used because of its simplicity and reasonably good effectiveness allowing safe preservation for up to 12 hours.[4,5]

Ischemia during transplantation

During insertion, rewarming of the graft occurs from the surrounding recipient tissues and can cause further damage. This can be partially prevented by protecting the graft from direct contact with the surrounding recipient tissues, by surface cooling of the graft, and by limiting the time taken by the insertion of the graft.

4.1.3.2. The role of the composition of the preservation fluid

Various preservation fluids are in use, which emphasizes that the ideal composition is not known. Originally Starzl et al.[21] as well as Calne et al.[26] used lactated Ringer's solution for preservation of donor livers but a delay in revascularization of these livers for more than a few hours was not compatible with life.

The main reason for this limitation was thought to be the composition of the preservation fluid and the experiments of Schalm tended to support this hypothesis.[27] Later on, however, Schalm et al. found in canine and porcine liver transplantation experiments no difference between the results obtained with 4 different types of preservation fluid, used for preservation times up to 6 hours, and concluded that the composition of the preservation fluid is of minor importance for a preservation period that long.[28] Using 3 different types of fluid in dogs for preservation of the liver for 9 hours, Benichou et al. obtained consistent survival, irrespective as to the composition of the preservation fluid. After a preservation period of 18 hours consistent survival was not anymore achieved with any of the types of preservation fluid. However, a higher survival rate was obtained when, instead of lactated Ringer's solution, plasma or Collins' solution was used. Moreover Collins' solution appeared to be slightly superior than plasma in terms of survival and liver function. These authors concluded that the composition of the preservation fluid is not important for preservation times of 9 hours, but that the composition does matter with longer times.[29] The study in dogs of Monden and Fortner support this conclusion. Consistent survival after a 24 hour period of preservation of the liver was only achieved when prostacyclin was added to the modified Sacks' solution. Unfortunately these authors did not test the effect of the addition of prostacyclin to lactated Ringer's solution, which was used for 24 hour preservation of the liver in the first group of dogs.[30]

In clinical liver transplantation a safe preservation time of up to 12 hours is claimed by world's two leading teams. Starzl et al. use Collins' solution which approaches the composition of intracellular fluid.[5] In contrast Calne et al. use Hartmann's solution, followed by a plasma protein fraction solution with additions.[4]

The latter solutions approach the composition of extra cellular fluid. In human kidney transplantation the use of Euro-Collins solution gives a safe preservation period for up to 50 hours.[31]

The gradual prolongation over the years of the safe preservation time of the liver, using basically the same types of preservation fluid, must be related to a more careful avoidance of normothermic hepatic ischemia and to better cooling of the organ. The composition of the preservation fluid for longer preservation times than are actually used may, however, prove to be critical.

4.2. The preoperative condition of the recipient

Because of the nature of the diseases for which liver transplantation is required and due to the lack of effective methods for preoperative correction of the effects of a failing liver, most recipients have been poor surgical risks. Moreover a number of patients, e.g. with biliary atresia, may have undergone extensive abdominal surgery before liver transplantation. Even in the absence of prior abdominal surgery, severe bleeding peroperatively and infections postoperatively are major problems when the procedure is carried out too late in the course of the disease, and patients with chronic liver failure in a moribund state should not be transplanted.[4,32-34] In view of the moderate results of clinical liver transplantation, the procedure should not be carried out too early either. Close monitoring of the potential candidates seems to make the risk of transplanting too early minimal.[35]

4.3. Anastomotic problems

The vascular anastomoses

Vascular anastomotic problems, though serious when they occur, do not seem to have played a major role in the over-all fatality associated with clinical liver transplantation.[5,36] The exact complication rate is not known, however, because the different types of anastomosis have not been monitored routinely.[37] In contrast, a high incidence of hepatic artery thrombosis has been noted in canine liver transplantation.[38] This complication can be largely prevented by the creation of an end-to-side arterial anastomosis with the use of a patch instead of an end-to-end anastomosis.[38] Krom et al. use this method of arterial reconstruction in human liver transplantation,[33] and a similar technique was employed in the experiments described in this thesis.

The biliary anastomosis

The reconstruction of the biliary tract has been called the Achilles' heel of clinical liver transplantation, complications accounting for more than one-third of the fatalities.[5,39-44] A major problem has been anastomotic leakage or obstruction. A multifactorial etiology for this problem is probable.

As early as 1960, Starzl et al. reported for canine liver transplantation that the gallbladder must be opened to prevent autolysis.[21] Almost 20 years later, Syrakos et al. found that in the rabbit the presence of bile in the gallbladder during cold preservation leads to extensive damage of the wall and that prior evacuation of the bile can prevent this damage.[45] In the pig, McMaster et al. found a relationship between cold ischemia and biliary-tree damage, but in these experiments the bile was left in situ during the 24-hour period of cold preservation. However, no damage was seen after one hour

of normothermic preservation in the presence of bile.[46]

Northover and Terblanche drew attention to the poor blood supply to the supra-duodenal portion of the common bile duct in man compared with the situation in the pig and baboon. Furthermore, the duodenal side takes a greater share in this blood supply than the liver side.[47] Long donor common bile ducts are therefore undesirable, and shortening has reduced the incidence of anastomotic leakage.[4]

Another problem concerning the biliary tract in liver transplantation is the development of biliary sludge or casts. When this complication was first reported, all of the patients had previously developed a biliary leak.[48] This suggests that infection and anastomotic scarring with subsequent stenosis are mainly responsible for this phenomenon. In fact, all cast cultures were found to be positive for Escherichia coli.[49] In Starzl's experience a mechanical etiology almost always plays a role.[41] An altered lithogenicity has been postulated to be co-responsible.[48] In 1979, in a series of 34 patients, McMaster et al. found a 60% incidence of early sludge formation leading to intraluminal obstruction and cholangitis.[49] Because this complication was much less common in Starzl's series, the question arises whether the gallbladder conduit technique, developed and used by Calne's team,[40] is responsible for this complication.

For the prevention of cholangitis, the advantage of retaining Oddi's sphincter has been clearly demonstrated in animals,[50,51] and the choledocho-choledochostomy over a T-tube is considered to be the anastomosis of choice.[4,5,33,51] Although Calne too retains Oddi's sphincter, he interposes the gallbladder as a conduit.[40] Unfortunately, a sphincter-retaining technique for biliary anastomosis is sometimes not feasible, for example in children with biliary atresia.[5] Under these conditions Starzl et al. prefer a choledocho-jejunostomy at present,[5] whereas Calne and Williams use an

anastomosis between the gallbladder conduit and the jejunum.[4] A cholecysto-intestinal anastomosis should be avoided in view of the high incidence of cholangitis and cystic duct obstruction.[5,39,42,49,51,52] Moreover the gallbladder should always be removed if a choledocho-intestinal anastomosis is performed because of the high incidence of cholecystitis in case of nonremoval.[51]

4.4. Rejection

The first clinical kidney-transplantation trials were performed in patients whose state had not been altered by therapy, because immunosuppressive drugs such as prednisolone and azathioprine were not yet available. As a result, the natural course of rejection of renal transplants in man is well known. Analogous information about liver-transplant rejection in man will never be obtained, because all human recipients receive immunosuppressive therapy. Therefore, the only recourse is to extrapolate data on rejection phenomena in non-immunosuppressed animal recipients to man.[53]

In unmodified unrelated canine recipients the survival time usually does not exceed 10 days.[54] For unmodified unrelated baboons Mybergh et al. reported a 14-day survival of 25%, and only one baboon lived for as much as 36 days.[55] Although various regimens of immunosuppressive therapy mitigated rejection, the long-term survival rate in dogs and baboons remained low. Moreover, in about one-third of the canine recipients, the course of rejection was not significantly changed by the use of immunosuppressive drugs.[56] In pigs a different natural course of rejection was first observed by Garnier et al. Changes in liver biochemistry were found to be less pronounced, and a few pigs lived for more than 25 days.[57,58] These findings were confirmed by Peacock,

Terblanche and Calne,[26,59-62] but consistent long-term survival in unmodified pigs has not been achieved.[50]

Clinical liver transplantation was given a trial because of the relatively great success of clinical kidney transplantation.[53] These trials showed clearly that acute rejection was less of a problem in liver than in kidney transplantation, even under violation of blood-group or histocompatibility-matching rules.[39,63] Moreover, rejection appeared to account for less than 10% of the deaths.[64,65] Thus, donor-recipient selection based on tissue-typing appeared to be much less important here than in clinical kidney transplantation, although a prospective study of the effect of typing has never been possible because of the urgent demand for and shortage of donor livers[66] and the small acceptor pool in the absence of an artificial liver. In view of above findings and the fact that significant extrahepatic infections were found in almost all deceased recipients, systematic over-immunosuppression was considered to be at least partially responsible for the high fatality rate.[64,65] Some authors have compared the course of rejection in man and the pig,[4,65] but this comparison is not valid because all human recipients have been given immunosuppressive therapy whereas most of the porcine recipients were not. Starzl et al. warned against lightening of the immunosuppressive regimens in clinical liver transplantation, and pointed out that some of the structural abnormalities in the hepatic grafts,thought to be nonspecific, could be subtle manifestations of hepatic rejection or recovery from rejection phenomena.[64] More recently, Starzl et al. stated that the tendency to ascribe most of the high fatality rate to non-rejection factors is probably not completely correct, and they thought that the next major improvement of survival would follow advances in the field of immunosuppression.[5] This prediction seems to have been fulfilled by the introduction of cyclosporin A.[67-70] However, the improved

techniques and the better recipient selection undoubtedly have to be taken into account for the better results achieved since 1980 (Chapter 5).

REFERENCES

1. Williams R: Transplantation of the liver in man. Br Med J 1:585-593, 1970
2. Fox PS, Failla JP,Kauffman HM, et al: The cadaver donor. Logistics of supply and demand in an urban population. JAMA 222:162-167, 1972
3. Slapak M: The immediate care of potential donors for cadaveric organ transplantation. Anaesthesia 33:700-709, 1978
4. Calne RY, Williams R: Liver transplantation. Curr Probl Surg 16:1-44, 1979
5. Starzl TE, Koep LJ, Halgrimson CG, et al: Fifteen years of clinical liver transplantation. Gastroenterology 77:375-388, 1979
6. Starzl TE, Iwatsuki S, Van Thiel DH, et al: Evolution of liver transplantation. Hepatology 2:614-636, 1982
7. Terasaki PI: Histocompatibility typing, in Starzl TE, Putnam CW (eds): Experience in Hepatic Transplantation. Philadelphia, Saunders, 1969, pp 22-23
8. Johnstone FRC: Acute ligation of the portal vein. Surgery 41:958-971, 1957
9. Raffuci FL, Wangensteen OH: Tolerance of dogs to occlusion of the entire vascular inflow to the liver. Surg Forum 1:191-195, 1951
10. Drapanas T, Becker DR, Alfano GS, et al: Some effects of interrupting hepatic blood flow. Ann Surg 142:831-835, 1955

11. Jolly PC, Foster JH: Hepatic inflow stasis. Surgery 54:45-65, 1963
12. Battersby C, Hickman R, Saunders SJ, et al: Liver function in the pig. I. Effects of 30 minutes normothermic ischemia. Br J Surg 61:27-32, 1974
13. Mackenzie RJ, Furnival CM, O'Keane MA, et al: The effect of hepatic ischaemia on liver function and the restoration of liver mass after 70 per cent partial hepatectomy in the dog. Br J Surg 62:431-437, 1975
14. Mackenzie RJ, Furnival CM, Wood CB, et al: The effects of prolonged hepatic ischaemia before 70 per cent partial hepatectomy in the dog. Br J Surg 64:66-69, 1977
15. Nordlinger B, Douvin D, Javaudin L, et al: An experimental study of survival after two hours of normothermic hepatic ischemia. Surg Gynecol Obstet 150:859-864, 1980
16. Huguet C, Nordlinger B, Bloch P, et al: Tolerance of the human liver to prolonged normothermic ischemia. Arch Surg 113:1448-1451, 1978
17. Bernhard WF, McMurrey JD, Curtis GW: Feasibility of partial hepatic resection under hypothermia. N Engl J Med 253:159-164, 1955
18. Bernhard WF, Cahill GF, Curtis GW: The rationale of surgery under hypothermia in certain patients with severe hepatocellular disease. Ann Surg 145:289-303, 1957
19. Farkouh EF, Daniel AM, MacLean LD: The effect of collateral circulation on survival in liver ischemia. J Surg Res 11:130-134, 1971
20. Humphries AL, Liu WP: Perfusates for preservation of kidneys, livers and hearts. Ann Anesth Franç 15:183-199, 1974
21. Starzl TE, Kaupp HA, Brock DR, et al: Reconstructive problems in canine liver homotransplantation with special reference to the postoperative role of

hepatic venous flow. Surg Gynecol Obstet 111:733-743, 1960

22. Moore FD, Smith LL, Burnap TK, et al: One-stage homotransplantation of the liver following total hepatectomy in dogs. Transplant Bull 6:103-106, 1959
23. Kestens PJ, Farelly JA,McDermott WV: A technique of isolation and perfusion of the canine liver. J Surg Res 1:58-63, 1961
24. Alican F, Hardy JD: Replantation of the liver in dogs. J Surg Res 7:368-375, 1967
25. Marchioro TL, Huntley RT, Waddell WR, et al: Extracorporeal perfusion for obtaining postmortem homografts. Surgery 54:900-911, 1963
26. Calne RY, White HJO, Yoffa DE, et al: Observations of orthotopic liver transplantation in the pig. Br Med J 2:478-480, 1967
27. Schalm SW: A Simple and Clinically Applicable Method for the Preservation of a Liverhomograft. Leiden, Doctoral Thesis, 1968
28. Schalm SW, Terpstra JL, Popescu DT, et al: Orthotopic liver transplantation after preservation without perfusion for up to six hours: a controlled trial evaluating different preservation fluids in dogs and pigs. Surgery 78:637-643, 1975
29. Benichou J, Halgrimson CG, Weil III R, et al: Canine and human liver preservation for 6 to 18 hr by cold infusion. Transplantation 24:407-411, 1977
30. Monden M, Fortner JG: Twenty-four- and 48-hour canine liver preservation by simple hypothermia with prostacyclin. Ann Surg 196:38-42, 1982
31. Squifflet JP,Pirson Y, Gianello P, et al: Safe preservation of human renal cadaver transplants by Euro-Collins solution up to 50 hours. Transplant Proc 13:693-696, 1981
32. Starzl TE: Liver transplantation. Johns Hopkins Med J 143:73- 83, 1979

33. Krom RAF, Gips CH, Kootstra G, et al: Six liver transplantations performed in Groningen. Ned Tijdschr Geneeskd 125:878-885, 1981
34. Schröter GPJ, Hoelscher M, Putnam CW, et al: Infections complicating orthotopic liver transplantation. Arch Surg 111:1337-1347, 1976
35. Gips CH, Krom RAF, De Groot EH: The fate of 30 patients for whom liver transplantation was considered during the period 1977 to 1979 included, and actually performed in 7 of them. Ned Tijdschr Geneeskd 125:868-875, 1981
36. Starzl TE, Putnam CW: Experience in Hepatic Transplantation. Philadelphia, Saunders, 1969, pp 144-158
37. Bax NMA, Meradji M, Molenaar JC: Pervenous hepatic transplant angiography using a new low osmolar contrast medium. An experimental study in dogs. Invest Radiol 17:299-304, 1982
38. Schalm SW, Terpstra JL, Achterberg JR, et al: Orthotopic liver transplantation: an experimental study on mechanisms of hemorrhagic diathesis and thrombosis. Surgery 78:499-507, 1975
39. Starzl TE, Ishikawa M,Putnam CW, et al: Progress and deterrents to orthotopic liver transplantation, with special reference to survival, resistance to hyperacute rejection, and biliary duct reconstruction. Transplant Proc 6:129-139, 1974
40. Calne RY: A new technique for biliary drainage in orthotopic liver transplantation utilizing the gall bladder as a pedicle conduit between the donor and recipient common bile ducts. Ann Surg 184:605-609, 1976
41. Starzl TE, Putnam CW,Hansbrough JF, et al: Biliary complications after liver transplantation: with special reference to the biliary cast syndrome and techniques of secondary bile duct repair. Surgery 81:212-221, 1977

42. Calne RY, McMaster P,Portmann B, et al: Observations on preservation, bile drainage and rejection in 64 human orthotopic liver allografts. Ann Surg 186:282-290, 1977
43. Calne RY: The present status of liver transplantation. Transplant Proc 9:209-216, 1977
44. Starzl TE, Koep LJ, Halgrimson CG, et al: Liver Transplantation-1978. Transplant Proc 11:240-246, 1979
45. Syrakos TP, Wight DGD, McMaster P, et al: Damage to the biliary tract during preservation. Transplantation 28:166-171, 1979
46. McMaster P, Walton RM, White DGD, et al: The influence of ischaemia on the biliary tract. Br J Surg 67:321-324, 1980
47. Northover JMA, Terblanche J: A new look at the arterial supply of the bile duct in man and its surgical implications. Br J Surg 66:379-384, 1979
48. Waldram R, Williams R, Calne RY: Bile composition and bile cast formation after transplantation of the liver in man. Transplantation 19:382-387, 1975
49. McMaster P, Herbertson BM, Cusick C, et al: The development of biliary "sludge" following liver transplantation. Transplant Proc 11:262-266, 1979
50. Dent DM, Hickman R, Uys CJ, et al: The natural history of liver allo- and autotransplantation in the pig. Br J Surg 58:407- 413, 1971
51. Krom RAF: De Bilio-Digestieve Anastomose. Een Experimenteel Onderzoek. Leiden, Doctoral Thesis, 1976
52. Martineau G, Porter KA, Corman J, et al: Delayed biliary duct obstruction after orthotopic liver transplantation. Surgery 72:604-610, 1972
53. Starzl TE, Brettschneider L,Putnam CW: Transplantation of the liver. Prog Liver Dis 3:495-542, 1970

54. Starzl TE, Putnam CW: Experience in Hepatic Transplantation. Philadelphia, Saunders, 1969. pp 176-192
55. Myburgh JA, Abrahams C, Mendelsohn D, et al: Cholestatic phenomenon in hepatic allograft rejection in the primate. Transplant Proc 3:501-504, 1971
56. Brettschneider L, Starzl TE: Impediments to successful liver transplantation in man, and possible solutions. Colston Papers 19:307-319, 1967
57. Garnier H, Clot JP, Bertrand M, et al: Biologie expérimentale: greffe de foie chez le porc; approche chirurgicale. CR Seances Acad Sci 260:5621-5623, 1965
58. Cordier G, Garnier H, Clot JP, et al: La greffe de foie orthotopique chez le porc; premiers résultats. Mém Acad Chir 92:799-807, 1966
59. Peacock JH, Terblanche J: Orthotopic homotransplantation of the liver in the pig, in Read AE (ed): The Liver. London, Butterworhts, 1967, pp 333-336
60. Terblanche J, Peacock JH, Bowes J, et al: The technique of orthotopic liver transplantation in the pig. J Surg Res 8:151-160, 1968
61. Terblanche J, Peacock JH, Hobbs KEF, et al: Orthotopic liver homotransplantation: an experimental study in the unmodified pig. S Afr Med J 42:486-497, 1968
62. Calne RY, White HJO, Yoffa DE, et al: Prolonged survival of liver transplants in the pig. Br Med J 2:645-648, 1967
63. Iwatsuki S, Iwaki Y, Klintmalm G, et al: Successful liver transplantation from crossmatch-positive donors. Transplant Proc. 13:286-288, 1981

64. Starzl TE, Porter KA, Putnam CW, et al: Orthotopic liver transplantation in ninety-three patients. Surg Gynecol Obstet 142:487-505, 1976
65. Calne RY, Williams R: Orthotopic liver transplantation: the first 60 patients. Br Med J 1:471-476, 1977
66. Terblanche J, Koep LJ, Starzl TE: Liver transplantation. Med Clin North Am 63:507-521, 1979
67. White DJG: Cyclosporin A. Clinical pharmacology and therapeutic potential. Drugs 24:322-334, 1982
68. Calne RY, White DJG: The use of cyclosporin A in clinical organ grafting. Ann Surg 196:330-337, 1982
69. Klintmalm GBG, Klingensmith III WC, Iwatsuki S, et al: 99 mTc - DTPA and 131 I-hippuran findings in liver transplant recipients treated with cyclosporin A. Radiology 142:199-202, 1982
70. Starzl TE, Iwatsuki S, Malatack JJ, et al: Liver and kidney transplantation in children receiving cyclosporin A and steroids. J Pediatr 100:681-686, 1982

CHAPTER 5. RESULTS OF ORTHOTOPIC LIVER TRANSPLANTATION IN CHILDREN

Of the two leading teams in the world in the field of clinical liver transplantation, only one has accumulated considerable experience with pediatric liver transplantation. In the past, Calne and his team were reluctant to transplant children because of the uncertain long-term prognosis, the frequent and often painful pre- and postoperative investigations, and the side effects of drugs.[1] The recent improvement of the prognosis and the availability of cyclosporin A for use as an immunosuppressive drug have changed this attitude, but the shortage of appropriately sized donor livers has hampered this team's work on the development of pediatric liver transplantation.[2]

In contrast, between 1963 and May of 1982 Starzl and his team transplanted 112 patients aged 18 years or younger, which amounts to about 50% of all patients transplanted by this team. Sixty- three of the 112 patients had biliary atresia, 21 an inborn error of metabolism, 15 chronic aggressive hepatitis, and the remaining patients a variety of conditions, as underlying disorders leading to end-stage hepatic disease. Only three pediatric patients received a transplant for hepatic malignancy. Of the 86 pediatric patients transplanted before 1980, 38% were alive at one year and 21% are still alive with a follow-up duration ranging from 2.5 to 12.5 years. Of the 26 pediatric patients transplanted since 1980, 70% are still alive with a follow-up period ranging from one to 21 months. The one-year survival rate has therefore been roughly

doubled. Starzl attributes this improvement to the use of cyclosporin A and a reduced steroid dosage.[3] The better recipient selection and the improved techniques for the biliary anastomosis have, however, certainly contributed to this improvement.[2]

Before 1980 the results in the pediatric age group were distinctly better than those obtained in adults, but this does not hold when the group of children with biliary atresia is compared with the group of adults. Since 1980, the discrepancy between the results in pediatric and adult recipients has disappeared, as has the discrepancy between those in pediatric recipients with and without biliary atresia.[3] The use of microsurgical techniques in recipients with biliary atresia, who are usually smaller than other pediatric liver-transplantation candidates,[4] together with the awareness that associated anomalies may be present in these patients[5] and the availability of better techniques for the biliary anastomosis in the absence of a recipient common bile duct, seem to explain the disappearance of the discrepancy between the results in pediatric recipients with and without biliary atresia. The present 5-year survival rate is not known, however.

In the Groningen University Hospital (The Netherlands) two children have been transplanted so far. One of these children was 11 years old and had cryptogenetic cirrhosis, the other was 4 years old and had biliary atresia. Both are doing well with follow-up durations of one year and three months, respectively.[6]

REFERENCES

1. Calne RY, Williams R: Liver transplantation. Curr Probl Surg 16:1-44, 1979

2. Calne RY: Liver grafting. Transplantation 35:109-111, 1983
3. Starzl TE, Iwatsuki S, Van Thiel DH, et al: Evolution of liver transplantation. Heptology 2:614-636, 1982
4. Starzl TE, Koep LJ, Schröter GPJ, et al: Liver replacement for pediatric patients. Pediatrics 63:825-829, 1979
5. Mihyamoto M, Nakamura Y, Kajimoto T: Biliary atresia and associated malformations. J Jpn Soc Pediatr Surg 17:885-894, 1981
6. Krom RAF: Personal communication. December 1983

CHAPTERS 6 - 11. THE EXPERIMENTS

CHAPTER 6. THE EXPERIMENTAL DESIGN

6.1. The experimental animal: the beagle

The ready availability of inbred strains of rats at relatively low cost has favored the use of this species in transplantation work. The use of isogeneic grafts has led to consistent long-term survival after liver transplantation, which argues for the soundness of the techniques used. The same techniques have been used in an allogeneic combination to assess the effect of various immunosuppressive regimens.[1,2]

Although the validity of this work is beyond doubt, the anesthesiological and surgical techniques used differ considerably from those applied in clinical liver transplantation, and a larger animal is necessary to mimic the clinical situation. However, consistent long-term survival has not been achieved in the dog, pig, or monkey (Section 4.4). For the evaluation of the techniques used in these species, long-term survival is essential and the role of immunological factors in the outcome must be eliminated as far as possible. Selection of donor and recipient among littermates matched for the major histocompatibility complex can bring this goal into sight, but the cost will unfortunately be high.

For the present study the beagle was chosen as experimental animal because of the availability of a large outbred colony (Centraal Proefdieren Bedrijf TNO, Zeist, The Netherlands), excellent tissue-typing facilities, and the know-how in the handling and care of the beagle in various kinds of organ transplantation

experiments in the Surgery Laboratory of Erasmus University in Rotterdam (The Netherlands).[3-5] In addition, much is known about the anatomy, physiology, and pathology of the beagle,[6] and dogs have been used for many years in experimental liver transplantation.[7-14]

6.2. Mitigation of rejection

Although in beagles from the indicated colony rejection after various kinds of organ transplantation can be mitigated considerably by donor-recipient selection of littermates which are matched for the major histocompatibility complex, rejection does occur.[4] The survival period after orthotopic liver transplantation in this donor-recipient combination appeared to be much longer than those reported for other kinds of organ transplantation, but the only series published was too small to permit definite conclusions.[14] In contrast, Chandler et al. were, unable to achieve long-term survival in tissue-typed identical beagles from a selectively bred colony.[12] However, long-term survival is essential for the evaluation of surgical transplantation models. On these grounds, the experiments were done in littermate pairs matched for the major histocompatibility complex, and prednisone and azathioprine were given to the recipients.

6.3. The design of a control model

To obtain an impression of the impact of the surgical trauma alone on the outcome, i.e., in the absolute absence of rejection, hepatic vascular exclusion experiments resembling autotransplantation, were performed as control experiments. The effect of the immunosuppressive drugs on the outcome of these

experiments was assessed by giving half of the control dogs the same immunosuppressive treatment as that given to the transplanted dogs.

6.4. The orthotopic nonauxiliary mode of partial liver transplantation

The shortage of appropriately sized donor livers for pediatric liver transplantation would be increased by a nonremoval of the native liver, because the space occupied by the latter would not become available. For auxiliary liver transplantation in children, even age-matched donor livers would be too large and livers from younger donors would be required. Apart from the problem of size, removal of the native liver may be indicated, e.g. in children with biliary atresia (Section 2.2). Furthermore, one should know what happens to a partial graft placed in an orthotopic nonauxiliary position, before one embarks on heterotopic auxiliary transplantation of such a partial graft and is confronted with the difficult problem of graft atrophy. Lastly, in the absence of the native liver, total liver function is graft dependent, which greatly simplifies evaluation of the functioning of the graft.

For all of these reasons, the orthotopic nonauxiliary mode of partial liver transplantation was chosen for the experimental study.

6.5. The age of the animals at operation and the size of the partial grafts

The ideal experimental set up would have been the transplantation of part of an adult canine liver into a puppy, the size of the graft equaling the size of the puppy's liver, but this would have made it impossible to

use littermate dogs. Moreover, it would have been extremely difficult to obtain an adequate number of comparable donor-recipient pairs. For these reasons, only adult beagles were used. The size of the graft was arbitrarily chosen to represent 60% of the donor liver. In the control experiments, 60% of the native liver was left in situ.

6.6. The duration of liver ischemia

To mimic the clinical situation, in which longer periods of ischemia of the donor liver are required, donor and recipient dogs were operated on sequentially. According to this policy, the total ischemic time of the graft was about 5 hours, but in the control experiments the same total ischemic time of the liver could not be achieved without a concomitant and equal prolongation of the anhepatic phase. As a debatable compromise, the duration of the anhepatic phase in these dogs was set at about 20 minutes longer than in the transplanted dogs.

6.7. The duration of the period under study

Dogs surviving for 169 days after surgery were killed. A longer survival has rarely been reported for canine orthotopic liver transplantation.[7-14] This period was thought to be sufficiently long for the evaluation of the techniques used.

REFERENCES

1. Lee S, Charters AC, Chandler JG, et al: Prolonged survival after orthotopic liver transplantation in inbred rats. Surg Forum 24:381-383, 1973
2. Wolff ED: Liver Transplantation in the Rat. Rotterdam, Doctoral Thesis, 1976
3. Vriesendorp HM, Bijnen AB, Westbroek DL, et al: Genetics and transplantation immunology of the DLA complex. Transplant Proc 9:293-296, 1977
4. Bijnen AB: Donor Selection for Renal Transplantation. A Study on Mixed Lymphocyte Reactions and Kidney Allograft Survival in Unimmunosuppressed Dogs. Rotterdam, Doctoral Thesis, 1978
5. Vriesendorp HM: Applications of transplantation immunology in the dog. Adv Vet Sci Comp Med 23:229-265, 1979
6. Andersen AC: The Beagle as an Experimental Dog. Iowa,Iowa University Press, 1970
7. Goodrich EO, Welch HF, Nelson JA, et al: Homotransplantation of the canine liver. Surgery 39:244-251, 1956
8. Moore FD, Wheeler HB, Demissianos HV, et al: Experimental whole-organ transplantation of the liver and the spleen. Ann Surg 152:374-387, 1960
9. Starzl TE, Kaupp HA, Brock DR, et al: Reconstructive problems in canine liver homotransplantation with special reference to the postoperative role of hepatic venous flow. Surg Gynecol Obstet 111:733-743, 1960
10. Stuart FP, Torres E, Hester WJ, et al: Orthotopic autotransplantation and allotransplantation of the liver: functional and structural patterns in the dog. Ann Surg 165:325- 340, 1967

11. Sivula A, Lempinen M, Salmenkivi K, et al: Orthotopic liver transplantation in the dog. Acta Chir Scand 137:437-444, 1971
12. Chandler JG, Villar H, Lee S, et al: The influence of histocompatibility matching according to lymphocyte types on orthotopic liver transplantation in dogs. Surgery 71:807-816, 1972
13. Schalm SW, Popescu DT, Van der Waay D, et al: Orthotopic liver transplantation: an experimental study on the prevention of infections with gram-negative organisms. Br J Surg 62:513-517, 1975
14. Otte JB, Lambotte L, Westbroek DL, et al: Long-term survival after orthotopic liver transplantation in DLA-identical beagles. Eur Surg Res 8 (Suppl):63, 1976

CHAPTER 7. THE SURGICAL TECHNIQUES AND OVER-ALL RESULTS

ORTHOTOPIC NONAUXILIARY HOMOTRANSPLANTATION OF PART OF THE LIVER IN DOGS

N.M.A. Bax,[1] B.M.J. Vermeire,[2] N. Dubois,[1] G. Madern,[1] M. Meradji,[3] and J.C. Molenaar.[1]
From the Departments of Pediatric Surgery,[1] Pediatric Anesthesiology,[2] and Pediatric Radiology,[3] of the School of Medicine of Erasmus University, Rotterdam, The Netherlands.
Supported in part by the Sophia Foundation for Scientific Research.
Presented at the 1982 Meeting of the American Pediatric Surgical Association. Phoenix, Arizona, May 29 - June 1, 1982.
Published in J Pediatr Surg 17:906-913, 1982.
Reproduced by permission.

Summary

An experimental study was carried out in dogs to investigate the feasibility of using only a portion of a donor liver in orthotopic nonauxiliary transplantation. After being totally hepatectomized, 20 immunosuppressed adult beagles received a 60% donor liver graft, originating from tissue-typed identical littermates. Preservation time was 5 hr. In control experiments, 10 beagles, only half of them immunosuppressed, underwent an operation involving complete liver mobilization, vascular

exclusion, cooling of the liver in situ, a 40% partial hepatectomy, revascularization of the liver remnant, and biliary anastomosis. The surgical procedure, including the method of cooling and preservation, is described in detail. The survival rate amounted to 90% for the control group and 50% for the transplanted group, while all surviving dogs were sacrificed 24 wk postoperatively. At sacrifice, the liver weight amounted to the weight of the total native liver at operation. Body-weight profiles reflect good general condition of the surviving dogs. No major differences were noted between the immunosuppressed and the nonimmunosuppressed control dogs. No complications were related to the fact that only a partial liver was grafted or left in situ.

Introduction

Shortage of donor livers of an appropriate size is a major problem in pediatric liver transplantation.[1,2] Juvenile donor livers are not readily obtainable and may also be grafted into adult recipients,[3] rendering the shortage of organs for pediatric transplantation even more acute. Adult donor livers, although more readily obtainable, are generally unacceptably large for pediatric recipients, even for orthotopic, nonauxiliary grafting.[4,5] This problem would be solved if a partial donor liver would suffice.

We investigated the feasibility of using only part of a donor liver instead of the whole liver in orthotopic nonauxiliary transplantation. We report the development of a successful canine model, including a control model to determine the effect of factors other than rejection.

Materials and methods

Our laboratory animals consisted of male adult beagles from an outbred colony. In a randomized sequence we carried out 20 transplantations and 10 control experiments. The surviving dogs were sacrificed 24 wk (169 days) after surgery. Age at operation and body weight of donor,recipient, and control dogs are given in Table 1. The donor-recipient pairs came from the same litter and identicalness was substantiated by serologic and mixed lymphocyte culture typing.[6,7] The heavier one of the pair always became donor. Standardized immunosuppression (Table 2) was added to prolong the survival period. Half of the control dogs underwent the same immunosuppressive treatment. No blood transfusions

Table 1. Age and body weight at operation.

	age: mean in days ± 1 SD	body weight: mean in kg ± 1 SD
control dogs	484.9 ± 81.10	13.5 ± 1.6
donor dogs	479.55 ± 78.81	14.1 ± 1.6
recipient dogs		12.8 ± 1.3

Table 2. Immunosuppression.

postoperative days	azathioprine* mg/kg per day	prednisolone mg/kg per day
0 - 99	1	2
100 - 107	0.75	1.5
108 - 127	0.50	1.0
128 - 149	0.25	0.5
> 150	0	0

* Kindly supplied by the Wellcome Foundation

were given preoperatively. Parenteral lincomycin and kanamycin were administered on the day of surgery and the following day.

Anesthesia was initiated with fentanyl, dehydrobenzperidol, and atropine. Following induction with fentanyl and muscle relaxation with pancuronium, endotracheal intubation was carried out and artificial ventilation was started. Anesthesia was maintained with N_2O, O_2, and enflurane. Intravenous fluids consisted of electrolyte solutions, fresh blood, and fresh plasma. At the end of the operation, anesthesia was maintained until rewarming up to 36°C had been accomplished.

A standardized protocol was followed. The right femoral artery was cannulated for pressure monitoring in all dogs and for bleeding purposes in controls. In recipient and control dogs, the right external jugular vein was used for central venous pressure monitoring and for blood sampling, while the right femoral vein and left external jugular vein were used for bypass purposes. Abdominal access was gained by an extended right subcostal laparotomy in all dogs. Euro-Collins solution at 4°C was used for cooling and preservation of the liver. The composition is given in Table 3.

The donor dog is operated on first. Manipulation of the liver proper is carefully avoided until the liver has been cooled in situ. Dissection of the hepatoduodenal ligament close to the duodenum is initiated by ligation and division of the right gastric vessels. The common bile duct is ligated and transected. The gastroduodenal artery is ligated close to the duodenum and cannulated retrogradely in the direction of the liver with a soft transparent catheter, which is sealed until the cold arterial infusion can begin. The portal vein is then freed. After 5 min of bleeding via the femoral artery, the gastroduodenal, splenic, and superior mesenteric veins are ligated and a short, large-caliber cannula is

Table 3. Composition of Euro-Collins solution.

components	g/liter		mmol/l
1. KH_2PO_4	2.05	K	115
2. K_2HPO_4	7.4	Na	10
3. K Cl	1.12	Cl	15
4. $NaHCO_3$	0.84	HCO_3	10
5. Glucose	35	HPO_4	85
		H_2PO_4	15

Osmolality: about 355 mmol/l
pH at 20°C : 7.33
pH at 4°C : 7.20

inserted into the portal vein. The cold infusion is initiated under a hydrostatic pressure of 75 cm of water.

Through a hole in the diaphragm, the infracardiac caval vein is occluded. At the level of the renal veins, the infrahepatic caval vein is also occluded, and an immediately proximal caval venotomy enables drainage of the perfusate. To prevent autolysis, the biliary system is flushed manually with 0.9% saline at 4°C through the gallbladder, until the common bile-duct effluent becomes clear. Next,the gallbladder is opened wide. Further dissection is delayed until 2 liters of cold infusion have passed through the portal vein, cooling the liver down to 12°C. The common hepatic artery is then mobilized and transected, including a patch of the celiac trunk.

The liver proper is now mobilized by dividing the various ligaments, first on the left and then on the right. The retrohepatic caval vein, which remains attached to the liver, is freed from the renal veins up to the diaphragm. The infrahepatic caval vein is transected distal to the ligated right adrenal vein. The suprahepatic caval vein is mobilized, including part of the cuff of the tendinous diaphragm, and transected 1 cm above the diaphragm. Upon removal the liver is packed in two sterile plastic bags, the inner one containing cold

preservation fluid and the outer one melting ice. Storage takes place in the refrigerator at 4°C.

The recipient dog is prepared to accept the graft and then bench surgery is carried out on the donor liver. At this stage the liver has cooled down to about 4°C. It is put into a sterile basin containing melting ice, and infused with 500 ml of cold normal saline solution to remove the potassium-rich Euro-Collins solution. An over and over 5x0 Prolene® suture is applied to the posterior diaphragmatic cuff to make certain that all phrenic vein inlets are closed. Next, 40% of the liver is removed, i.e., the left liver lobes. This is achieved by careful hilar dissection as described by Sigel.[8] All individual structures are ligated separately and transected as peripherally as possible. With the back of a surgical blade the parenchymal bridge is divided, and all larger structures are divided between ligatures until the left hepatic vein is reached. The hepatic parenchyma is stripped off this vein, which is ligated with a heavy silk ligature. After removal of the gallbladder, a soft splint with one terminal and one lateral opening is passed through the common bile duct (Fig.1).

In the recipient dogs, the portal vein is extensively mobilized with preservation of the gastroduodenal vein. Subsequently the celiac trunk is freed. Mobilization of the liver lobes and retrohepatic caval vein proceeds as in donor dogs, but the right adrenal vein is left intact. Hepatic arteries and bile ducts are ligated and divided close to the liver. The largest duct, which drains the central lobes and the gallbladder, is marked for subsequent anastomosis.

Decompression of the distal venous systems during the anhepatic phase is achieved by a slightly modified Cooperman technique.[9] Three siliconized, gauge-18 nasogastric tubes, cut to appropriate lengths, are assembled in an inverted Y fashion, and the system is filled with 0.9% saline solution. The top end of the

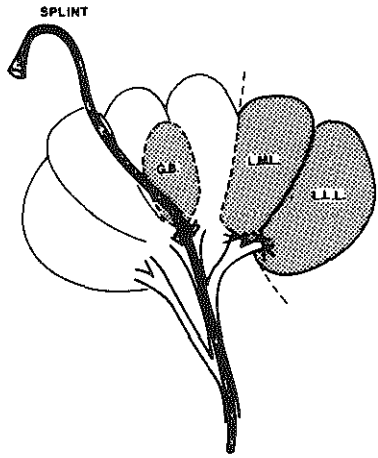

Fig. 1. Bench surgery on the donor liver.
The left medial and lateral lobes are removed as well as the gallbladder. A splint is inserted in the common bile duct via the cystic duct.

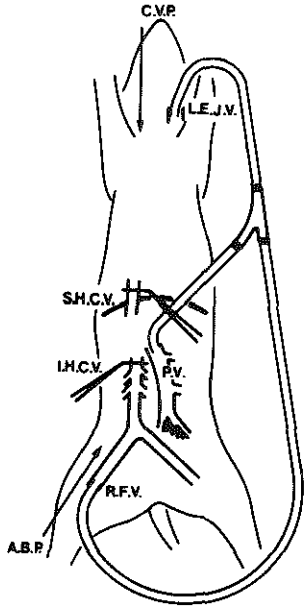

Fig. 2. The femoro-porto-jugular bypass during the anhepatic phase.
SHCV = suprahepatic caval vein; IHCV = infrahepatic caval vein; PV = portal vein; LEJV = left external jugular vein; RFV = right femoral vein; CVP = central venous pressure; ABP = arterial blood pressure

inverted Y is inserted into the left jugular vein, and the end of one of the arms into the right femoral vein. The portal vein is transected as close to the liver as possible, and the end of the other arm of the inverted Y is inserted into the proximal portal vein, distal to the entrance of the gastroduodenal vein.[10] The portal bypass clamp is then removed. The infrahepatic caval vein is clamped just above the entrance of the renal veins and at the liver level, after which it is transected immediately distal to the proximal clamp. The liver is now pulled downwards and the suprahepatic caval vein is occluded with a large Satinsky clamp involving a portion of the diaphragm (Fig. 2). The remaining bypass clamps are removed.

The liver is stripped from the caval vein until the cloaca of the hepatic veins is encountered, at which level the caval vein is once more transected, completing the recipient hepatectomy. Cold moist gauze is placed in the hepatic bed to receive the transplant. The suprahepatic caval anastomosis is performed in an end-to-end manner, using continuous 5 x 0 Prolene. Next, the portal vein is anastomosed. With the portal limb of the bypass in situ, the posterior layer is sutured with continuous 6 x 0 Prolene. Under total portal vein occlusion, after removal of the portal bypass, the anterior layer is closed rapidly to be followed by portal revascularization.[10] Approximately 200 ml blood is drained off through the infrahepatic caval vein, to allow discharge of waste products and air. The donor infrahepatic caval vein is then clamped and the clamp on the suprahepatic caval vein is released. Once continuity of the infrahepatic caval vein has been achieved, the remaining parts of the bypass are removed. Attention is now focused on the arterial reconstruction (Fig. 3).

After amputation of the recipient common hepatic artery, including a slice of the recipient celiac trunk, an end-to-side anastomosis is performed with continuous

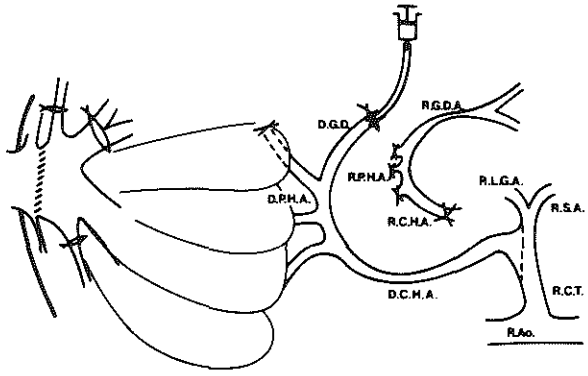

Fig. 3. The arterial anastomosis.

DGDA = donor gastroduodenal artery; DPHA = donor proper hepatic arteries; DCHA = donor common hepatic artery; RGDA = recipient gastroduodenal artery; RPHA = recipient proper hepatic arteries; RCHA = recipient common hepatic artery; RLGA = recipient left gastric artery; RSA = recipient splenic artery; RCT = recipient celiac trunk; RAo = recipient aorta.

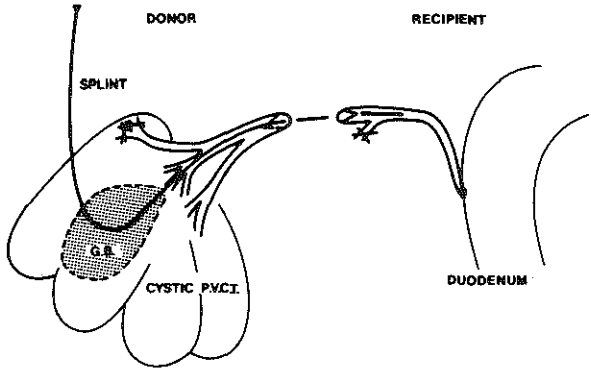

Fig. 4. The biliary anastomosis.

GB = gallbladder

6 x 0 Prolene. The anastomosis is flushed via the catheter in the gastroduodenal artery and patency is confirmed, after which the catheter is removed. Lastly, biliary drainage is achieved by a splinted choledocho-choledochostomy, using interrupted 6 x 0 Prolene sutures (Fig. 4). Upon hemostasis the abdomen is closed in one layer, using interrupted silk. The end of the biliary splint is sealed with a plastic cap and buried in a subcutaneous position distal to the incision for subsequent puncture cholangiography.

Control experiments

For the control dogs our surgical procedure was a derivative of Dent et al.'s experimental autografts in pigs.[11] The hepatoduodenal ligament is transected as in the donor dogs, including the common bile duct but excluding the portal vein and hepatic artery which have been freed. The gastroduodenal artery is transected too and cannulated retrogradely, leaving the vein intact. Mobilization of the liver proceeds as in the recipient dogs, except that the left phrenic vein is doubly ligated and severed. This permits suprahepatic caval vein occlusion without compromising the left hepatic vein outlet. Bypassing is achieved as in the recipient dogs. The portal branch is introduced into the proximal portal vein through a longitudinal venotomy distal to the entrance of the gastroduodenal vein and secured without compromising the latter vein. Through the same venotomy the distal portal vein is connected to the cold infusion system (Fig. 5). Clamping of the hepatic artery completes the interruption of the afferent blood supply.

The caval vein is clamped above and below the liver as in the recipient dogs, and an infrahepatic venotomy permits perfusate drainage. The cold arterial and portal infusion is initiated and the biliary tree is flushed as in the donor dogs. After 2 liters of cold infusion, the left liver lobes are removed. A cholecystectomy is performed and a splint inserted through the proximal

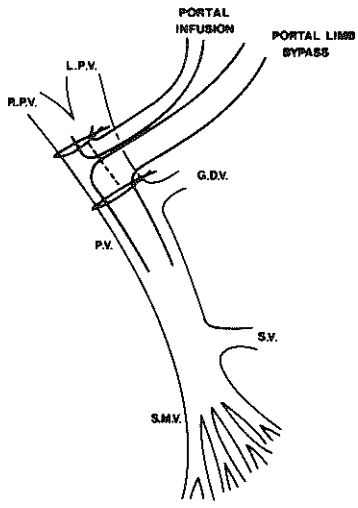

Fig. 5. Portal infusion and bypass in control dogs.
PV = portal vein; RPV = right portal vein; LPV = left portal vein; GDV = gastroduodenal vein; SV = splenic vein; SMV = superior mesenteric vein

common bile duct. After 1 hr of vascular exclusion, the portal branch of the bypass is removed and the portal venotomy is closed transversely with continuous 6-0 Prolene sutures. The portal vein and hepatic artery clamps are removed and the liver is revascularized. Approximately 200 ml blood is drained off, the infrahepatic caval vein is occluded and the suprahepatic caval vein reopened. The caval venotomy is closed with continuous 5 x 0 Prolene sutures, upon which the clamps and remaining bypass are removed. Finally, a splinted choledocho-choledochostomy is performed as in the recipient dogs.

Postoperative follow-up

To check the various vascular anastomoses, pervenous angiography was carried out on the 4th and 11th post-

operative day and also on indication. The angiographic technique and results have been published.[12] For the biliary anastomosis, percutaneous catheter cholangiography was performed on the 32nd postoperative day, at sacrifice, and on indication.

Results

None of the recipient or control dogs succumbed during surgery. The mean duration of the entire procedure, with specification of the individual phases, is given in Table 4. A 5-hr duration of hepatic ischemia could not be achieved in the control group without a concomitant prolongation of the anhepatic phase.

Table 4. Peroperative results.

duration	transplantation experiments mean in minutes ± 1 SD	control experiments mean in minutes ± 1 SD
total operation		
donor	116.20 ± 11.40	
recipient or control	334.05 ± 21.96	287.60 ± 21.82
bench surgery	32.25 ± 7.46	
anhepatic phase	53.05 ± 4.47	70.40 ± 5.73
total liver ischemia	308.05 ± 19.62	70.40 ± 5.73

Ten of the 20 recipients and 9 of the 10 control dogs survived for the duration of the experiment and were sacrificed 169 days postoperatively. Two recipients and one control dog died within 2 days; 4 recipients succumbed during the second week, 3 during the second month and one died more than 4 months postoperatively. The postoperative survival period, cause of death, and gross appearance of the liver at autopsy are given in Table 5.

Three recipient dogs and one control dog developed overt morbidity. One of these 3 recipients as well as the

Table 5. Postoperative survival period, cause of death, and liver appearance at autopsy in dogs dying before the 169th day.

	survival period	cause of death	liver appearance at autopsy
Transplantation group.			
1.	3 hr	air embolism	unremarkable
2.	24 hr	hypoglycemia	reddish-blue, hard
3.	8 days	peritonitis (leak biliary anastomosis)	thickening of Glisson's capsule, parenchyma unremarkable
4.	9 days	peritonitis (leak dislodged biliary splint)	thickening of Glisson's capsule, parenchyma unremarkable
5.	11 days	hemorrhage (bleeding duodenal ulcer)	unremarkable
6.	14 days	peritonitis (hepatic artery thrombosis)	total liver necrosis
7.	32 days	peritonitis (leak after withdrawal biliary splint)	thickening of Glisson's capsule, parenchyma unremarkable
8.	37 days	sacrifice (poor general condition)	central lobes: necrosis, right lobes: unremarkable
9.	39 days	sacrifice (liver failure)	generalized atrophy
10.	127 days	peritonitis (peripheral biliary leak after percutaneous biopsy)	thickening of Glisson's capsule, parenchyma unremarkable
Control group.			
1.	16 hr	sacrifice (neurologic sequellae after anesthetic accident)	unremarkable

Table 6. Clinical morbidity and autopsy findings in recipient dogs surviving for 169 days.

	clinical morbidity	ascites	transplant appearance
1.	persistent jaundice	100 ml	firm, micronodular
2.			slightly increased consistency
3.			unremarkable
4.			edematous, yellowish speckled
5.	wound infection		right lobes: unremarkable, central lobes: few pseudocysts
6.			slightly increased consistency
7.			unremarkable
8.	leak biliary anastomosis	50 ml	subphrenic abscess
9.			unremarkable
10.			unremarkable

control dog had to be reoperated for leakage of the biliary anastomosis, bringing the total incidence of anastomotic leakage up to 10% for both groups. The control dog recovered without sequellae, but the recipient developed a subphrenic abscess. Another recipient became permanently jaundiced after removal of the biliary splint on the 32nd postoperative day. At sacrifice no obstruction could be identified, but the liver had a firm micronodular aspect and there was also some ascites. The remaining recipient developed a severe wound infection, which healed spontaneously. Several weeks later the dog became febrile and vomited for a few days, but this subsided. At sacrifice, however, a few small pseudocysts were noted in the central lobes of the liver. Grossly, most of the other grafts looked healthy (Table 6). In the control dogs, a slight increase in liver density was sometimes noted, but never ascites.

The recipients showed a mean gain of 0.36 kg in body weight, and in the control dogs this amounted to 1.58 kg (Table 7). Although the weight gain was statistically significant in the control group, the difference in weight gain between the two groups was not significant.

Table 7. Changes in body weight in dogs surviving for 169 days.

	recipient dogs	control dogs
	n = 10	n = 9
mean in kg	+ 0.36	+ 1.58
± 1 SD	± 1.95	± 1.28

The weight of the liver at sacrifice was compared with the weight of the native liver at operation (Table 8). In control dogs the weight of the total native liver was estimated from the weight of the severed left lobes, according to the following formula:

$$\text{total native liver weight} = \frac{\text{weight of the left lobes} \times 100}{40.68}$$

These estimates can be considered reasonably accurate, since the weight of the left lobes of the 20 donor and 20 native recipient livers amounted to 40.68% (SD 2.72) of the individual total liver weight. At autopsy, the weight of the transplanted liver amounted to 102% of the native recipient liver. In the control dogs the liver weight at autopsy amounted to 105% of the peroperative total liver weight. The difference between the two groups is not significant. The same holds for the comparison of immunosuppressed and nonimmunosuppressed control dogs.

Table 8. Liver weight at sacrifice on day 169 as a percentage of the native liver weight.

	recipient dogs	control dogs	
	n = 10	n = 9	
	%	%	
	95.69	185.18	
	89.88	98.29	
	101.65	134.49	
	110.59	103.46	
	116.51	75.59	
	96.03	112.37	no immunosuppression
	85.58	65.15	
	127.24	90.81	
	97.61	86.95	
	105.09		
mean	102.58	105.81	
SD	12.64	36.01	

Discussion

Consistent long-term survival is required before accrediting a surgical model in organ transplantation. In our series 50% of the recipient and 90% of the control dogs survived for the duration of the experiment, amounting to 169 days. While the peroperative survival can be attributed largely to the quality of the

anesthesiologic and surgical techniques, long-term survival demonstrates the soundness of the whole procedure, particularly the efficacy of the control of rejection. The effectiveness of the cooling and preservation techniques is demonstrated by the fact that only one animal, the first recipient dog, died as a result of early liver failure. This was due to an inadvertently high temperature of the refrigerator containing the preservation fluids and donor liver. Cooling of the donor liver in situ prior to mobilization has been generally abandoned in clinical practice since the acceptance of heart-beating donors. In our pilot experiments, however, we found that extensive mobilization prior to cooling, no matter how carefully carried out, always resulted in superficial liver bruises. In addition, some areas would take longer to become bloodless during cold infusion, and post-transplantation revascularization was delayed in these areas. Perhaps this might explain Starzl's report of hopelessly damaged livers having been transplanted from apparently good donors.[13] With the technique for early premobilization cooling of the liver developed in our experiments, bruises were avoided and a much more homogeneous cooling and revascularization process was obtained. The decompression of the occluded venous systems achieved during the anhepatic phase was sufficient to prevent uncontrollable hemodynamic changes, although urine output almost ceased during the bypassing period and most of the dogs had a loose stool mixed with some blood upon awakening.

The systematic checking of all vascular anastomoses by pervenous angiography,[12] and of all biliary anastomoses by percutaneous catheter cholangiography, permitted exact assessment of the anastomotic complication rate in our series. Although arterial thrombosis is a common and fatal complication in canine liver transplantation,[14] it occurred only once in our series. The diagnosis was the

result of routine pervenous angiography in the absence of any clinical manifestation. Unfortunately, reclotting occurred after initially successful thrombectomy. No other vascular anastomotic complications occurred in the entire series.

As in clinical liver transplantation, the biliary anastomosis represented the Achilles' heel in our experimental series.[13,15-17] Anastomotic leakage occurred with a 10% incidence in both groups. Of the 3 dogs 2 were reoperated and both survived, which underscores the importance of early diagnosis and reintervention. Severe biliary splint complications occurred in 3 transplanted dogs, with fatal results in 2 of them. A more careful insertion of the splint, which was left in situ for the entire survival period, prevented complications in the second half of the series.

The notion of transplanting a portion of a donor liver is not new. Experimental heterotopic auto-, and homotransplantation of canine liver lobes inside as well as outside the abdominal cavity has been reported.[18-21] Heterotopic partial liver homotransplantations have even been carried out in patients.[22,23] In animals auxiliary homotransplantation of a portion of a donor liver, with partial removal of the native liver, has also been reported.[24,25] None of these models proved entirely successful. Orthotopic nonauxiliary homotransplantation of a partial liver has not been reported before. In our model the animal survival equals the graft survival, and the hepatic function is entirely dependent on the graft. Our model also bypasses the difficult and ongoing discussion on the etiology of graft atrophy in auxiliary liver transplantation.[26-31] In our series no complications occurred, which were related to the fact that only a portion of the liver was transplanted, and the transplants functioned well enough to sustain long-term survival of good quality. This is demonstrated by the body-weight profiles.

Consequently, orthotopic nonauxiliary homotransplantation of 60% of a donor liver, including 5 hr of ischemia, is technically feasible in immunosuppressed, tissue-typed identical littermate beagles. The long-term good-quality survival is reproducible. In surviving recipients the graft does not atrophy and functions in much the same way as the liver remnant in vascular exclusion experiments.

REFERENCES

1. Calne RY: Surgical aspects of clinical liver transplantation in 14 cases. Br J Surg 56:729-736, 1969
2. MacDougall BRD, Williams R: The indications for orthotopic liver transplantation. Transplant Proc 11:247-251, 1979
3. Calne RY: Transplantation of the liver. Ann Surg 188:129-138, 1978
4. Marshall VC: Selection of donors for liver transplantation, in Calne RY(eds): Clinical Organ Transplantation, Oxford, Blackwell, 1971, pp 347-349
5. Starzl TE, Putnam CW: Experience in Hepatic Transplantation. Philadelphia, Saunders, 1969, pp 133
6. Vriesendorp HM, Bijnen AB, Westbroek DL, et al: Genetics and transplantation immunology of the DLA complex. Transplant Proc 9:293-296, 1977.
7. Bijnen AB: Donor Selection for Renal Transplantation. A Study on Mixed Lymphocyte Reactions and Kidney Allografts Survival in Immunosuppressed Dogs. Rotterdam, Doctoral Thesis, 1978
8. Sigel B: Partial hepatectomy in the dog. Arch surg 87:788- 791, 1963

9. Cooperman AM, Woods JE, McIlrath DC: Simplified method of canine orthotopic hepatic transplantation. Am J Surg 122:797-810, 1971
10. Calne RY, Yoffa DE, White HJO, et al: A technique of orthotopic liver transplantation in the pig. Br J Surg 55:203- 206, 1968
11. Dent DM, Hickman R, Uys CJ, et al: The natural history of liver allo- and autotransplantation in the pig. Br J Surg 58:407- 413, 1971
12. Bax NMA, Meradji M,Molenaar JC: Pervenous hepatic transplant angiography using a new low osmolar contrast medium. An experimental study in dogs. Invest Radiol 17:299-304, 1982
13. Starzl TE, Koep LJ, Halgrimson CG, et al: Fifteen years of clinical liver transplantation. Gastroenterology 77:375-388, 1979
14. Schalm SW, Terpstra JL, Achterberg JR, et al: Orthotopic liver transplantation: an experimental study on mechanisms of hemorrhagic diathesis and thrombosis. Surgery 78:499-507, 1975
15. Starzl TE, Ishikawa M, Putnam CW, et al: Progress in and deterrents to orthotopic liver transplantation, with special reference to survival, resistance to hyperacute rejection, and biliary duct reconstruction. Transplant Proc 6:129-139, 1974
16. Starzl TE, Putnam CW, Hansbrough JF, et al: Biliary complications after liver transplantation: with special reference to the biliary cast syndrome and techniques of secondary duct repair. Surgery 81:212-221, 1977
17. Calne RY: A new technique for biliary drainage in orthotopic liver transplantation utilizing the gall bladder as a pedicle conduit between the donor and recipient common bile ducts. Ann Surg 184:605-609, 1976

18. Aoki H, Wang TC, Matsumura H, et al: Partial liver transplantation with primary revascularization in dogs. Preliminary report on new method and its theoretical background. Keio J Med 16:205-221, 1967
19. Corry RJ, Chavéz-Peón F, Miyakuni T, et al: Auxiliary partial liver transplantation in the dog. Arch Surg 98:799-802, 1969
20. Lavarello RJ, Kinne DW, Kim DK, et al: Life-sustaining canine hepatic autotransplants. Arch Surg 107:878-881, 1973
21. Sigel B, Baldia L, Dunn MR: Studies on liver lobes autotransplantated outside the abdominal cavity. Surg Gynecol Obstet 124:525-530, 1967
22. Shumakov VI, Galperin EI: Transplantation of the left liver lobe. Transplant Proc 11:1489, 1979
23. Fortner JG, Kim DK, Shiu MH, et al: Heterotopic (auxiliary) liver transplantation in man. Transplant Proc 9:217-221, 1977
24. Smith B: Segmental liver transplantation from a living donor. J Pediatr Surg 4:126-132, 1969
25. Mizumoto R, Yokata T, Ohsawa J, et al: Orthotopic partial hepatic transplantation in dog. Jpn J Surg 4:121-129, 1974
26. Schalm L: Heterotopic auxilary liver transplantation. I. A physiological concept of functional competition. Arch Chir Neerl 18:283-284, 1966
27. Marchioro TL, Porter KA, Dickinson TS, et al: Physiological requirements for auxiliary liver homotransplantation. Surg Gynecol Obstet 121:17-31, 1965
28. Van der Heyde MN, Schalm L, Vink M: The role of functional competition in auxiliary liver transplantation. Transplantation 5:78-80, 1967

29. Jerusalem C, Van der Heyde MN, Smidt WJ, et al: Heterotopic liver transplantation. II. Unfavorable outflow conditions as a possible cause for late graft failure. Eur Surg Res 4:186-197, 1972
30. Hess F, Willemsen A, Jerusalem C: Survival of auxiliary rat liver grafts with decreased portal blood flow. Eur Surg Res 10:444-455, 1978
31. Starzl TE, Francavilla A, Halgrimson CG, et al: The origin , hormonal nature and action of hepatotrophic substances in portal venous blood. Surg Gynecol Obstet 137:179-199, 1973

Discussion following the presentation of the paper at the 1982 meeting of the American Pediatric Surgical Association.

E. Fonkalsrud (Los Angeles): Fifty percent survival for 6 mo with an orthotopic canine liver homograft is perhaps the best reported in the world literature thus far. I would like to ask Dr. Bax how he avoids some of the serious problems that others have encountered with coagulopathy after placing a homograft in the canine model. The studies presented here today perhaps take on a little greater significance now that cyclosporin-A and other new immunosuppressive regimens have reduced the incidence of rejection. Liver transplants are now being done more frequently than they were a few years ago, with increasing success. Could you give us some estimate, Dr. Bax, of the function of the liver grafts that you have transplanted? Do you see, for example, reasonably normal alkaline phosphatase, SGOT, and bilirubin levels? Some authors have indicated that when one takes a large organ from an adult and places it in an infant, there are certain physiologic problems with the blood supply.

For example, the mean blood pressure for an adult may be somewhere around 110-130, whereas in the infant who might be the recipient the mean blood pressure might be only 50-60. The liver is particularly sensitive to changes in blood pressure and I wonder if you can conceive of any problems that this might cause in the studies that you have carried out in which a liver is transferred from an adult dog to another adult model. It would be of interest to transplant an adult liver into a puppy to simulate more closely the clinical situation posed by the authors.

N.M.A. Bax (closing): No complications were related to the use of a partial liver. Premobilization cooling avoids inadvertant hypoperfusion and warm ishemic damage of the graft. The precarious blood supply of the donor common bile duct, preservational damage, autolysis by bile, infection by stasis, and rejection all play a role in the etiology of biliary complications and have to be dealt with, for example by trimming the common bile duct until good bleeding is obtained. Dr. Fonkalsrud, hemorrhagic diathesis, though never uncontrollable, occurred in most experiments, starting before revascularization and coinciding with a major drop in the platelet count. Clotting-factor activities dropped only after revascularization, intensifying the blood loss. Concomitant with a rise in the platelet count during further surgery, the bleeding ceased in spite of persistent low clotting-factor activities. A more optimal bypass might have prevented these events to a large extent. The transaminase levels rose sharply after revascularization but had largely normalized by the fourth day. The alkaline phosphatase levels took longer to recover. I do not think that a partial adult graft in a child will be hypoperfused, because that graft will receive the total hepatic blood supply of that child. Moreover, the portal venous system is a low pressure one, irrespective of age.

CHAPTER 8. PEROPERATIVE COMPLICATIONS

PEROPERATIVE COMPLICATIONS DURING ORTHOTOPIC NONAUXILIARY ALLOTRANSPLANTATION OF PART OF THE LIVER IN DOGS

N.M.A. Bax,[1] B.M.J. Vermeire,[2] and A.C.P. Zondag.[3]
From the Departments of Pediatric Surgery,[1] Anesthesiology, Subdivision Pediatric Anesthesiology,[2] and Pediatrics, Subdivision Hematology,[3] of the School of Medicine of Erasmus University, Rotterdam, The Netherlands.
Supported in part by the Sophia Foundation for Scientific Research.
Submitted for publication.

Summary

The peroperative complications in a series of 20 canine liver transplantations and 10 control experiments, using partial livers, are described. The same protocol for peroperative management was used for both surgical groups. All dogs survived the procedure. The most important changes occurred during the one- hour anhepatic phase of the operation and were related to insufficient decompression of the infradiaphragmatic venous systems despite use of a bypass rather than to the absence of the liver. Although severe hypotension and disturbance of the acid-base balance could be prevented, congestional bowel and renal damage occurred in all cases. The sharp reduction of the number of circulating

platelets during the anhepatic phase, which appeared to initiate the observed hemorrhagic diathesis, may well have been related to the congestion in the infradiaphragmatic venous systems. The two surgical groups behaved similarly, which implies that these changes were not due to immunological factors. Nor can these changes be attributed to the use of partial instead of total livers, since these changes occurred during the anhepatic phase. It is concluded that the degree of decompression of the infradiaphragmatic venous systems achieved during the anhepatic phase, at least in dogs, has repercussions on not only the hemodynamics but also on coagulation. The use of a partial instead of a total liver did not seem to require any modification of the peroperative management.

Introduction

Liver transplantation, particularly during the anhepatic phase,has been called the acme of liver disease management.[1] After years of animal and human liver transplantation, a number of guidelines for peroperative management have emerged. For adjustment of these guidelines, fully standardized animal experimental work is needed. The purpose of the present study is to assess the peroperative complications that occurred in a standardized series of canine liver transplantation experiments, using partial livers.

Material and methods

All experiments were conducted in accordance with the laws of The Netherlands governing animal experiments. The animals were male beagle dogs from an outbred colony. The mean age at operation was 481 (SD 78) days and the mean

body weight 12.8 (SD 1.9) kg.

Thirty experiments were performed. Twenty dogs received 60% of a donor liver orthotopically and ten dogs were given a control operation consisting of vascular exclusion and cooling of the liver in situ, a 40% partial hepatectomy, and revascularization of the liver remnant. During the anhepatic phase, which lasted 53 (SD 4) minutes in the transplantation and 70 (SD 5) minutes in the control experiments, decompression of the infradiaphragmatic venous systems was achieved by a femoro- and porto-jugular bypass. Total liver ischemia lasted 308 (SD 19) and 70 (SD 5) minutes in the transplantation and control groups, respectively. The control group was used to study the effects of surgical trauma and liver ischemia on the outcome in the absence of immunological factors. Surgical details have been published elsewhere.[2]

The same anesthesiological protocol was used for both groups. On the day before surgery, the regular kennel diet was replaced by a liquid diet. At 5 p.m. all leftover food was removed, leaving only water. Premedication consisted of i.m. fentanyl 0.01 mg per kg, dehydrobenzperidol 0.5 mg per kg, and atropine 0.02 mg per kg. After induction of anesthesia with i.v. fentanyl 0.02 mg per kg and subsequent muscle relaxation with pancuronium 0.1 mg per kg, a cuffed Portex^{R} tube nr. 9 was inserted into the trachea and artificial ventilation with a Siemens 900^{R} ventilator was started. The end-expiratory CO_2 volume concentration was determined with a Meinhardt^{R} capnograph. A tidal volume of 24 ml per kg at a rate of 12 per minute and an inspiratory-to-expiratory ratio of 1 to 2 were used for a 2-to-1 N_2O-O_2 gas mixture.

The dogs were put on a warm-water mattress in a supine position. Two infusion systems were connected to a right cephalic vein cannula, one for a 4.2% $NaHCO_3$ solution given at an hourly dosage of 4 ml per kg body weight and

the other for a solution containing 112.5 mmol KCl and 80 mmol glucose per liter given at an hourly dosage of 8.5 ml per kg body weight. A left cephalic vein cannula was kept open with lactated Ringer's solution at a rate of 5 drops per minute. This cannula served for the administration of any additional fluids. A nasogastric tube, esophageal temperature probe, urine catheter, and intradermic ECG electrodes were inserted. The right external jugular vein and right femoral artery were cannulated for pressure measurements and for blood sampling. A 1.18% NaCl, 3.4% glucose solution containing 2 U heparin per ml was used for flushing of the central lines. The dogs were not heparinized during the anhepatic phase.

At the start of the operation enflurane was given in a concentration of 1%, but during surgery this concentration was gradually reduced to 0.6%. At the beginning of the anhepatic phase and again at revascularization of the liver, enflurane administration was temporarily stopped. When muscle relaxation was needed, pancuronium was given i.v. in a dose of 0.05 mg per kg. All dogs received 10 ml per kg fresh packed cells immediately before and 20 ml per kg fresh plasma immediately after the start of the anhepatic phase. Additional plasma as well as lactated Ringer's solution were rapidly infused in the event of hypotension. At the end of the anhepatic phase fresh blood was brought into readiness to cope with any major loss of blood after revascularization. Per 100 ml citrated blood or blood components, 1 ml Ca Cl_2 10% was slowly injected.

After surgery, the dogs were kept anesthetized until rewarming up to 36^{o}C had been effected. Persisting muscle relaxation was reversed with prostigmine 0.05 mg per kg i.v. under cover of atropine 0.025 mg per kg i.v. Enflurane and N_2O were stopped and extubation was carried out in the dog's preheated cage. A hypodermic infusion of 20 ml glucose 10% containing 1.5 mmol KCl per kg was

administered. One cephalic vein cannula was sealed off and left in situ till the next morning for intravenous fluid administration, if required.

Blood sampling and observation recording were done at standardized time-points during the course of the experiments (Table 1).

Table 1. Time-points of blood sampling and observation recording.

0 = base line (no starvation, anesthesia, or surgery)
1 = under premedication, before induction of anesthesia
2 = 10 minutes after the start of surgery
3 = 30 minutes after the start of surgery
4 = 60 minutes after the start of surgery
5 = immediately after the start of the anhepatic phase
6 = immediately before the end of the anhepatic phase
7 = 30 minutes after revascularization
8 = at the end of surgery
9 = at the end of the anesthetic period
10 = 3 hours after the end of the anesthetic period
11 = 9 a.m. on the day after surgery
12 = 4 p.m. on the day after surgery
13 = 9 a.m. on the 4th day after surgery

For points 2 - 9 sampling concerned arterial blood, points 0 - 1 and points 10 - 13 venous blood.

Statistical analysis

The significance of the changes observed within each group was tested with the rank sign test at $P < 0.05$. Significant changes are indicated by a plus sign and non-significant ones by a minus sign in the Tables and Figures. Differences in changes between the two groups were assessed with Wilcoxon's test, also at $P < 0.05$. Significant differences between the two groups are also indicated (⊙) in the Figures and Tables.

Results

Survival

None of the transplanted or control dogs died during surgery and all but one dog awoke promptly at the conclusion of the anesthetic period. One control dog was, however, prematurely extubated and developed a respiratory and then cardiac arrest. Although resuscitation was successful in re-establishing cardiac action and spontaneous breathing, severe neurological damage had occurred and the dog was killed the next morning.

Duration of anesthesia

The mean duration of the periods under anesthesia is given for both groups in Table 2. For the transplanted group the preanhepatic surgical time includes the time needed for bench surgery on the donor liver, which was on average 32.25 (SD± 7.46) minutes. For the control group the duration of the anhepatic phase was taken about 20 minutes longer than for the transplanted group as a debatable compromise for the shorter total ischemic time of the liver in the control group. In the latter group the durations of the anhepatic phase and of liver ischemia were obligatorily equal. The longer postanhepatic surgical time in the transplanted group is explained by the need for an arterial anastomosis in this group. The greater loss of blood in this group also contributed to the duration differences. The time needed to bring the dogs up to temperature after surgery was about the same in both groups.

Table 2. Duration of anesthesia (mean in minutes ± 1 SD), not including the period under premedication.

	transplanted dogs	control dogs
pre-surgical	25.70 ± 6.56	23.40 ± 5.37
- preanhepatic	139.15 ± 12.23	115.60 ± 12.03
surgical - anhepatic	53.05 ± 4.47	70.40 ± 5.73
- postanhepatic	141.85 ± 13.47	101.60 ± 13.65
post-surgical	82.25 ± 24.74	84.50 ± 25.76
total	442.00 ± 35.50	395.50 ± 21.40

Body temperature

As shown in the body-temperature profiles (Fig. 1), the temperature dropped by about 2°C during premedication. During surgery, in spite of a high room temperature and the use of a warm-water mattress, the body temperature dropped progressively and with a sharper decline during the anhepatic phase, when the 4°C transplant was inserted or the liver of the control dog was cooled in situ.

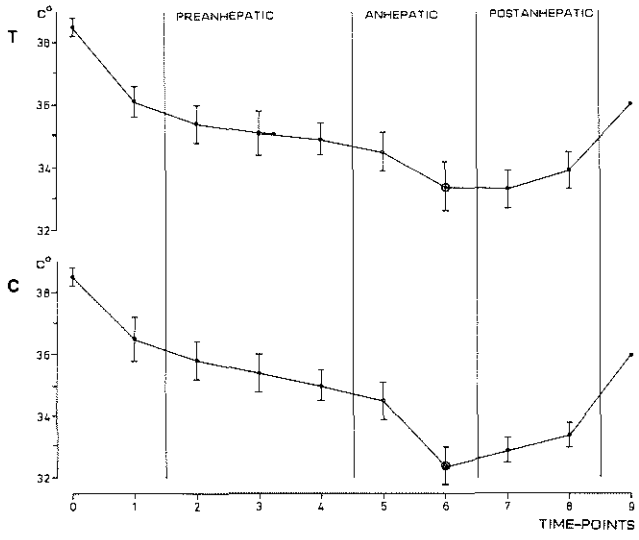

Fig. 1. Body temperature (mean ± 1 SD).
The body temperature dropped about 2°C during the period under premedication, further during surgery, and more sharply during the anhepatic phase.

Artificial ventilation

At the described ventilator settings, only minor tidal volume adjustments were required occasionally. The blood pCO_2 and the end expiratory CO_2 volume concentration showed a transient decrease at the beginning of the anhepatic phase, but then increased to reach peak values 30 minutes after revascularization (Fig. 2).

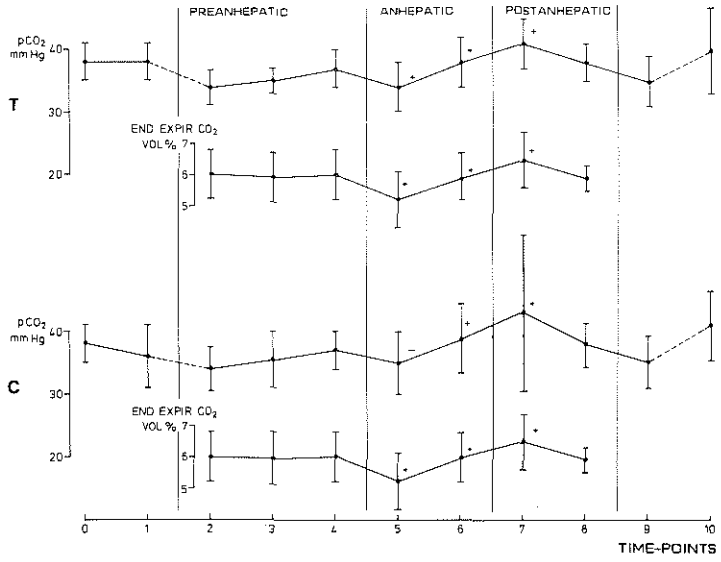

Fig. 2. Blood pCO_2 and end expiratory CO_2 volume concentration (mean $\pm$ 1 SD).
Blood samples 2 - 9 were arterial blood, the others were venous blood.
The arterial pCO_2 and the end expiratory CO_2 volume concentration decreased at the beginning of the anhepatic phase and then increased reaching peak values 30 minutes after revascularization.

Hemodynamics

The mean pulse rate and the mean systolic as well as the mean diastolic arterial pressure throughout the preanhepatic, anhepatic, and postanhepatic phases are given in Table 3, which also shows the mean values for the first blood-pressure recordings made during the anhepatic and postanhepatic phases. It is evident that there was a slight increase in the mean pulse rate at the close of the preanhepatic phase, whereas the mean systolic arterial pressure decreased slightly. The mean diastolic arterial pressure decreased only in the postanhepatic phase. All with-in group changes reached significance but the between-group changes did not.

The mean of the first arterial pressure recordings during the anhepatic and postanhepatic phases did not differ from the mean for the entire corresponding period with the exception of the mean of the first systolic arterial pressure values in the postanhepatic phase in the transplantation group, which was significantly lower. Again, differences between the groups were not significant.

An isolated drop in the arterial blood pressure during the anhepatic phase sometimes occurred because of accidental kinking of the bypass. Severe prolonged hypotension occurred in only one dog, a control whose systolic blood pressure fell from 140 to 60 mmHg at the start of the anhepatic phase. After about 20 minutes of rapid fluid loading with plasma and lactated Ringer's solution, the systolic blood pressure rose to about 100 mmHg and then remained at that level.

There were no major changes in the central venous pressure, even when overt bypass dysfunction occurred.

Evidence of suboptimal decompression of the infradiaphragmatic portal and systemic venous systems was seen in all experiments. Although the use of a bypass during the anhepatic phase together with fluid loading

Table 3. Hemodynamic monitoring

	pulse rate per minute		blood pressure in mmHg			
			systolic		diastolic	
	T	C	T	C	T	C
preanhepatic						
mean of all values	149.50	139.60	151.20	151.00	103.05	105.20
SD	17.72	10.26	11.45	13.84	10.85	13.29
anhepatic						
mean of all values	167.35^{+}	153.20^{+}	145.05^{+}	137.40^{+}	104.85^{-}	97.10^{-}
SD	14.98	10.26	16.55	18.62	12.77	14.88
mean of first values only			144.35	136.00^{-}	108.50^{-}	98.60^{-}
SD			18.46	32.64	15.13	23.60
postanhepatic						
mean of all values	163.15^{-}	155.30^{-}	147.40^{-}	145.10^{-}	92.80^{+}	89.50^{+}
SD	14.90	13.88	17.26	5.87	14.40	10.73
mean of first values only			129.50^{+}	138.50^{-}	84.25^{-}	84.80^{-}
SD			29.55	23.69	25.09	25.19

T = transplanted dogs

C = control dogs

+ = significant, - = not significant (rank sign test, $P<0.05$).

The mean of all values for a given phase was compared with that of the preceding phase, whereas the mean of the first recordings was compared with that of all values for the same phase. (Application of Wilcoxon's test to both groups showed no significant differences).

and enflurane withdrawal prevented prolonged hypotension, urine production virtually ceased during the bypassing period (Table 4) and the subsequently produced urine contained increasing amounts of blood and protein. Concomitantly, there was a transient increase in the serum creatinine level (Fig. 3). The absence of a corresponding increase of BUN may have been due to the absence of the liver, since in dogs BUN is produced exclusively by this organ.[3] During the bypassing period the bowel and spleen always became slightly engorged, and all dogs passed a loose stool mixed with some blood upon awakening.

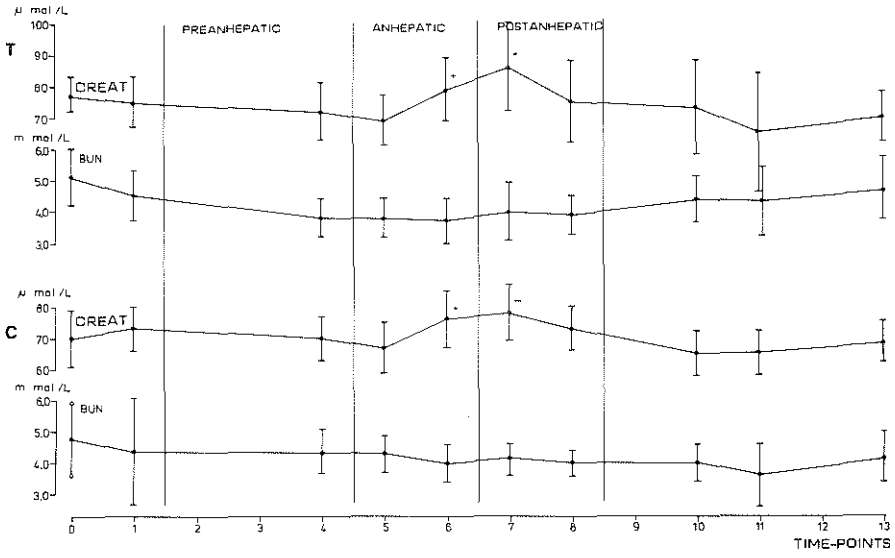

Fig. 3. Serum creatinine and blood urea nitrogen (mean ± 1 SD).
The serum creatinine values increased by the end of the anhepatic phase in the absence of a concomitant increase in the BUN values.

Fluid balance

The intake and output of fluid are shown in Table 4. The two groups of dogs received the same amounts of the standard, $NaHCO_3$ and lactated Ringer's solutions. A larger amount of blood was given in the transplantation group. Urine production was similar in the two groups and showed a significant reduction during the anhepatic phase.

Table 4. Fluid balance.

	transplantation group	control group
INTAKE		
maintenance fluid (per kg per hour)	mean ± 1 SD	mean ± 1 SD
standard solution	7.02 ml ± 0.78	6.90 ml ± 0.47
$NaHCO_3$ 4.2%	2.83 ml ± 0.59	3.04 ml ± 0.43
total	9.86 ml ± 0.59	9.95 ml ± 0.67
Na^+	1.41 mmol	1.52 mmol
K^+	0.82	0.81
Cl^-	0.82	0.81
$NaHCO_3^-$	1.41	1.52
glucose	0.56	0.56
extra fluid (per kg)	mean ± 1 SD	mean ± 1 SD
packed cells	29.0 ml ± 10.0 ⊙	16.0 ml ± 5.8 ⊙
plasma	35.9 ml ± 12.9 ⊙	25.2 ml ± 13.3 ⊙
lactated Ringers' solution	21.2 ml ± 6.7	19.6 ml ± 14.6
total	86.2 ml ± 23.8 ⊙	61.0 ml ± 30.0 ⊙
OUTPUT		
urine (per kg per hour)	mean ± 1 SD	mean ± 1 SD
preanhepatic phase	3.70 ml ± 1.71	3.10 ml ± 1.06
anhepatic phase	0.94 ml ± 1.12	0.97 ml ± 0.61
postanhepatic phase	5.35 ml ± 3.29	7.05 ml ± 3.10
total	3.94 ml ± 1.83	3.90 ml ± 1.15

Acid-base balance

All blood samples were analysed at 37°C. No gross pH changes were observed (Fig. 4). The initial rise of the base excess values is in all probability related to the fact that after the induction of anesthesia, arterial blood was analysed instead of venous blood (Fig. 5). The base excess values remained relatively constant during surgery. At the end of the operation and during the early postoperative hours there was, however, a relative increase of the pH and base excess values (Figs. 4 and 5).

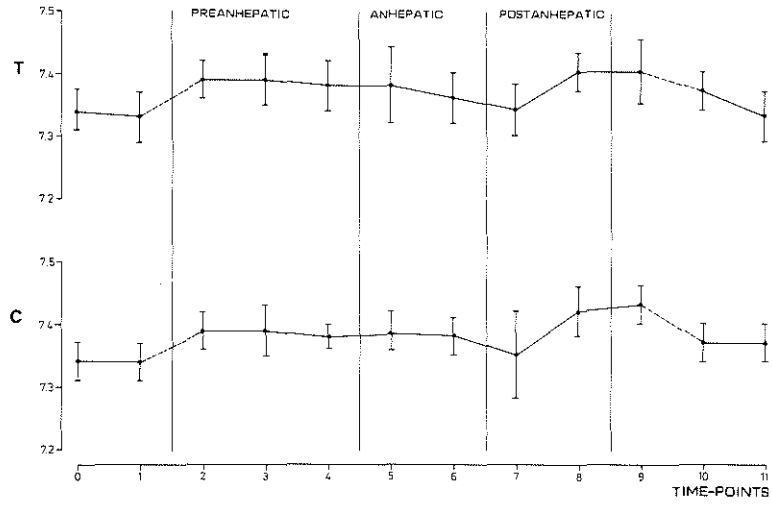

Fig. 4. Blood pH (mean ± 1 SD).
Samples 2 - 9 were arterial blood, the others were venous blood.
The pH level was maintained well during surgery but showed a relative increase at the end of the operation and in the early postoperative period.

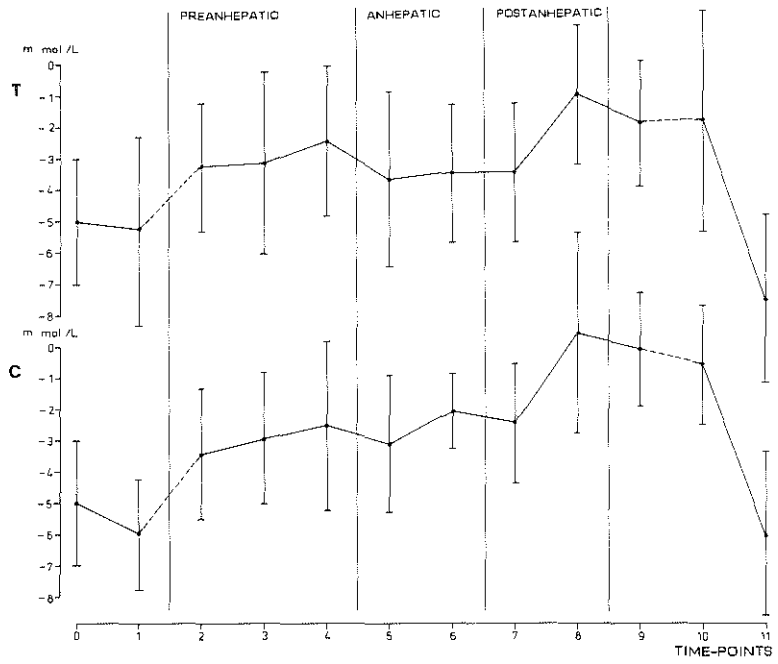

Fig. 5. Base excess (mean ± 1 SD).
Samples 2 - 9 were arterial blood, the remaining ones were venous blood.
During surgery the base excess values were slightly above normal and even higher values were seen at the end of the procedure and during the early postoperative course.

Serum sodium and potassium changes

The serum sodium values remained within a range of 140 to 150 mmol per liter. The serum potassium values rose during the anhepatic phase and reached peak values at the end of that phase. No electrocardiographic changes were observed. After the anhepatic phase the serum potassium levels dropped and tended to lie slightly under the base-line values in the early postoperative period (Fig. 6).

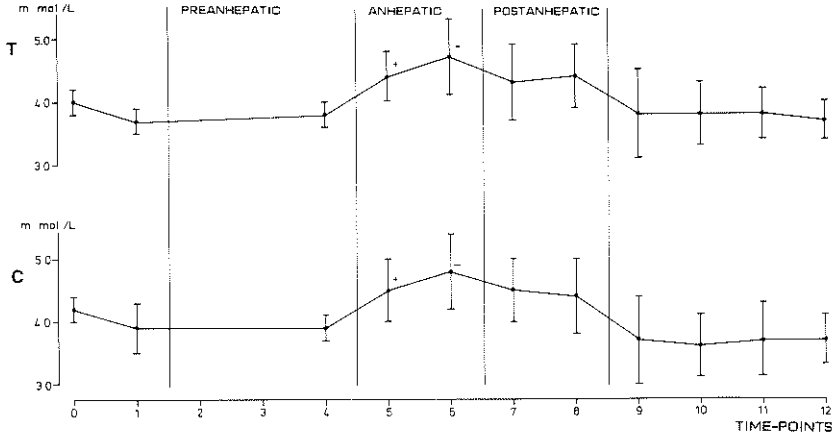

Fig. 6. Serum potassium (mean ± 1 SD).

The serum potassium values increased during the anhepatic phase but no increase occurred after revascularization.

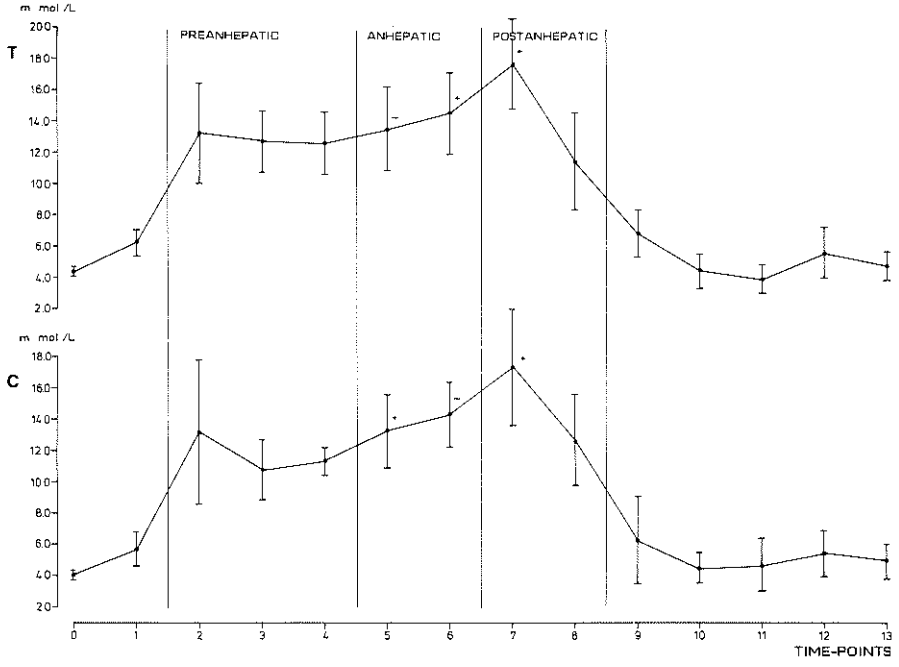

Fig. 7. Blood glucose (mean ± 1 SD).

Hyperglycemia occurred consistently throughout the procedure and there was no increased glucose need during the anhepatic phase.

Blood-sugar changes

Despite the administration of only small doses of glucose, hyperglycemia and glucosuria occurred consistently throughout the anesthetic period. The blood-sugar levels did not decline during the anhepatic phase and peak levels were measured shortly after revascularization (Fig. 7).

Serum total protein changes

The serum total protein content declined progressively during the operation. During the anhepatic phase a slight increase or a plateau was seen after plasma administration (Fig. 8).

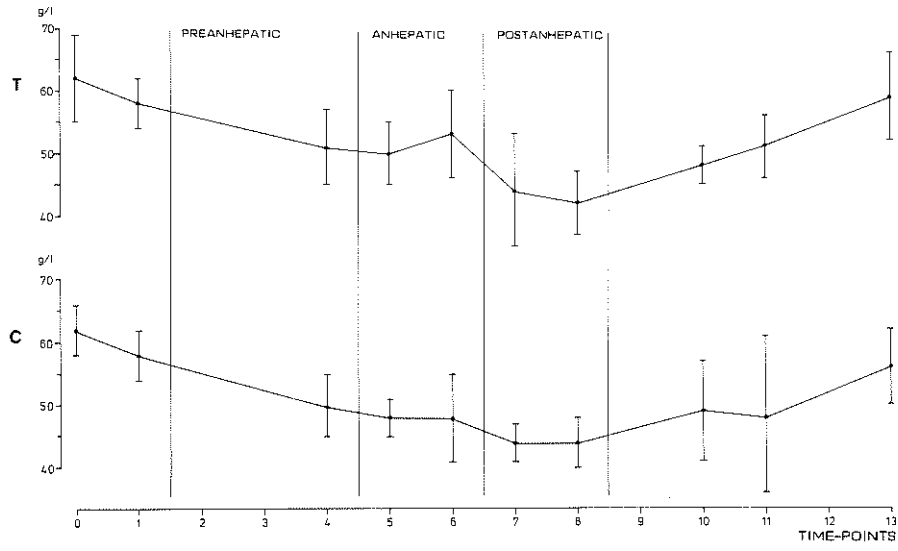

Fig. 8. Total serum protein (mean ± 1 SD).
The progressive decrease during the procedure was interrupted by a slight increase or plateau after plasma administration during the anhepatic phase.

Hemostasis and coagulation

Clinical evidence of hemorrhagic diathesis was found in all experiments,but the signs were most pronounced in the transplantation group. At the end of the anhepatic phase the incisions started to ooze, but the loss of blood, which was diffuse, only became important shortly after revascularization of the liver. During further surgery the loss of blood decreased progressively and had always stopped by the end of the anesthetic period.

Concomitantly, marked changes in the platelet count were observed. The anhepatic phase showed a rapid and major decrease, the lowest counts occurring at the end of the phase in controls and 30 minutes after revascularization in transplanted dogs. At the end of the operation a relative increase of the platelet count occurred (Fig. 9).

For the monitoring of the coagulation activity during the experiments, use was made of Normotest^{R}.[4] This test, designed for coagulation assessment in man, measures the combined activity of factors II, VII, X, and, possibly, factor IX. In healthy beagles it gives extremely short coagulation times, which makes the sensitivity of the test too low. However, dilution of the canine plasma samples by a factor of three with Veronal buffer containing citrate in the same concentration as in the undiluted plasma gave a "modified" Normotest coagulation time of about 23 seconds, which corresponds with a normal or 100% value for human plasma. With this modification, the test was found to be sufficiently sensitive, covering levels between 20% and 120%. Compared with the preanesthetic values, the preanhepatic phase showed a 20% decrease occurring progressively. During the anhepatic phase the administration of fresh plasma was followed by an increase of about 5%. Shortly after revascularization, the values dropped further and no increase was observed during the rest of the operation. The lowest values were

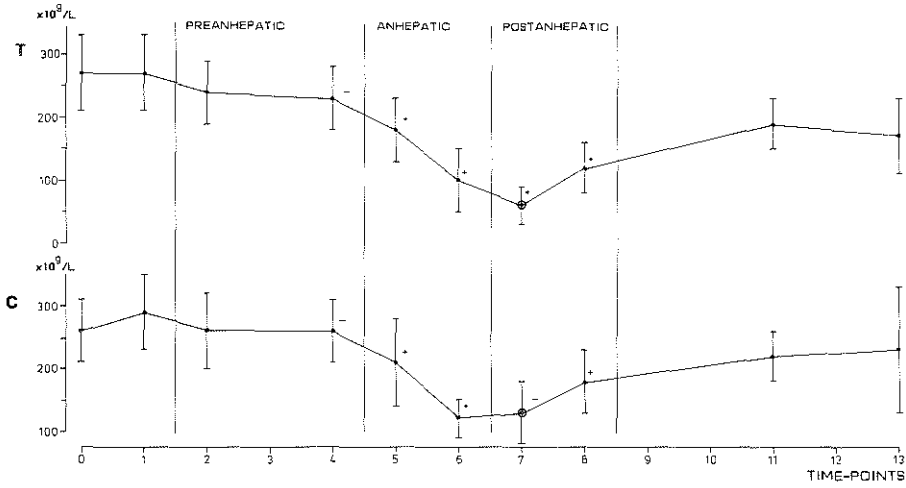

Fig. 9. Platelet count (mean ± 1 SD).

A strong and progressive drop in the number of circulating platelets occurred during the anhepatic phase. The lowest counts were noted 30 minutes after revascularization in the transplantation group and at the end of the anhepatic phase in the control group.

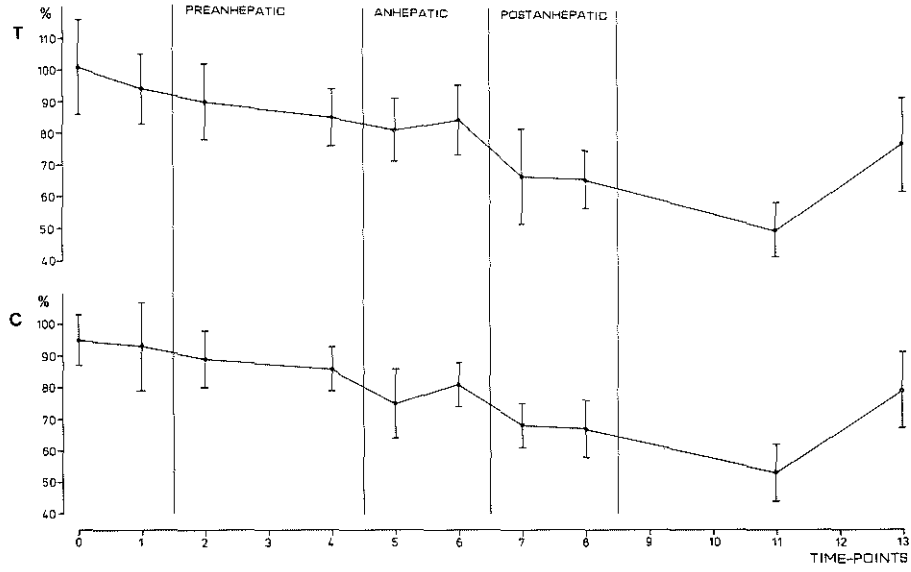

Fig. 10. "Modified Normotest" results (mean ± 1 SD).

The progressive decrease during the procedure is apparent. A small increase followed plasma administration in the anhepatic phase. The lowest values were found on the morning after the operation.

measured the morning after the operation (Fig. 10).

Discussion

All but one of the dogs regained consciousness promptly at the end of the period under anesthesia, thus confirming the beneficial value of the peroperative management protocol used. Under premedication the dogs were easy to handle but the body temperature decreased by about 2°C, which means that more attention should have been paid to the temperature of the environment. During surgery the body temperature continued to decrease progressively, as reported for clinical liver transplantation.[5,6]

An inhalation type of anesthetic agent was chosen because this category permits quick adjustments. Enflurane was used because no major hepatotoxic effect has been reported for this drug.[7] However, the choice of the anesthetic agent seems less important than the peroperative management and the surgical technique as factors affecting the over-all outcome.[8]

Unlike Popescu, we do not feel that the beagle is difficult to ventilate correctly.[9] With the described ventilator settings, adjustments were rarely required. Like Popescu, we too observed a decrease in the end expiratory CO_2 volume concentration during the anhepatic phase.[10] At the same time there was a small decrease in the arterial pCO_2. Since these changes occurred immediately after the start of the anhepatic phase and normalized spontaneously during the rest of that phase, they cannot be ascribed to the absence of the liver or hypothermia. The changes in the venous return to the heart may offer a better explanation. At the start of the anhepatic phase the infradiaphragmatic venous systems were temporarily occluded for the insertion of the bypass, and this reduced the venous return considerably.

Release of the bypass clamps restored the venous return at least partially. Because some degree of congestion in spite of the bypass was unavoidable, it may be assumed that the mobilization of stagnant blood after removal of the bypass was responsible for the postrevascularization increase in the capnographic and arterial pCO_2 readings.

The intravenous fluid regimen we used is susceptible to criticism from various points of view. In the first place, substantial plasma losses into the peritoneal cavity occurred from the beginning of the operation, as reflected by the progressive decrease of total serum protein in the absence of changes in hematocrit values. Therefore, the systematic administration of fresh frozen plasma from the beginning of the operation, as advocated by Fortner,[11] seems recommendable, and might have prevented the preanhepatic prolongation of the coagulation time.

On the basis of the canine studies done by Popescu, who found some of the deaths to be related to hypokalemia, potassium was given throughout the procedure.[12] Even though electrocardiography showed no toxic effects, the serum potassium levels invariably rose during the anhepatic phase, as observed in human liver transplantation.[6] This phenomenon can be explained by an insufficient infradiaphragmatic venous decompression resulting in impaired kidney function and portal venous congestion. During acute total portal vein occlusion in the dog the serum potassium levels are known to rise rapidly,[13] which makes it seem unwise to continue potassium administration during the anhepatic phase.

Since a venous base deficit of about 5 mmol per liter is normal in nonfasting, nonanesthetized dogs, it can be concluded that the dogs were in a relatively alkalotic state during most of the procedure. This raises questions about the need for routine continuous administration of large amount of sodium bicarbonate.

In human liver transplantation, the policy of using large amounts of sodium bicarbonate has already been discredited.[8]

In the initial period of orthotopic liver transplantation large amounts of exogenous glucose were administered, especially during the anhepatic phase.[14] This policy was based on the knowledge that hepatectomized dogs usually die from hyperglycemia in the early postoperative period,[15] and that hypoglycemia is a common finding in acute liver failure in man.[16] Later, it was recognized that hypoglycemia was a consistent finding during human liver transplantation, even during the anhepatic period, and that the degree of posttransplantation hypoglycemia was related to the quality of the donor liver.[6,8] Although small amounts of glucose were given in the present experiments, hyperglycemia was a consistent finding even during the anhepatic phase. Therefore, less glucose should have been given, and perhaps none at all.

Pathological bleeding remains a common and serious problem in clinical and experimental liver transplantation.[6,8,17-19] The most important factor in the severity of the bleeding is said to be the quality of the donor organ;[20] the preoperative coagulation status of the recipient plays a far less important role.[19] However, little is known about the pathogenesis of the bleeding disorder. In this series of experiments the course of the hemorrhagic diathesis was closely related to the changes in the platelet count. The sharp decrease of the platelet count during the anhepatic phase marked the onset of the hemorrhagic diathesis which became clinically manifest at the end of the anhepatic phase in spite of a reasonable coagulation time at that time. Compared with the control group, the transplantation group showed a significantly lower platelet count 30 minutes after revascularization. This phenomenon, together with the much greater loss of blood after revascularization in the transplantation

group, might be related to the difference between the preservation time of the liver in the two groups. But a greater loss of blood and consumption of platelets can be anticipated in the transplantation group, because more vascular suturing was needed in this group. During futher surgery a gradual cessation of the bleeding was accompanied by a rise of the platelet count despite a further prolongation of the coagulation time. According to Groth and Böhmig, the platelet-count drop during orthotopic liver transplantation is due mainly to the induction of platelet aggregation and intravascular coagulation within the graft.[17,19] In the present study, however, the platelet-count decrease was related more closely to the anhepatic phase than to the revascularization of the liver. Trapping and perhaps destruction of platelets in the insufficiently decompressed infradiaphragmatic venous systems might be responsible for this phenomenon. Our findings suggest that the course of the hemorrhagic diathesis is related to changes in the platelet-count rather than to changes in the coagulation activity. It is not known whether the administration of platelet concentrates during the anhepatic phase, which is current practice in human liver transplantation, would have changed the course of the hemorrhagic diathesis seen in our study.[8]

Many of the peroperative difficulties we encountered seemed to reflect insufficient decompression of the portal and systemic infradiaphragmatic venous systems. Although less pronounced and not lethal, the changes observed during the anhepatic phase resembled those seen during canine total portal[13,21] and suprarenal caval vein occlusion.[22] Similar changes also occur during clinical liver transplantations where no bypass is used. Although in man bypassing is stated to be unnecessary and even dangerous,[23] congestion undoubtedly occurs in the infradiaphragmatic venous systems. Furthermore, the effects of clamping of the infradiaphragmatic venous

systems in the absence of a bypass are poorly tolerated by patients without preexistent portal hypertension. For such cases the use of a partial cardiopulmonary bypass has been advocated.[24] The systematic use of a safe and effective bypassing system might therefore avoid many of these problems and thus render orthotopic liver transplantation a much less risky form of treatment.

The peroperative complications cannot be ascribed to immunological factors, because the transplantation and control groups behaved similarly.

The use of a partial instead of a total liver did not seem to require any modification of the peroperative management.

REFERENCES

1. Brown BR: Editorial comment. Contemp Anesth Pract 4:111, 1981
2. Bax NMA, Vermeire BMJ, Dubois N, et al: Orthotopic nonauxiliary homotransplantation of part of the liver in dogs. J Pediatr Surg 17:906-913, 1982
3. Mann FC, Magath TB: Studies in the physiology of the liver. III. The nitrogen constituents of the blood following removal of the liver. Am J Physiol 55:286-287, 1921
4. Owren PA: Normotest in the evaluation of the liver function, in Hemker JC, Loeliger EA, Veltkamp JJ (eds): Human Blood Coagulation, Leiden University Press, 1969, p 369
5. Aldrete J, Clapp HN, Starzl TE: Body temperature changes during organ transplantation. Anesth Analg 49:384-388, 1970
6. Farman JV, Lines JG, Williams RS, et al: Liver transplantation in man. Anaesthetic and biological management. Anaesthesia 29:17- 32, 1974
7. Black GW: Enflurane. Br J Anaesth 51:627-640, 1979

8. Aldrete JA, Goldman E, De Campo T: Anesthetic implications in hepatic transplantation. Contemp Anesth Pract 4:111-112, 1981
9. Popescu DT: Anaesthetic and Homeostatic Problems in Experimental Orthotopic Liver Transplantation in Dogs. Leiden, Doctoral Thesis, 1973, p 67
10. Popescu DT: Anaesthetic and Homeostatic Problems in Experimental Orthotopic Liver Transplantation in Dogs. Leiden, Doctoral Thesis, 1973, pp 72-75
11. Fortner JG, Beattie EJ, Shiu MH, et al: Orthotopic and heterotopic liver homografts in man. Ann Surg 172:23-32, 1970
12. Popescu DT: Anaesthetic and Homeostatic Problems in Experimental Orthotopic Liver Transplantation in dogs. Leiden, Doctoral Thesis, 1973, pp 93-97
13. Bergan J, Teixera E, Yu H, et al: Hyperkalemia accompanying canine portal venous occlusion. Surgery 64:629-633, 1968
14. Aldrete J: Anaesthesia and intra-operative care, in Starzl TE, Putnam CW (eds): Experience in Hepatic Transplantation. Saunders, Philadelphia, 1969, pp 83-111
15. Mann FC, Magath TB: Studies in the physiology of the liver. III. The liver as a regulator of the glucose concentration of the blood. Am J Physiol 55:285-286, 1921
16. Saunders SJ, Seggie J, Krisch RE, et al: Acute liver failure, in Wright R, Alberti KGMM, Karran S, et al (eds): Liver and Biliary Disease. Saunders, London, 1979, pp 569-584
17. Groth CG, Pechet L, Starzl TE: Coagulation during and after orthotopic transplantation of human liver. Arch Surg 98:31-34, 1969

18. Schalm SW, Terpstra JL, Achterberg JR, et al: Orthotopic liver transplantation: an experimental study on mechanisms of hemorrhagic diathesis and thrombosis. Surgery 78:499-507, 1975
19. Böhmig HJ: The coagulation disorder of orthotopic hepatic transplantation. Semin Thromb Hemost 4:57-82, 1977
20. Starzl TE, Putnam CW: Experience in Hepatic Transplantation. Saunders, Philidelphia, 1969, pp 65-80
21. Johnstone FRC: Acute ligation of the portal vein. Surgery 41:958-971, 1957
22. Whittenberger JL, Huggins C: Ligation of the inferior vena cava. Arch Surg 41:1334-1343, 1940
23. Starzl TE,Putnam CW: Experience in Hepatic Transplantation. Saunders, Philidelphia, 1969, pp 112-143
24. Calne RY, McMaster P, Smith DP, et al: Use of partial cardiopulmonary bypass during the anhepatic phase of orthotopic liver grafting. Lancet 2:612-614, 1979

CHAPTER 9. VASCULAR AND BILIARY COMPLICATIONS

9.1. Vascular complications

9.1.1. Methods of diagnosis

Vascular complications were diagnosed angiographically and at autopsy. Pervenous angiography was performed routinely on the 4th and 11th postoperative days and additionally on indication. The technique used has been published and the text is reproduced here by permission.

PERVENOUS HEPATIC TRANSPLANT ANGIOGRAPHY USING A NEW LOW OSMOLAR CONTRAST MEDIUM - AN EXPERIMENTAL STUDY IN DOGS -

N.M.A. Bax,[1] M. Meradji,[2] and J.C. Molenaar.[1]
From the Departments of Pediatric Surgery,[1] and Radiology,[2] of the School of Medicine of Erasmus University, Rotterdam, The Netherlands.
This study was financially supported by the Sophia Foundation for Scientific Research.
Published in Invest Radiol 17:299-304, 1982.
Reproduced by permission.

Summary

The patency of the vascular anastomoses in a canine liver transplantation model was imaged by serial pervenous angiography using ioxaglate, which is a new

ionic low osmolar contrast medium. No premedication or general anesthesia was administered. No major adverse reaction was noted, not even after repeated angiography. Except for the occurrence of one hematoma formation and one paravenous injection which resolved without sequellae, no local complications occurred. Sufficient information regarding the patency of the hepatic artery anastomosis was obtained in 94% of the angiographic studies, and in 70% the patency of the caval anastomoses could be determined. In addition, portal vein patency was imaged in 21% of the angiograms. Even better visualization results can be expected by combining this method with video subtraction techniques, although patient cooperation or general anesthesia would then be mandatory.

Introduction

Orthotopic liver transplantation involves one biliary and four vascular anastomoses. The biliary anastomosis is considered to be the Achilles' heel of liver transplantation,[1] and many complications have been reported. However, biliary anastomotic complications can easily be detected and interpreted by T-tube cholangiography. Surprisingly enough, complications resulting from the vascular anastomoses have been reported less frequently in clinical liver transplantation, but the patency of the various types of vascular anastomosis was not monitored routinely. In contrast, an incidence of hepatic artery thrombosis of up to 38% has been reported in canine liver transplantation, in studies where the arterial anastomosis was angiographed on a more routine basis.[2]

Whereas thrombosis of the allograft arterial supply results in the death of canine recipients within a few hours, the effects of arterial thrombosis in transplanted

children may be delayed for several days. What is more, the results of e.g. serum transaminase determinations or liver scans may well remain normal or at least nondiagnostic until the situation has become irreversible, and it is likely that aortography will prove to be the only really decisive way of consistently establishing the diagnosis while there is still time for repair.[3]

Although accurate from the diagnostic point of view, classic arteriography has serious drawbacks mainly related to the need for arterial catheterization and to the hyperosmolality of the commonly used contrast media.[4-6] Since the injection is painful, premedication or general anesthesia is mandatory in children or laboratory animals such as dogs, which may be perilous in cases with poor graft function. Consequently, classic arteriography is unsuitable as a routine evaluation procedure.

To evaluate the patency of the various vascular anastomoses, and particularly of the hepatic artery, in a new canine liver transplant model, use was made of a less invasive angiographic technique based on the principle of pervenous angiography but using a new contrast medium of lower osmolality.

Methods

Laboratory animals and surgical procedures

The laboratory animals consisted of 38 dogs, predominantly beagles, of both sexes. They were approximately 1 year old, and their body weight averaged 12.7 kg, ranging from 6.7 to 15.3 kg. Twenty-five dogs received an orthotopic, partial liver transplant consisting of 60% of a donor liver, the left lateral and medial lobes having been removed. The venous anastomoses

were performed end-to-end. The donor hepatic artery with a celiac trunk patch was anastomosed end-to-side to the recipient celiac trunk once the recipient hepatic artery had been disconnected (Fig. 1).

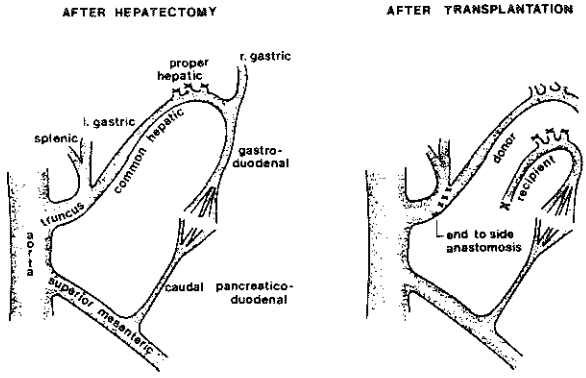

Fig. 1. Arterial anatomy in the recipient (lateral projection) after removal of the native liver and after insertion of the allograft.

Thirteen dogs had a sham operation. In these operations the liver was temporarily excluded from the circulaton by vascular clamping and cooled in situ, after which a 40% partial hepatectomy was carried out. Revascularization was achieved by releasing the vascular clamps. The sham operations served to evaluate the combined effects of surgical trauma, liver ischemia, an anhepatic phase, partial hepatectomy, biliary anastomosis, and immunosuppressive therapy, on animal survival, liver function, and liver regeneration in the absence of an allograft. Even though no vascular anastomosis was carried out in the sham operations, these 13 dogs were subjected to pervenous angiography as in the transplanted animals, in order to standardize procedures in both groups of dogs.

Contrast medium

Use was made of a new ionic, water-soluble agent, ioxaglate, which has a monoacid, dimeric structure with six iodine atoms per molecule. In a concentration of 320 mg iodine per ml, the osmolality amounts to 600 mmol/l. Figure 2 summarizes the chemical structure and the physicochemical properties of the agent.

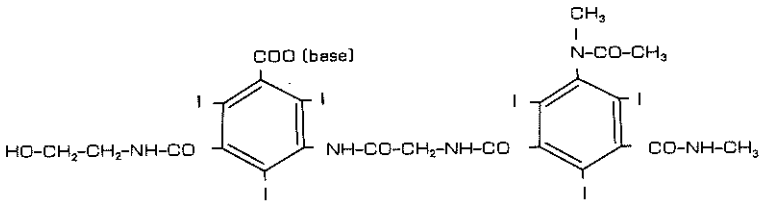

Fig. 2. Chemical formula of ioxaglate (Guerbet, Paris, France).

Composition:

molecular weight = 1269		iodine content	= 32 g%
salt content	= 59 g%	viscosity at 37°C	= 7.5 m Pa s
meglumin salt	= 39.4 g%	osmolality	= 600 mmol/l
sodium salt	= 19.7 g%	pH	= 6.5 - 7.6

Radiologic technique

A gauge 16.50 mm cannula (Braunula^{R}) was inserted into the cephalic vein and connected to an automatic pressure injector (Mark II Medrad, Inc.). A dose of 2.5 ml ioxaglate per kg body weight was injected in 4 seconds, and serial angiograms were taken at 0,6,8,10,12, and 14 seconds.

The dogs were not starved, and no premedication or anesthesia was administered. Because our main objective was the visualization of the celiac trunk and its major branches, a lateral decubitus positon was chosen. The dogs were kept in position on the table by two assistants, one holding the head and pelvis, the other holding the paws. The dogs were examined routinely on the

4th and the 11th postoperative days and, in addition, on indication.

All experiments were conducted in accordance with the regulations on animal experiments as prescribed by the Netherlands' law.

Results

Pervenous hepatic angiography was carried out 79 times in 38 dogs and resulted in 70 examinations that were technically satisfactory (Table 1).

Table 1. Assessment of pervenous angiography*.

	number of dogs	number of angiographic examinations	
		total	technically satisfactory
transplantation group	25	55	52
control group	13	24	18
total	38	79	70

* Pervenous angiography was carried out 79 times in a series of 38 dogs, resulting in 70 technically satisfactory examinations. In nine instances the angiographic examination did not succeed due to technical difficulties.

In one instance, the dog was too aggressive to be examined and in another instance, the cephalic vein could not be cannulated as a result of multiple previous venipunctures. Hematoma formation at the cannulation site, paravenous injection, and cannula disconnection each occurred once. Defective X-ray apparatus was responsible for the remaining four failures.

Seventy angiographic examinations were technically satisfactory.Each dog was examined one to five times (Table 2). An assessment of the quality of visualization of the main hepatic artery, the retrohepatic caval vein, and the portal vein is presented in Table 3.

Table 2. Specification of technically satisfactory angiographic examinations.

number of angiographic examinations per dog	number of dogs	total number of angiographic examinations
1	13	13
2	20	40
3	4	12
5	1	5
total	38	70

Table 3. Quality of visualization *

		transplantation group			control group			total		
			adequate			adequate			adequate	
		n	n	%	n	n	%	n	n	%
hepatic artery	excellent	14			3			17		
	good	30	51	98	9	15	83	39	66	94
	medium	7			3			10		
	poor	1			3			4		
caval vein	excellent	3			1			4		
	good	15	37	71	1	12	66	16	49	70
	medium	19			10			29		
	poor	15			6			21		
portal vein	excellent	1			0			1		
	good	2	12	21	0	3	16	2	15	21
	medium	9			3			12		
	poor	40			15			15		

* Assessment of 70 pervenous angiographs of 38 dogs, a total of 52 angiographs in the transplantation group and 18 in the control group. Quality of visualization is termed adequate when sufficient for diagnosis, ranging from medium (sufficient for determining patency) to excellent. Poor visualization includes no visualization.

The classification ranged from poor (insufficient or no visualization) to excellent, and from medium upwards the quality was adequate in that it was sufficient for determining patency. In the 6- and 8-second angiographic examinations, the rate of successful visualization of the patency of the main hepatic artery was as high as 94%. Retrograde filling of the recipient gastroduodenal artery and the amputated hepatic artery was seen on the angiograms of the transplanted dogs. The superior mesenteric artery was also clearly visible as were the renal arteries, albeit in a lateral projection with superimposition of the renal parenchyma (Fig. 3). Thrombosis of the hepatic artery was easily detectable as shown in Fig. 4 and Fig. 5. A retrohepatic cavogram was obtained in 70% of the 10- and 12-second angiographic examinations, and delineation of the portal vein was in the range of 21% in the 12- and 14-second angiograms (Fig.6).

On the whole, the dogs remained quiet during the injection of contrast media and the subsequent angiography procedure, though in a few instances there was some movement, resulting in partial blurring of the serial angiograms. There were two local complications, one hematoma and a paravenous injection, but these resolved without sequellae; the paravenous injection had subsided completely within 24 hours. Mild vomiting occurred in two instances, without any lasting effect on the general condition or appetite of the dogs concerned. Apart from this, no adverse reaction occurred, not even in the dog that had just been transplanted or in the dog with poor liver function, despite multiple angiographic examinations. As a matter of interest, we may add that in two dogs, dosages up to 7.5 ml ioxaglate per kg body weight were administered without any adverse reaction. These nonoperated dogs were used in a few pilot experiments performed to standardize the radiographic method.

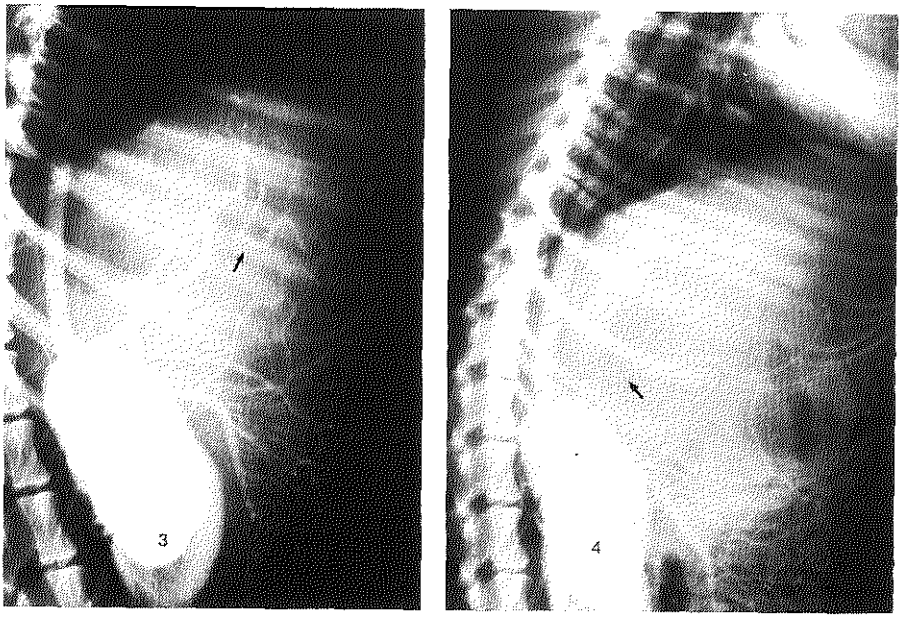

Fig. 3. Pervenous angiogram at 6 seconds.
The aorta, celiac trunk, and superior mesenteric artery are clearly visible. The transplanted hepatic artery has a dilated aspect, and retrograde filling of the gastroduodenal artery is visible (arrow). The renal parenchymas are seen in a superimposed lateral position.

Fig. 4. Pervenous angiogram at 8 seconds.
The transplanted hepatic artery shows a filling defect (arrow). The defect is not complete, since the distal artery is still visualized. Immediately caudal to the filling defect, the amputated retrograde-filled recipient hepatic artery can be seen. A cavogram was also obtained. At autopsy, the next day, the thrombus could be identified.

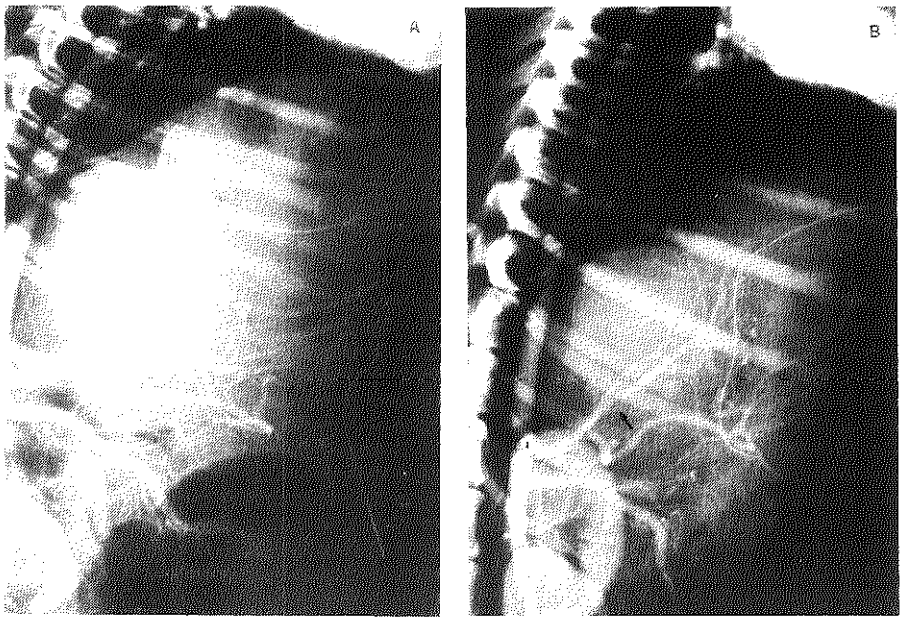

Fig. 5. Pervenous angiograms at 6 seconds.

(A) The transplanted hepatic artery is not visible. At repeat laparotomy, the hepatic artery was opened and a thrombus was extracted.

(B) On a pervenous angiogram made the next day, restoration of the hepatic arterial blood supply is shown, although the site of the arteriotomy is narrowed (arrow). Note the transcholedochal splint.

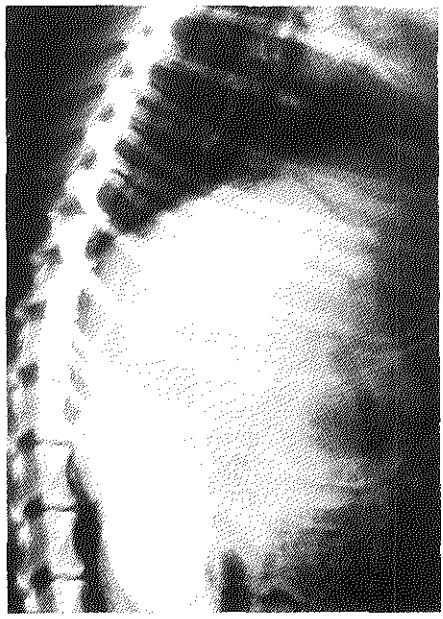

Fig. 6. Pervenous angiogram at 12 seconds.
While visualization of the arterial tree is fading, the retrohepatic caval vein shows up as does the portal vein.

Discussion

The technique of pervenous arteriography is not new. It was first described by Robb and Steinberg in 1939.[7] Other reports followed,[8-13] though the technique did not become widely accepted. The reason for this was that large quantities of strongly hypertonic contrast media had to be injected rapidly, which not only resulted in important hemodynamic changes, but also caused a great deal of pain during the actual injection.[4-6] What is more, the visualizaton of the peripheral arteries was very poor. In order to improve the visualization of the peripheral arteries and slow down the rate of injection of highly hypertonic contrast media, the usefulness of xeroradiography has been tested, with promising

results.[14] More recently, video subtraction techniques have become available, renewing the interest in pervenous arteriography[15-19] as lower doses of the classic contrast media can be used. A requisite for satisfactory examination, however, is patient cooperation, which cannot be expected in small children or laboratory animals such as dogs.

With the introduction of contrast media with lower osmotic concentration, new possibilities have arisen in pervenous arteriography. These products cause less-pronounced hemodynamic changes and considerably less pain reaction during injection,[20-25] reducing the need for premedication or anesthesia. While the osmolality of the classic agents at an iodine concentration of 320 mg per ml at least quadruples the serum osmolality,[26] this is only doubled when ioxaglate is used at an identical iodine concentration. Consequently, higher doses might be injected, improving the quality of the visualization of the blood vessels. Although patient cooperation would certainly improve the quality of the x-rays, satisfactory results can be obtained in an anxious dog without premedicaton or anesthesia.

In combination with video subtracton techniques in the cooperative patient, further improvement in visualization can be expected, not only of the arterial but also of the venous phases and perhaps also of the portal circulation.

REFERENCES

1. Starzl TE, Koep LJ, Halgrimson CG, et al: Fifteen years of clinical liver transplantation. Gastroenterology 77:375-388, 1979
2. Schalm SW, Terpstra JL, Achterberg JR, et al: Orthotopic liver transplantation: an experimental study on mechanisms of hemorrhagic diathesis and thrombosis. Surgery 78:499-507, 1976

3. Starzl TE, Putnam CW: Experience in Hepatic Transplantation. Philadelphia, Saunders, 1969, pp 145-148
4. Friesinger GC, Schaffer J, Criley JM, et al: Hemodynamic consequences of the injection of radiopaque material. Circulation 31:730-740, 1965
5. Hilak SK: Hemodynamic changes associated with intra-arterial injection of contrast media. Radiology 86:615-633, 1968
6. Lindgren P: Hemodynamic responses to contrast media. Invest Radiol 5:424-435, 1970
7. Robb GP, Steinberg I: Visualization of the chambers of the heart, the pulmonary circulation, and the great blood vessels in man. A practical method. AJR 41:1-17, 1939
8. Steinberg I, Finby N, Evans JA: A safe and practical intravenous method for abdominal aortography, peripheral arteriography, and cerebral angiography. AJR 82:758-772, 1959
9. Ronderos A, Branton EW, Carlin JT, et al: Venoangiography. AJR 85:1097-1105, 1961
10. Steinberg I, Evans JA: Technique of intravenous carotid and vertebral arteriography. AJR 85:1138-1145, 1961
11. Steinberg I, Marshall V: Intravenous abdominal aortography in urologic diagnosis. J Urol 86:456-469, 1961
12. Steinberg I: Bilateral simultaneous intravenous angiocardiography. AJR 88:38-48, 1962
13. Scatliff JH, Greenspan RH: Radiographic evaluation of major arteries of the neck by intravenous aortography. Acta Radiol (Diag) 1:404-416, 1963
14. Von Kramann B, Christen N: Die Intravenöse Arteriographie im Xeroxbild: eine nicht invasive Methode zur Darstellung peripherer Gefässe. Forthschr Röntgenstr 128:319-323, 1978
15. Kruger RA, Mistretta CA, Houk TL, et al:

Computerized fluoroscopy in real time for noninvasive visualization of the cardiovascular system. Radiology 130:49-57, 1979

16. Crummy AB, Strother CM, Sackett JF, et al: Computerized fluoroscopy: digital subtraction for intravenous angiocardiography and arteriography. AJR 135:1131-1140, 1980
17. Ovitt TW, Christenson PC, Fisher HD III, et al: Intravenous angiography using digital video subtraction: x-ray imaging system. AJR 135:1141-1144, 1980
18. Christenson PC, Ovitt TW, Fisher HD III, et al: Intravenous angiography using digital video subtraction: intravenous cervico-cerebrovascular angiography. AJR 135:1145-1152, 1980
19. Meaney TF, Weinstein MA, Buonocore E, et al: Digital subtraction angiography of the human cardiovascular system. AJR 135:1153-1160, 1980
20. Björk L, Erikson V, Ingelman B: Clinical experience with a new type of contrast medium in peripheral arteriography. AJR 106:418-424, 1969
21. Almén T, Trägardh B: Effects of non-ionic contrast media on the blood flow through the femoral artery in dog. Acta Radiol (Suppl) 335:197-202, 1973
22. Almén T, Boisen E, Lindell SE: Metrizamide in angiography. Acta Radiol (Diagn) 18:33-38, 1977
23. Petitier H, Crochet D, Bizais Y, et al: Etude des effects hémodynamiques d'un nouveau produit de contrast á structure dimère mono-acide. Ann Radiol 21:297-300, 1978
24. Tillman U, Adler R, Fuchs WA: Pain in peripheral arteriography - a comparison of a low osmolality contrast medium with a conventional compound. Br J Radiol 52:102-104, 1979
25. Holm M, Praestholm J: Ioxaglate, a new low osmolar contrast medium used in femoral angiography. Br J Radiol 52:169-172, 1979

26. Golman K: Urography. Physiological considerations on the excretion of contrast media. J Belge Radiol 60:229-238, 1977

9.1.2. Results

In the present series of experiments, 54 angiographies were performed,i.e, 37 in the transplantation group and 17 in the control group. Five of these studies were performed on indication, all of them in transplanted dogs. The others were performed according to the protocol, on the 4th and 11th postoperative days.

Vascular anastomotic problems occurred in one dog only. Hepatic artery thrombosis in this transplanted dog (T 6) was diagnosed on a routine angiogram on the 4th postoperative day, when no obvious clinical morbidity was present (Fig. 5A). Patency was reestablished surgically, as confirmed by the angiographic study performed on the day after thrombectomy (Fig. 5B). However, the angiography performed on the 11th day after transplantation confirmed the clinical suspicion of recurrence of thrombosis, and this was substantiated at autopsy on the 14th day after transplantation.

All remaining anastomoses remained patent and stenosis did not occur.

9.1.3. Discussion

The angiographic and autopsy findings allowed correct determination of the vascular anastomotic complication rate. Since vascular anastomotic problems occurred in only one dog , it may be concluded that the surgical techniques used for the vascular anastomoses were satisfactory. It is of interest to mention that the initial diagnosis of hepatic artery thrombosis in the one dog that eventually died from this complication, was made

on a routine angiogram, on the 4th postoperative day, in the absence of obvious clinical morbidity.

The described technique of pervenous angiography, using a low osmolar contrast medium, proved to be a valuable tool for the assessment of the patency of the hepatic artery and of the retrohepatic caval vein. However, patency of the portal vein could only be evaluated in 21% of the angiographic studies. In combination with digital video subtraction better visualization can be expected, not only of the arterial but also of the venous phases and perhaps also of the portal circulation.

9.2. Biliary complications

9.2.1. Methods of diagnosis

Biliary complications were diagnosed clinically, cholangiographically, and at autopsy.

Cholangiographic studies were carried out routinely on the 32nd and 169th days after surgery and additionally on indication. Indications were: fever, jaundice or abnormal behaviour. In-vivo studies were performed under sedation with fluanisone in a dose of 3 mg per kg i.m. Studies on the 169th day were performed immediately after the animal was killed. All dogs were examined in a left lateral position on the X-ray table, and Conray 60^{R} was used as a contrast medium. All studies on the 32nd day or earlier were carried out by percutaneous puncture of the subcutaneous end of the biliary splint. The same technique was used after sacrifice on the 169th day if the splint had not been removed; otherwise, a retrograde cholangiographic study was performed through the papil of Vater. Initially, all splints were removed after the routine cholangiographic study on the 32nd postoperative day, and this was the case for the first 7 transplanted

and the first 3 control dogs, but after one dog (T 7) died from biliary leakage and one (T 11) became permanently jaundiced after removal of the splint, it was decided to leave the splint in situ in the other dogs for the entire survival period. Nevertheless two more splints, had to be removed because of biliary splint complications (T 8, T 14): the first because of severe infection around the splint and the second because of a discrete, cholangiographically detected biliary leak from the lateral hole in the splint.

The numbers and timing of the cholangiographic studies are given in Table 4.

Table 4. Number and timing of the cholangiographic studies.

	transplanted dogs	control dogs	total
	n	n	n
on indication (all percutaneously)	9*	1	10
routinely			
on day 32 (all percutaneously)	14	9	23
on day 169	10	9	
- percutaneously	7	6	13
- via the papil of Vater	3**	3***	6
total	33	19	52

* 5 dogs examined once each, 2 dogs examined twice
** one splint had been removed according to the original protocol, two because of splint-related complications
*** all three splints had been removed according to the original protocol

9.2.2. Results

Diagnosis on clinical grounds

One dog (C 10) was reoperated on the first postoperative day because of fever, jaundice, and clinical signs of peritonitis. At laparotomy a leaking anastomosis was found and repaired.

Cholangiographic studies

Studies on indication

Nine studies were carried out in transplanted dogs, one each in five dogs and two each in two. In the former group, only one study gave abnormal results. In this dog (T 10) the biliary splint had shifted partially into the duodenum and the biliary tree was not visualized. The severe jaundice in this dog was explained by blockage of the papil of Vater by the splint. The splint was surgically repositioned and the jaundice cleared up rapidly. One of the dogs (T 3) had a normal cholangiogram on the first postoperative day, but at autopsy eleven days later was found to have had an anastomotic leak. The two studies in dog T 19 were normal, but in T 18 an anastomotic leak was documented cholangiographically on day 18. A repeat study performed three days after repair showed an irregular but non-leaking anastomosis.

The only control dog (C 10) examined on indication had been reoperated for an anastomotic leak diagnosed clinically on the first postoperative day. Five days later a discrete leak was still present cholangiographically, but this leak healed spontaneously.

Studies on day 32:

On day 32, 14 transplanted and 9 control dogs were alive and available for study. The results of 12 of the 14 studies in transplanted dogs, were normal. In one dog (T 14) a discrete leak from the lateral hole in the splint was seen and the splint was removed. Marked anastomotic narrowing was seen on the cholangiogram of T 18, which had undergone anastomotic repair on day 18.

Two of the nine studies performed in the control group gave abnormal results. In one dog (C 3) visualization was not obtained due to occlusion of the splint. In the remaining dog (C 8) a narrowed right hepatic duct without proximal dilatation was seen.

Studies on day 169:

Two of the 19 studies gave abnormal results. In T 18, whose anastomosis had been repaired on day 18, the tip of the splint had become dislodged and was now in an intraperitoneal position. In control dog C 8, the known constriction of the right hepatic duct showed no change. All of the other studies gave normal results except for some narrowing at the anastomotic side, but without proximal dilatation. Even the transplanted dog, which became permanently jaundiced after removal of the splint on day 32 (T 11), showed no obstruction on the cholangiogram obtained via the papil of Vater.

Autopsy findings:

Biliary peritonitis was present in four transplanted dogs (T 3, T 4, T 7, T 10). One dog (T 3) had an anastomotic leak. T 4 showed leakage from the lateral hole of the splint into the peritoneal cavity, due to dislodgement of the splint. T 7 developed leakage from the cystic duct stump after withdrawal of the splint on the 32nd postoperative day, and T 10 died from a peripheral bile-duct leak after a percutaneous biopsy procedure.

In dogs surviving until the end of the study period the anastomosis looked well healed at autopsy, and severe narrowing with proximal dilatation was not seen. Even T 11, which became permanently jaundiced after removal of the biliary splint, showed no obstruction at autopsy and the cause for the jaundice was cholangitis as could be determined microscopically.

Minimal biliary slush was found in one transplanted dog (T 18) and one control dog (C 7).

The total biliary complication rate is shown in Table 5.

Table 5. Total biliary complication rate based on clinical, cholangiographic, and autopsy diagnosis.

	transplantation group		control group	
	n total	fatality n total	n total	fatality n total
anastomotic complications	3	1	2	0
leakage	2	1	1	0
stenosis	1	0	±1	0
formation of slush (minimal)	1	0	1	0
splint-related complications	7	2	1	0
splint infection	1	0	0	0
splint occlusion	0	0	1	0
splint dislodgement				
- anterogradely	1	0	0	0
- retrogradely + peritonitis	1	1	0	0
- peritonitis	2	0	0	0
after splint withdrawal				
- biliary leakage	1	1	0	0
- biliary obstruction	1	0	0	0
accidental biliary perforation (percutaneous liver biopsy)	1	1	0	0
total	12	4	4	0

9.2.3. Discussion

The biliary reconstruction was the Achilles' heel of this experimental series too (Section 4.3). Leakage of the anastomosis occurred in two transplanted dogs and one control dog, giving an incidence of 10% for both groups. Only one transplanted dog died. The other two were saved by early diagnosis and prompt reintervention.

Substantial stenosis at the site of the anastomosis did not occur, and minimal slush formation was seen at autopsy in only one dog of each group.

Splint-related complications occurred frequently in the transplantation group and were responsible for the death

of two dogs. Moreover, there seems to have been a relationship between the presence of a biliary splint and the long-term biochemical as well as the long-term histological findings, as will be discussed below (Chapters 10 and 11). The use of an appropriately sized T-tube, left in situ for a long time as is done in clinical liver transplantation, might have prevented these problems.

CHAPTER 10. LIVER FUNCTION

10.1. Clinical assessment

Liver failure was the primary cause of death in two dogs, both belonging to the transplantation group: one of them (T 2) died from acute liver failure 24 hours after surgery and the other (T 9) was killed on the 39th postoperative day because of manifestations of chronic liver failure. In the latter an atrophied transplant was found at autopsy. In the other dogs death was due to other causes (Chapter 7). Most of the dogs that survived the period of the study did well clinically, as can be deduced from the over-all body weight changes during the survival period (Chapter 7).

10.2. Biochemical assessment

10.2.1. Methods

The levels of serum potassium, blood sugar, serum bilirubin, serum glutamic pyruvic transaminase (GPT), alkaline phosphatase, and serum albumin were determined at standardized time-points during the period under study (Table 1).

Table 1. Timing of biochemical analysis.

preoperatively at 9 a.m. on the 7th day before surgery.
postoperatively at 9 p.m. on the day of surgery
at 9 a.m. on days 1, 4, 6, 11, 18, 25, 32, 46, 60, 74, 95, 123, and 151.

The elimination of two intravenously injected substances from the plasma by the liver was studied. The bromsulphthalein test is considered to be a sensitive indicator of liver function, at least in the absence of jaundice. Because bromsulphthalein is taken up by the liver cells, conjugated with glutathione, and excreted in the bile, the classic test does not discriminate between hepatocellular and cholestatic conditions. Due to the relatively high intrinsic clearance of the substance, the over-all clearance is dependent on the blood flow through the liver. Since a large proportion of injected bromsulphthalein binds to the plasma proteins, the distribution is largely determined by the plasma volume.[1-3]

Antipyrine is considered to be an ideal substance for the study of liver metabolism. It is distributed rapidly and proportionally over the total body water, is almost totally and exclusively metabolized by the liver, gives very few side effects, and is relatively simple to determine in plasma samples. Because of its extremely low intrinsic clearance, the total clearance is to a great extent independent of the blood flow through the liver. The antipyrine clearance gives a good idea of the integrity of the microsomal enzyme system of the hepatocyte.[2,3]

Bromsulphthalein and antipyrine elimination tests were performed preoperatively and 4, 18, 25, 32, 46, 60, 74, 123, and 151 days after surgery. The dogs were starved the night before the test. At 9 a.m., bromsulphthalein was injected intravenously in a dose of 5 mg per kg body weight. Two-milliliter samples of venous blood were drawn into heparinized tubes at 0, 1, and 15 minutes. The samples were centrifuged and the plasma was stored at -20°C until analysed. The determination of bromsulphthalein in the samples was carried out spectrophotometrically, according to Seligson et al.[4] After completion of the bromsulphthalein test, antipyrine

was injected intravenously in a dose of 50 mg per kg body weight. Two-milliliter samples of venous blood were drawn into heparinized tubes at 0, 60, 90, 120, and 300 minutes. The samples were centrifuged and the plasma was stored at -20°C until analysed. The determination of the antipyrine concentration in the plasma samples was carried out gas-chromatographically according to Prescott et al.[5]

10.2.2. Results

Serum potassium

During the first 24 hours after surgery, the serum potassium levels tended to be slightly below the preoperative values (Chapter 8). The lowest value during this period was 2.7 mmol per liter, the highest 5.1 mmol per liter.

Blood sugar

During the first 24 hours after surgery, values below 3 mmol per liter were found in two transplanted dogs and one control dog. The hypoglycemia was, however, symptomatic in only one of these dogs, i.e., the transplanted dog that died at 24 hours due to acute liver failure.

Total serum bilirubin

The total serum bilirubin levels are given in the Tables 2, 3, and 4. None of the preoperative levels were higher than 2 µmol per liter.

A slight temporary rise of the levels during the first 24 hours after surgery was a common finding in the first 15 experiments, even in the absence of obvious biliary-tract pathology. Early high increases or increases which had not returned to normal by the 4th or 6th postoperative day usually indicated biliary-tract

Table 2. Total serum bilirubin (μmol/l).

Transplantation group: dogs dying before the 169th day.

	pre-operatively	postoperative days													
		0 9 p.m.	1 9 a.m.	4	6	11	18	25	32	46	60	74	95	123	151
T 1*	1	6													
2*	0		10												
3*	1	10	8	6	7										
4*	1	13	14	108	139										
5*	1	5	2	1	2	2									
6*	1	10	3	9	58	43									
7*	0	4	1	1	1	1	1	1							
8	1	1	21	56	28	6	4	56							
9	1	1	1	1	1	1	1	112							
10	1	36	41	42	17	1	1	1	1	1	1	1	1	1	

T = transplanted dogs * = first 10 experiments

Table 3. Total serum bilirubin (μmol/l).

Transplantation group: dogs surviving for 169 days.

	pre-operatively	postoperative days													
		0 9 p.m.	1 9 a.m.	4	6	11	18	25	32	46	60	74	95	123	151
T 11*		5	4	2	1	0	1	1		4	47	109	95	72	86
12*		2	2	1	1	2	1	1	1	1	2	1	2	1	2
13*	1	5	7	1	2	1	2	1	2	2	1	2	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1		1	1	1
18	1	1	1	1	1	1	69	8	30	9	1	1	1	1	14
19	1	1	44	17	4	1	1	1	1	1	1	1	1	1	1
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

T = transplanted dogs * = first 10 experiments

Table 4. Total serum bilirubin (umol/l).

Control group.

	pre-operatively	postoperative days													
		0 9 p.m.	1 9 a.m.	4	6	11	18	25	32	46	60	74	95	123	151
C 1	1	1													
2*	2	2	5	0	2	1	1	1	1	3	3	2	2	1	2
3*	1	3	1	1	1	2	1	2	2	6	4	4	4	3	3
4*	1	1	1	1	1	1	1	1	2	1	1	1	2	2	1
5*	1	2	1	1	2	2	3	1	1	1	1	1	1	2	2
6*	2	1	2	2	4	3	3	3	1	1	2	2	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	7	11
8	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
10	1	22	29	3	1	1	1	1	1	1	1	1	1	1	1

C = control dogs * = first 5 experiments (with immunosuppressive treatment)

pathology. In three transplanted dogs, (T 6, T 8, T 19),however, no biliary tract pathology could be demonstrated. Dog T 6 had a thrombosed hepatic artery, which was diagnosed on a routine pervenous angiogram performed on the 4th postoperative day. At that stage only a small increase in the total serum bilirubin level was noted. Dogs T 8 and T 19 were febrile during the period of hyperbilirubinemia, suggesting an infectious etiology. T 8 subsequently developed wound infection and eventually died from lobar necrosis of the graft.

Elevated total serum bilirubin levels after the first two postoperative weeks were found in four transplanted (T 8, T 9, T 11, T 18) and two control dogs (C 3, C 7). T 8 died from lobar necrosis of the graft and T 9 from biliary peritonitis after withdrawal of the biliary splint on the 32nd postoperative day. T 11 became permanently jaundiced after removal of the biliary splint on the 32nd postoperative day. At autopsy on day 169, no obstruction was found but cholangitis was seen histologically. T 18 developed a late leak at the site of

the biliary anastomosis, which was repaired. At autopsy, a subphrenic abscess was found. In the two control dogs which developed discrete hyperbilirubinemia (C 3 and C 7), no obvious cause was found.

Serum liver enzymes

The serum GPT and alkaline phosphatase levels are given in Tables 5-7 and 8-10, respectively.

After revascularization, the GPT levels showed a sharp rise. Peak levels were noted at 9 p.m. on the day of surgery or, more often, at 9 a.m. on the next day. The rise was much higher in the first 15 experiments and more pronounced in the transplantation group. By the fourth postoperative day the levels had dropped markedly, and in most of the surviving dogs they continued to decrease until the 18th postoperative day. The further course of the GPT profiles differed considerably from dog to dog. However, more hectic profiles were seen in transplanted dogs and complete longstanding normalization of the GPT levels was noted in only a few of the control dogs.

Table 5. Serum GPT(IU/l).

Transplantation group: dogs dying before the 169th day.

	pre-operatively	postoperative days 0 9 p.m.	1 9 a.m.	4	6	11	18	25	32	46	60	74	95	123	151
T 1*	23	834													
2*	19	1229	1498												
3*	21	1298	2148	943	636										
4*	24	645	838	348	222										
5*	34	659	700		157	128									
6*	22	1357	1098	487	678	1200									
7*	29	1462	1680	642	384	124	125	471	246						
8	28	1054	928	403	252	210	86	583	429						
9		813	849	322	152	55	33	177	322						
10		914	909	497	274	209	172	191	342	297	329	313	230	117	

T = transplanted dogs

* = first 10 experiments

Table 6. Serum GPT (IU/l).

Transplantation group: dogs surviving for 169 days.

	pre-operatively	postoperative days													
		0 9 p.m.	1 9 a.m.	4	6	11	18	25	32	46	60	74	95	123	151
T 11*	34	1410	834	550	277	86	38	66	34	426	1255	1426	477	271	953
12*	32	709	921	510	252	155	61	47	204	168	126	214	296	173	157
13*	25	706	727	309	288	124	44	65	65	204	60		102	26	33
14	37	868	807	21	127	399	55	413	155	183	363	149	155	490	722
15	26	885		359	282	144	127	46	51	52	68	24	55	80	100
16	32	450	522		374	46	31	171	120	388	842	449	738	345	156
17	21	487	608	201	105	63	92	65	74	63	58		289		30
18		649	655	303	136	51	26	60	465	220	154	251	263	139	226
19	31	447	568	304	181	66	363	407	398	369	190	380	393	151	64
20	24	115		115	159	82	25	52	213	207	102	60	66	35	45

T = transplanted dogs

* = first 10 experiments

Table 7. Serum GPT (IU/l).

Control group.

	pre-operatively	postoperative days													
		0 9 p.m.	1 9 a.m.	4	6	11	18	25	32	46	60	74	95	123	151
C 1	26	100													
2*	32	591	509	449	496	136	45	70	50	1345	170	832	560	174	119
3*	42	477	594	291	104	77	30	48	30	844	1606	1321	430	733	493
4*	18	169	183	131	72	26	8	20	19	39	30	117	73	14	55
5*	28	129	212	83	70	186	110	120	437	108	101		273	249	88
6*	30	311	417	777	898	202	47	24	25	26	38	50	71	82	91
7	16	518	485	219	110	163	97	47	47	78	41	54	116	261	
8	20	326	325	143	91	46	24	34	26	30	24	28	33	20	371
9	32	276	322	104	11	28	15	5	30	28	22	24	24	23	47
10		332	408	170	78	44	24	42	37	58	27	38	16	19	25

C = control dogs

* = first 5 experiments (with immunosuppressive treatment)

The serum alkaline phosphatase levels started to rise after the completion of the operation. The highest values were usually measured on the 4th or 6th postoperative day, after which the levels usually declined progressively until the 18th or 25th day. The further course of these profiles also differed considerably from dog to dog and was usually more abnormal in transplanted dogs.

Table 8. Serum alkaline phosphatase (IU/l).

Transplantation group: dogs dying before the 169th day.

	pre-operatively	postoperative days 0 9 p.m.	1 9 a.m.	4	6	11	18	25	32	46	60	74	95	123	151
T 1*	110	398													
2*	133	114	238												
3*	103	213	948	6607	5208										
4*	60	88	323	3978	5868										
5*	109	135	495		2576	311									
6*	81	214	627	1941											
7*	78	98	196	414	376	207	263	613	486						
8	60	62	178	1065	752	1189	671	1793	1822						
9		124	242	504	494	416	274	696	9350						
10		183	856	9240	7188	5346	3100	2381	2409	2646	2192	1886	1435	1111	

T = transplanted dogs

* = first 10 experiments

Table 9. Serum alkaline phosphatase (IU/l).

Transplantation group: dogs surviving for 169 days.

	pre	postoperative days													
	operatively	0 9 p.m.	1 9 a.m.	4	6	11	18	25	32	46	60	74	95	123	151
T 11*	92	87	289	706	596	388	201	189	138	935	8102				
12*	115	150	266	244	237	2169	1200	572	827	560	523	751	974	678	356
13*	241	140	279	1719	1953	1030	468	331	464	675	631		424	54	241
14	127	64	62	338	320	323	401	859	731	905	1301	522	554	1017	567
15	162	167	742	1459	1512	1097	478	387	289	256	330	242	470	452	1377
16	88	156	252		2450	246	224	598	539	878	1186	124	1153	935	478
17	66	62	192	809	654	456	304	209	148	56	95		272		84
18		153	840	1063	882	3194	2454	6380	6646		4873	6695	6556	6526	6843
19	131	151	88	2734	2034	1320	1399	1409	1457	2182	1039		1214	328	200
20	87	186			864	1731	512	245	243	295	173	205	115	52	69

T = transplanted dogs * = first 10 experiments

Table 10. Serum alkaline phosphatase (IU/l).

Control group.

	pre-	postoperative days													
	operatively	0 9 p.m.	1 9 a.m.	4	6	11	18	25	32	46	60	74	95	123	151
C 1	57	65													
2*	114	144	329	353	369	612	385	267	255	2206	704	837	871	501	265
3*	103	125	343	513	277	291	185	160	139	949	940	3037	1272	1576	492
4*	75	131	168	1555	1144	462	219	158	156	387	160	153	135	108	115
5*	188	223	322	226	221	392	286	307	338	284	233		246	211	168
6*	163	166	515	3810	3509	1642	626	382	266	221	463	239	266	330	305
7	106	153	386	1852	1140	749	442	234	159	435	102	93	773	412	4123
8	179	447	205	205	242	321	211		302	185	175	165	192	193	3144
9	111	140	285	549	428	238	157	161	123	112	116	100	103	115	172
10		261	426	2012	4437	1763	626	307	271	349	200	140		103	123

C = control dogs * = first 5 experiments (with immunosuppressive treatment)

Serum albumin

The serum albumin levels are given in Tables 11, 12, and 13. The decrease seen during surgery was corrected to a large extent postoperatively, and reasonable levels were maintained in dogs that survived the study period, at least in the absence of overt morbidity.

Table 11. Serum albumin (g/l).

Transplantation group: dogs dying before the 169th day.

	pre-	postoperative days													
	opera-	0	1	4	6	11	18	25	32	46	60	74	95	123	151
	tively	9 p.m.	9a.m.												
T1*	42	26													
2*	35		36												
3*	35	25	28	30											
4*	41	30	33	37											
5*	36	28	36	27		20									
6*	42	30	34	37		25									
7*	41	34	35	38		37	36	38	39						
8	40		32	33			25	25	29						
9		31	35	34		35	38	36	38						
10	46	25	30	27		26	29	37	36	37	35	35	35	37	

T = transplanted dogs

* = first 10 experiments

Table 12. Serum albumin (g/l).

Transplantation group: dogs surviving for 169 days.

	pre-operatively	postoperative days: 0 9 p.m.	1 9 a.m.	4	6	11	18	25	32	46	60	74	95	123	151
T 11*	42	27	29	32		35	40	39	39	34	33	34	29	23	28
12*	32	28	29	32		28	32	36	38	38	37	38	36	37	33
13*	36	30	29	29		34	37	37	37	36	37	37	35	35	36
14	38	25	28	35		36	38	38	38		40	40	39	43	40
15	40	27	31	39		36	37		34	42	42	30	31	38	
16	39	31	29	31		36	36	35	38	40	38	35	37		39
17	39	31	32	32		33		36	39	35	37		36		
18		30	31	34		41	26	28	34	30	28	30	39	27	23
19			35	33		34	37		37	38	38		36	37	
20	40	28	36	33		34	35	36	37		43	41	39	33	31
mean	38.2	28.5	30.9	33.0		34.7	35.2	35.6	37.1	36.6	37.3	35.6	35.7	34.1	32.8
SD	3.0	2.0	2.7	2.6		3.2	4.1	3.3	1.7	3.7	4.3	4.1	3.3	6.4	6.0

T = transplanted dogs

* = first 10 experiments

Table 13. Serum albumin (g/l).

Control group.

	pre-operatively	postoperative days: 0 9 p.m.	1 9 a.m.	4	6	11	18	25	32	46	60	74	95	123	151
C 1	30	33													
2*	43	34	34	33		33	40	39	37	36	38	35	38	39	37
3*	37	27	29	29		36	38	38	37	36	35	37	37	39	35
4*	38	31	34	30		36	35	39	35	35	35	36	37	36	37
5*	36	32	33	31		33	37	37	36	35	35	34	39	34	38
6*	37	29	25	29		30	33	35	36	36	34	34	35	36	31
7	38	32	42	29		28	34	34	37	37	39	36	34	34	36
8	38	16	11	29		34	35	40	37	40	39	38	40	41	35
9		35	33	29		32	35		36	39	25	38	39	44	
10		30	25	27			31	36		35	37		39	35	34
mean	37.1	29.9	29.5	29.5		32.7	35.3	37.2	36.3	36.5	35.2	36.0	37.5	37.5	35.3
SD	3.5	5.4	8.6	1.6		2.7	2.6	2.1	0.7	1.8	4.2	1.6	2.0	3.4	2.1

C = control dogs

* = first 5 experiments (with immunosuppressive treatment)

Bromsulphthalein and antipyrine handling

The half-life values for both substances are given in Tables 14-16 and 17-19, respectively. As could be expected, the half-life values for bromsulphthalein were increased in jaundiced animals. In dogs which survived for the entire period of the study, the bromsulphthalein handling was maintained well, although temporary increases above a value of four minutes were sometimes seen in the absence of jaundice.

Table 14. Bromsulphthalein ($t_{\frac{1}{2}}$ in minutes).

Transplantation group: dogs dying before the 169th day.

	pre-operatively	postoperative days 0	1	4	6	11	18	25	32	46	60	74	95	123	151
T 1*	3.2														
2*	3.1														
3*	3.3			5.5											
4*	3.0			11.4											
5*	3.2			3.6											
6*	3.0			18.0											
7*	3.2			5.2			3.6								
8	3.3			8.8			4.0								
9	3.1			4.5			3.4		>30.0						
10	3.2			8.8			3.4		4.0	4.0	3.6	3.5			

T = transplanted dogs

* = first 10 experiments

Table 15. Bromsulphthalein ($t\frac{1}{2}$ in minutes).

Transplantation group: dogs surviving for 169 days.

	pre	postoperative days													
	operatively	0	1	4	6	11	18	25	32	46	60	74	95	123	151
T 11*	3.2			3.2			3.2		3.5	3.6	16.6	24.4		21.0	14.1
12*	3.5			4.6					3.3	3.8		6.2		3.3	4.8
13*	3.2			4.2			3.4		3.3	3.4	3.0	3.4		3.0	3.2
14	3.2			4.1			4.2		4.5	3.2	3.8	5.0		3.3	3.1
15	3.2			4.2			3.4		3.0	3.1	3.4	3.3		3.3	3.3
16	3.4			3.8			3.2		3.6	3.4	4.2	3.1		3.0	3.2
17	3.4			3.7			3.3		3.5	3.9	3.2	3.8		3.5	3.6
18	3.3						4.0		13.1	5.3	3.8	5.3		6.4	16.4
19	3.0			4.5			4.4		5.7	3.4	3.9	3.3		3.9	3.6
20	3.0			3.8			3.2		3.0	3.2	3.2	3.1		3.3	

T = transplanted dogs

* = first 10 experiments

Table 16. Bromsulphthalein ($t\frac{1}{2}$ in minutes).

Control group.

	pre-	postoperative days													
	operatively	0	1	4	6	11	18	25	32	46	60	74	95	123	151
C 1	3.2														
2*	3.2			3.2			3.6		5.7	5.0	5.4	5.7		3.2	3.4
3*	3.3			3.8			3.6		4.8	17.0	3.6	8.6		4.1	3.3
4*	3.0			4.0			3.4		3.3	3.3	3.2	3.5		4.2	2.9
5*	3.5			3.6			3.5		3.0	2.8	3.2	3.0		3.0	3.5
6*	3.0			3.3			3.4		3.5	3.2	3.3	3.0		3.0	3.0
7	3.3			5.0			4.0		3.1	3.3	3.0	3.4		8.9	8.0
8	2.9						3.7		3.2	3.0	3.4	3.4		3.0	3.1
9	3.0			3.0			3.1		2.8	3.1	3.5	3.0		3.1	3.0
10	2.8						3.3		3.3	3.3	3.1	3.2		3.0	2.9

C = control dogs

* = first 5 experiments (with immunosuppressive treatment)

The preoperative half-life values for antipyrine varied considerably, but the mean values for the control group remained about the same throughout the survival period. In contrast, the transplantation group showed a considerable decrease of the half-life values postoperatively and these lowered values persisted in surviving dogs until after the 74th postoperative day. Later, the values rose in this group.

Table 17. Antipyrine ($t_{\frac{1}{2}}$ in minutes).

Transplantation group: dogs dying before the 169th day.

	pre-operatively	postoperative days													
		0	1	4	6	11	18	25	32	46	60	74	95	123	151
T 1*	119														
2*	88														
3*	92			71											
4*	136			61											
5*	127			91											
6*	160			84											
7*	117			77			118								
8	90			89			112								
9	129			91			89		192						
10	110			106			74		66						

T = transplanted dogs

* = first 10 experiments

Table 18. Antipyrine ($t_{\frac{1}{2}}$ in minutes).

Transplantation group: dogs surviving for 169 days.

	pre-operatively	postoperative days												
		0	1	4	6	11	18	25	32	46	60	74	95	123	151
T 11*	132			73			86		94	86	71	78		123	131
12*	67			64							65				72
13*	72			72			75		83	71	73	69		78	107
14	68			60			80		76	72	65	68		74	80
15	88			73			71		72	69	73	68		90	126
16	79			74			75		59	63	63	49		72	84
17	76			65			78		71	77	69	65		81	82
18	94			73					79	100	74	77		112	161
19	91			63			81		80	73	88	77		121	90
20	76			65			82		75	103	80	80		86	90
mean	84			68			78		76	79	72	70		93	102
SD	19			5			4		9	14	7	9		20	28

T = transplanted dogs

* = first 10 experiments

Table 19. Antipyrine ($t_{\frac{1}{2}}$ in minutes).

Control group.

	pre-operatively	postoperative days												
		0	1	4	6	11	18	25	32	46	60	74	95	123	151
C 1	135														
2*	110			114			124		105	107	84	93		88	76
3*	86			81			72		74	85	93	91		72	62
4*	72			71			88		82	77	74	78		79	102
5*	90			69			80		85	101	72	73		67	67
6*	99			85			131		88	109	102	88		87	81
7	100			110			94		125	107	95	112		107	124
8	81			78			71		84	77	88	89		68	106
9	54			65			67		69	80	73	53		85	73
10	56						68		66	81	76	78		100	90
mean	88			84			88		86	90	84	83		83	86
SD	24			18			24		18	15	11	16		13	20

C = control dogs

* = first 5 experiments (with immunosuppressive treatment)

10.3. Discussion

None of the 20 transplanted and 10 control dogs died during surgery, and there was only one death due to acute liver failure within the first week after surgery. In the event of massive ischemic damage to the liver, the dog dies within a few hours after surgery from acute liver insufficiency and liver outflow block.[6] Persistent bleeding, hypoglycemia, hyperkalemia and hyperbilirubinemia form part of the picture.[6,7] Clinical liver outflow block was not observed in this series of experiments, and pathological bleeding, though constantly present during surgery, was not uncontrollable. Symptomatic hypoglycemia only occurred in the dog which died from acute liver failure. In 27 of the 29 other dogs, the blood sugar level remained above 3 mmol per liter.

Postoperative serum potassium concentrations higher than 6 mmol per liter were not observed. In contrast, there was a tendency toward early postoperative hypokalemia. This phenomenon is considered to be an early postoperative manifestation of liver function, because it indicates that the intracellular potassium content has been restored after depletion due to ischemia.[7]

An early postoperative rise of the total serum bilirubin level above 10 umol per liter usually indicated biliary-tract pathology. A slight temporary rise of the levels during the first 24 hours was, however, a common finding in the first 15 experiments, even in the absence of obvious biliary-tract pathology, and may be a manifestation of ischemic damage to the liver. This is supported by the fact that the postoperative increase of the serum GPT levels was also more pronounced in these experiments. The aminotransferases are known to be sensitive indicators of hepatic injury.[1,3] The whole cooling and preservation procedure was therefore checked

critically. It was found that the refrigerator used for storage of the preservation fluids and the livers was only maintaining a temperature of 10°C at its minimal setting and that the maximum setting was required to obtain a temperature of 4°C. This change in the storage temperature may well explain why early increases of total serum bilirubin became rare during the second 15 experiments and why the GPT rises became less pronounced. However, the rise of GPT remained more marked in the transplantation group, which is not surprising in view of the much longer total ischemic time to which the livers in this group were exposed.

Eight of the deaths in the present series occurred after one week and 5 were clearly related to technical complications. Chronic liver failure was only the cause of death in one case, a dog in the transplantation group which was killed because of this condition on the 39th day. An atrophied graft was found at autopsy. Histology revealed multilobular necrosis and local purulent cholangitis but no rejection.

In the 10 transplanted and 9 contol dogs which survived for the entire period the liver obviously functioned well enough to sustain life that long. In the majority of these dogs the quality of life was clinically excellent, as can be deduced from the over-all body-weight changes. In the survivors the synthesis of albumin and handling of bromsulphthalein were well preserved, at least in the absence of overt morbidity. Measurement of the serum albumin levels provides a guide to the severity of chronic liver disease,[3] and the bromsulphthalein test is considered to be a sensitive indicator of liver function in the absence of jaundice.[1-3] The microsomal function of the liver, as reflected by the half-life of antipyrine,[2,3] also was well preserved. While the mean half-life values remained relatively constant in the control group, lowered values persisted postoperatively in the transplantation group

until after the 74th day. This could be explained as a steroid effect.[3] However, this phenomenon, was not observed in the five control dogs, which were on the same immunosuppressive regimen.

The first part of this discussion has dealt with the positive aspects; the second part concerns the shortcomings. Although usually not lethal,hepatic damage always occurred. The tendency to develop early postoperative hypokalemia although not a bad prognostic sign, as well as the early rise of the GPT and alkaline phosphatase levels, are expressions of hepatic damage. The difference between the transplantation and control groups, with respect to the magnitude of the early postoperative rise of the levels of the enzymes in question indicates that the liver grafts were more severely damaged than the livers in control dogs. This is not surprising in view of the difference between the two groups as to the total ischemic time of the liver.

The restlessness of the GPT and alkaline phosphatase profiles in the dogs surviving for the entire study period is worrying, as is the late increase in the half-life values for antipyrine in the transplantation group. One wonders what would have happened if the study period had been longer. No clear relationship was found between the restlessness of these profiles and the percutaneous biopsy procedures. The transanastomotic biliary splint may, however, have contributed to these events by causing intermittent obstruction and recurrent ascending cholangitis. Microbiological screening might have given more information, but unfortunately was not included in the protocol.

10.4. Conclusions

After revascularization, all of the livers showed biochemical evidence of damage, and this was more pronounced in the transplantation group. Nevertheless, all but one of the livers were able to sustain life during the early postoperative period, and late liver failure occurred only once as a primary cause of death. The remaining 9 dogs died from other causes.

Most of the 19 dogs which survived the entire study period had a good quality of life. Although the liver of these dogs was able to synthetize albumin and to handle bromsulphthalein and antipyrine reasonably well, the fluctuating enzyme profiles indicated that hepatic damage occurred repeatedly during the survival period. The transanastomotic biliary splint may well have been co-responsible for this.

References

1. Rosoff L jr., Rosoff L sr.: Biochemical tests for hepatobiliary disease. Surg Clin North Am 57:257-273, 1977
2. Hoorntje SJ: Leverfunctieproeven, in Gips CH, Wilson JHP (eds): Lever en Galwegendiagnostiek en Therapie. Utrecht, Bohn Scheltema Holkema, 1979, pp 29-31
3. Price CP, Alberti KGMM: Biochemical assessment of liver function, in Wright R, Alberti KGMM, Karran S, et al (eds): Liver and Biliary Disease. London, Saunders, 1979, pp 381-416
4. Seligson D, Marino J, Dodson E: Determination of sulphobromophthalein in serum. Clin Chem 3:638-645, 1957
5. Prescott LF, Adjepon-Yamoah KK, Roberts E: Rapid gasliquid chromatographic estimation of antipyrine in plasma. J Pharm Pharmacol 25:205-207, 1973

6. Starzl TE,Putnam CW: Experience in Hepatic Transplantation. Philadelphia, Saunders, 1969, pp 65-80
7. Calne RY, Williams R: Liver transplantation. Curr Probl Surg 16:1-44, 1979

CHAPTER 11. PATHOLOGY OF THE LIVER

11.1. Methods

11.1.1. Sampling

Three wedge biopsies were performed peroperatively: the first sample was taken from the left lateral lobe of the liver immediately after entering the abdomen of donor and control dogs, the second from the left medial lobe of the same liver at the end of the preservation period, and the third from the right medial lobe of the same liver after revascularization. The wedges were cut with a pair of scissors, the first and last under digital compression of the part of the liver to be biopsied, to prevent loss of blood. Compression was maintained until the cut surfaces of the organ had been electrocoagulated.

Percutaneous biopsies were carried out on the 8th, 22nd, 43rd, 85th, and 126th days after surgery, with a Menghini-type needle (Hepafix^{R},1.6 mm in diameter, 90 mm long, and with a sleeve length of 20 mm). The dogs were starved overnight before the procedure. As sedative, fluanisone was given i.m. in a dose of 3 mg per kg. The dogs were placed in a supine position, and the needle was introduced subcostally on the right side above the laparotomy scar, at a point lying on the nipple line, and was advanced in a vertical plane upward and posteriorly at an angle of 45^{o}. At most 3 punctures were performed per session.

Final samples were taken at autopsy. All autopsies were performed within 18 hours after the last time the

dog had been seen alive. In dogs that were killed, fresh material was taken. After removal of the liver, all lobes were transected through the largest diameter. A biopsy specimen was routinely taken from the right lateral lobe. Additional samples were taken from other areas of the organ if differences in pathology were suspected.

11.1.2. Processing of the samples

All specimens were divided into three parts, one of which was immersed in formalin for routine histological assessment, one in liquid nitrogen for immunofluorescence studies, and one in glutaraldehyde for electron-microscopy. The first was then embedded in paraffin and sections were stained with haemotoxylin and azoflavin; haemotoxylin, azoflavin and saffran; Gomori's reticulin stain; periodic acid Shiff (PAS); PAS after pretreatment with diastase (D/PAS); Perl's iron stain; and the Gieson-Lawson elastin stain. The other two parts were not routinely examined but stored for further analysis in case routine histological assessment indicated a need for that. In this context only one post-revascularization biopsy specimen was additionally analysed electron-microscopically.

11.2. Results

11.2.1. Methods of sampling

No complications associated with the peroperative sampling were seen. Of the total of 105 percutaneous liver biopsies ,62 were in the transplantation group and 43 in the control group. One transplanted dog died from a peripheral bile-duct leak on the 127th postoperative day, one day after a percutaneous biopsy had been performed. There were no other obvious complications, but the tissue

collected was often fragmented and included few portal fields. It was therefore impossible to draw definite conclusions regarding the portal fields, which is unfortunate because the most important pathological changes in the liver of long-term survivors concerned the portal fields.

11.2.2. The microscopical findings

The first biopsy specimen of two dogs already showed pathological changes. A dense polymorphonuclear-cell infiltrate was found in the portal fields of a transplanted dog and moderate mononuclear-cell infiltration without invasion of the bile ducts was seen in the portal fields of a control dog.

All post-revascularization biopsy specimens showed signs of congestion, albeit varying in intensity from liver to liver. Liver cells adjacent to strongly dilated sinusoids had a compressed appearance and an angular shape. Other liver cells had an edematous appearance. Accumulation of fat droplets in the mid-sinusoidal liver cells was noted in about 40% of the transplanted livers but was rare in the livers of control dogs. Liver-cell necrosis was seen in only one dog (T 2). The necrosis in this dog was minimal and restricted to the pericentral area. Glycogen depletion was seen in all livers; the degree varied from liver to liver, but was greater in the transplantation group. The sinusoids contained an increased number of polymorphonuclear cells. The activity of Kupffer cells had increased and many of these cells contained inclusions which stained positively with PAS after pretreatment with diastase. Another consistent finding in the post-revascularization biopsy specimens was the intrahepatocellular presence of smooth homogeneous bodies of different sizes which stained positively with PAS after pretreatment with diastase (Fig. 1). Electron-microscopically, these bodies were

homogeneously black and had no surrounding membranes (Fig. 2). Marked swelling of the mitochondria was also seen electron-microscopically. Light-microscopically, the portal areas appeared normal except that the cells of the epithelium of the smaller bile ducts were no longer cuboidal but had become high-cylindrical and showed basal vacuolization.

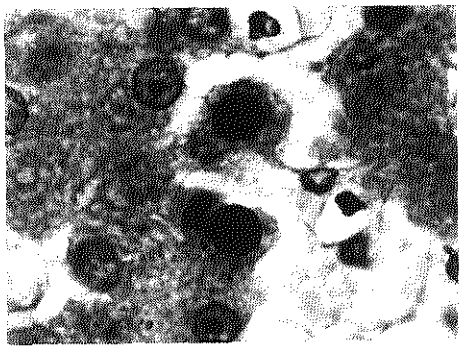

Fig. 1. Biopsy specimen of a liver graft, taken shortly after revascularization. Hepatocytes with inclusion bodies in the cytoplasm are clearly visible (PAS stain after pretreatment with diastase, x 250).

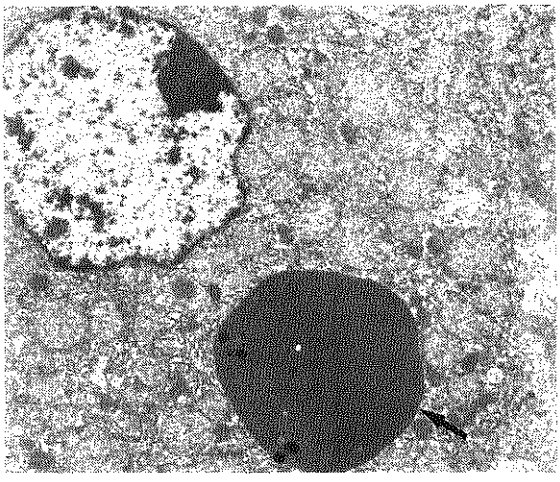

Fig. 2. Electron-micrograph (x 16,000) of the same biopsy specimen showing part of a hepatocyte with a nucleus and an inclusion body (arrow). (Courtesy of V.D. Vuzevski. M.D.).

In view of the differences in survival duration for a considerable number of the dogs, a short description of the microscopical appearance of the liver at autopsy is given for each animal. Three groups of dogs are considered: group A, in which death occurred within 24 hours; group B, in which death occurred later than 24 hours; and group C, in which the animals were still alive at the end of the study period. The macroscopic autopsy findings are given in Chapter 7.

Group A: Microscopical appearance of the liver at autopsy in dogs which died within 24 hours after the operation (n = 3).

T 1 (survived 8 hr, died of air embolism):

Over-all architecture intact. The hepatocytes are angular and the cytoplasm is dark and eosinophilic. Bodies showing D/PAS positivity have disappeared from liver cells. Extensive hemorrhagic areas are present in the parenchyme, especially around the portal fields. Polymorphonuclear cells have accumulated in the sinusoids. Kupffer cell activity is slightly increased. Almost no signs of inflammatory cell infiltration are seen in the portal areas. The bile-duct epithelium is of the high-cylindrical type and is vacuolated basally.

Conclusion: hepatic tissue showing marked signs of ischemia.

T 2 (survived 24 hr, died of hypoglycemia):

Over-all architecture intact. Marked signs of acute congestion. The hepatocytes still contain inclusion bodies. Severe glycogen depletion is noted.

Conclusion: acute congestion of the liver.

C 1 (survived 16 hr, killed because of neurological damage secondary to an anesthetic accident):

Over-all architecture intact. There are signs of acute congestion. No intrahepatocellular inclusion bodies are seen. Kupffer cells loaded with D/PAS +

stained material are scattered throughout the sinusoids. The portal veins are dilated. The bile duct epithelium is high cylindrical and is vacuolated basally.

Conclusion: acute congestion of the liver.

Group B: Microscopical appearance of the liver at autopsy in dogs dying later than 24 hours after the operation (n = 8).

T 3 (survived 8 days, died of biliary peritonitis):

Over-all architecture intact. There are signs of congestion. Kupffer cells loaded with D/PAS + stained material and some iron pigment are scattered throughout the sinusoids. The portal veins are dilated. The bile-duct epithelium is of the high-cylindrical type and is vacuolated basally. Glisson's capsule is thickened and infiltrated by polymorphonuclear cells.

Conclusion: congestion of the liver and peritonitis.

T 4 (survived 9 days, died of biliary peritonitis):

Over-all architecture intact. The sinusoids are dilated. Pericentral hepatocytes show necrosis. More remote hepatocytes are angular shaped and have cytoplasm with an eosinophilic appearance. Accumulations of polymorphonuclear cells lie scattered in the sinusoids. There are increased numbers of Kupffer cells containing D/PAS + stained material. The portal fields are enlarged by fibrosis, but cellular infiltration is absent. The portal veins are dilated. Glisson's capsule is thickened and infiltrated by polymorphonuclear cells.

Conclusion: congestion of the liver with pericentral necrosis, peritonitis.

T 5 (survived 11 days, died of a bleeding duodenal ulcer):

Over-all architecture intact. Signs of acute

congestion with pericentral necrosis. Kupffer cells loaded with D/ PAS + stained material are scattered throughout the sinusoids. Some fibrosis is often seen in the portal fields. The bile ducts have a high-cylindrical type of epithelium with basal vacuolization.

Conclusion: acute congestion with pericentral necrosis.

T (survived 14 days, died of hepatic artery thrombosis):

Over-all architecture intact. Extensive pericentral necrosis. Extensive cholestasis. The sinusoids contain Kupffer cells loaded with D/PAS + stained material and some iron pigment. The portal fields appear almost normal. The bile ducts have a high-cylindrical type of epithelium with basal vacuolization.

Conclusion: strong signs of ischemia, secondary cholestasis.

T 7 (survived 32 days, died of biliary peritonitis):

Autopsy material lost; no data available.

T 8 (survived 37 days, killed because of poor general condition):

The biopsy specimen taken from the right liver lobe shows an intact over-all architecture. There are signs of extensive congestion and pericentral liver-cell necrosis. The hepatocytes contain some iron and occasionally also bile pigment. The sinusoids have been invaded by polymorphonuclear cells and Kupffer cells loaded with D/PAS + stained material and iron pigment. The portal fields do not show any cellular infiltrate. The bile-duct epithelium is of the high-cylindrical type and is vacuolated basally. The additional biopsy specimens taken from the central lobes show extensive necrosis.

Conclusion: congestion with signs of pericentral

necrosis in the right lobes and extensive necrosis in the central lobes.

T 9 (survived 39 days, killed because of liver failure):
Liver tissue shows multilobular necrosis. Numerous Kupffer cells, loaded with D/ PAS + stained material and iron pigment are seen in the necrotic areas. The portal fields are enlarged by fibrosis and show a polymorphonuclear-cell infiltrate which has occasionally reached and invaded the bile ducts. The bile-duct epithelium is of the high-cylindrical type and is vacuolated basally.
Conclusion: multilobular necrosis of the liver with moderate fibrosis, local purulent cholangitis; no evidence of rejection.

T 10 (survived 127 days, died of biliary peritonitis):
Over-all architecture intact. Signs of severe congestion. The portal fields contain a few lymphocytes, but bile ducts have not been invaded. The bile-duct epithelium shows a tendency to stratification, and basal vacuolization is seen. Glisson's capsule is thickened and infiltrated by polymorphonuclear cells.
Conclusion: congestion, peritonitis; no signs of rejection.

Group C: Microscopic appearance of the liver at autopsy in dogs still alive at the end of the study (n = 19).

Transplantation group (n = 10)

T 11 (permanent jaundice after day 32):
Over-all architecture intact. The hepatocytes have a feathery appearance and some are necrotic. Scattered accummulations of polymorphonucelear cells are seen in the sinusoids, and there is also an increased number of Kupffer cells loaded with D/PAS + stained material and sometimes also showing iron pigment. The portal fields are edematous and contain

polymorphonuclear and mononuclear cells as well as macrophages loaded with D/PAS + stained material. There is local invasion of the bile-duct epithelium, which shows a tendency to stratify and is vacuolated basally.

Conclusion: purulent cholangitis, perhaps also septicemia.

T 12:

Over-all architecture intact. Sparse polymorphonuclear cells in the sinusoids. Kupffer-cell activity is low. Slightly increased fibrosis in the portal fields , which show slight infiltration of polymorphonuclear and mononuclear cells. Limited invasion of the high-cylindrical, basally vacuolated bile-duct epithelium.

Conclusion: slight signs of cholangitis and perhaps of rejection.

T 13:

Over-all architecture intact. Signs of congestion and pericentral fibrosis. The sinusoids show very few polymorphonuclear cells. Kupffer cells loaded with D/PAS + stained material, are rare. The portal fields show some increased fibrosis and the portal veins are slightly congested. The bile-duct epithelium is predominantly of the high-cylindrical type and often vacuolated basally. The cellularity of the portal fields is minimal.

Conclusion: signs of longstanding congestion but not of rejection.

T 14:

Over-all architecture intact. Small aggregates of polymorphonuclear cells are seen throughout the parenchyme. Scattered Kupffer cells loaded with D/PAS + stained material are also present. The portal fields are enlarged by fibrosis and contain a mixed polymorphonuclear- and mononuclear- cell infiltrate which sometimes invades the bile ducts.

The bile-duct epithelium is of the high-cylindrical type and is vacuolated basally. Bile-duct proliferation has occurred.

Conclusion: cholangitis, perhaps minor signs of rejection.

T 15 (temporary wound infection):

Over-all architecture intact. The sinusoids contain polymorphonuclear cells, sometimes aggregated. Scattered Kupffer cells loaded with D/PAS + stained material and sometimes showing iron pigment. The portal fields show some fibrosis and infiltration of mixed polymorphonuclear and mononuclear inflammatory cells occasionally also invading the bile ducts. The bile-duct epithelium is of the high-cylindrical type and is vacuolated basally.

Conclusion: minor signs of rejection with an infectious component.

T 16:

Over-all architecture intact. Polymorphonuclear cells as well as Kupffer cells loaded with D/PAS + stained material are found scattered throughout the sinusoids. Many portal fields are edematous and show fibrosis. A dense mononuclear-cell infiltrate is seen, occasionally invading the high-cylindrical, basally vacuolated bile-duct epithelium.

Conclusion: portal hepatitis and cholangitis, rejection.

T 17:

Over-all architecture intact. The sinusoids contain a mixed polymorphonuclear- and mononuclear-cell infiltrate as well as scattered Kupffer cells loaded with D/PAS + stained material. The portal fields are enlarged by fibrosis. A small amount of cellular infiltrate is present, but invasion of the high-cylindrical, basally vacuolated bile-duct epithelium has not occurred.

Conclusion: pericholangiolar fibrosis, aspecific

changes, no signs of rejection.

T 18 (repaired biliary anastomotic leak, subphrenic abscess):

Over-all architecture intact. Portal and periportal infiltration by mainly mononuclear cells. Invasion of the high- cylindrical, basally vacuolated epithelium is seen occasionally. Glisson's capsule is thickened and there is a dense subcapsular infiltrate containing mixed polymorphonuclear and mononuclear cells.

Conclusion: portal and periportal hepatitis, localized peritonitis, no signs of rejection.

T 19:

Over-all architecture intact. A few degenerated hepatocytes are present. Kupffer cells loaded with D/PAS + stained material, are found in the sinusoids. The portal fields show lymphocytic and plasmocytic cell infiltrate which has occasionally invaded the high-cylindrical, basally vacuolated bile-duct epithelium. Some fibrosis is seen around the bile ducts.

Conclusion: minor signs of rejection.

T 20:

Same picture as in T 19.

Control group (n = 9)

C 2:

Over-all architecture intact. The hepatocytes have a low glycogen content. A sparse mixed poly-morphonuclear and mononuclear cell reaction is observed in the sinusoids. Kupffer cells loaded with D/ PAS + stained material are seen occasionally. The bile-duct epithelium is of the high-cylindrical type and is basally vacuolated.

Conclusion: minor aspecific changes, glycogen depletion.

C 3:

Same picture as in C 2, but without glycogen depletion.

Conclusion: minor aspecific changes.

C 4:

Same picture as in C 3, but without cellular infiltration of the sinusoids.

Conclusion: minor aspecific changes.

C 5:

Some fibrosis around the bile ducts. Sparse mixed polymorphonuclear- and mononuclear-cell infiltrate in the portal fields but no invasion of the bile ducts.

Conclusion: minor aspecific changes.

C 6:

Over-all architecture intact. There is evidence of congestion. Some pericentral fibrosis. Sparse mixed cellular infiltrate in the sinusoids and portal fields. Scattered Kupffer cells loaded with D/PAS + stained material. The bile ducts occasionally show an invading lymphocyte and are often surrounded by fibrotic tissue. The epithelium is of the high-cylindrical type and is vacuolated basally.

Conclusion: signs of longstanding congestion, minor cholangitis.

C 7:

Over-all architecture intact. Signs of congestion. The sinusoids contain a mixed cellular infiltrate and sparse Kupffer cells loaded with D/PAS + stained material. The cellularity of the portal fields is clearly increased and of a mixed type occasionally seen to have infiltrated the bile ducts. The epithelium is of the high-cylindrical type and is vacuolated basally. Some fibrosis is present around the bile ducts.

Conclusion: signs of congestion, minor purulent cholangitis.

C 8:

Over-all architecture intact. The sinusoids contain a low-grade mixed cellular infiltrate as well as scattered Kupffer cells loaded with D/PAS + stained material. The portal fields too show a low-grade mixed cellular infiltrate which has occasionally invaded the bile ducts. The epithelium is of the high-cylindrical type and is vacuolated basally.
Conclusion: minor cholangitis.

C 9:

Over-all architecture intact. Some signs of congestion. Sparse mixed cellular infiltration in the sinusoids. Some Kupffer cells loaded with D/PAS + stained material. The bile ducts are locally surrounded by fibrotic tissue. The epithelium is of the high-cylindrical type and shows basal vacuolization.
Conclusion: some congestion, minor aspecific changes.

C 10:

Over-all architecture intact. Kupffer cells loaded with D/PAS + stained material are seen in the sinusoids. The bile-duct epithelium is of the high-cylindrical type and basally vacuolated.
Conclusion: minor changes.

11.3. Discussion

The method of sampling of the liver by wedge biopsies performed during the operation proved to be simple and without complications. Digital compression of the part of the liver to be biopsied, combined with electro-coagulation of the cut surfaces, resulted in a bloodless procedure even in the presence of coagulopathy.

The percutaneous-biopsy method for sampling of the liver proved unsatisfactory. The material obtained was

often fragmented and contained only a few portal fields. Since most of the relevant long-term microscopical changes concerned the portal fields, the percutaneous biopsy specimens had limited value for the assessment of long-term microscopical changes.

Although liver outflow block did not appear to be a clinical problem, all post-revascularization biopsy specimens showed signs of sinusoidal congestion. Hepatocyte necrosis was rare, but accumulation of fat droplets in the hepatocytes of the middle zone was seen in about 40% of the transplanted livers. The combination of centrilobular sinusoidal congestion, pericentral hepatocyte necrosis, and accumulation of fat droplets in the hepatocytes of the middle zone, are known histological features of liver outflow block in the dog.[1] It must therefore be concluded that some degree of liver outflow block always occurred, even in the control group. The lowered arterial diastolic bloodpressure in the postanhepatic period is consistent with this interpretation. The use of partial instead of total livers may well have been co-responsible for this phenomenon because the reduced livers received an unreduced portal venous supply after revascularization. Alican and Hardy[2] and Starzl et al.[3] postulated a relationship in dogs between the occurrence of liver outflow block and liver damage. Moreover the combination of liver damage with an augmented portal venous supply is known to promote the occurrence of liver outflow block.[3]

The presence of intrahepatocellular inclusion bodies in the post-revascularization biopsy specimens is undoubtedly a preservation-related phenomenon, because these bodies were already present at the end of the preservation period. The responsible substance is neither glycogen nor fat, but the exact nature is not known. This phenomenon has not been described, but Krom has seen it in canine liver transplantation and very occasionally in human liver transplantation.[4] It appears to be related to

the composition of the preservation fluid, but species-related differences also seem to play a role. The intrahepatocellular inclusion bodies disappeared rapidly. In two of the three dogs, whose death occurred within 24 hours after the operation, no inclusion bodies were found in the autopsy specimen of the liver. In dog T 2, which died from acute liver failure, the inclusion bodies were still present in the autopsy specimen. The rapid clearance of these bodies therefore seems to be an expression of early hepatocellular function.

The percutaneous biopsy specimens taken 8 days after the operation showed remarkable signs of recovery in the liver. The dilated sinusoids had largely disappeared and the shape of the hepatocytes had normalized. Hepatocyte necrosis was absent and the accumulation of fat droplets had disappeared. Moreover, glycogen storage had markedly improved, at least in dogs without overt morbidity in that stage. The intrahepatocellular inclusion bodies had also disappeared. Cellular infiltration of the portal areas was absent. The cells of the bile-duct epithelium were of a high-cylindrical type and showed basal vacuolization, and this feature was seen in all subsequent biopsy specimens.

The microscopical appearance of the liver at autopsy of dogs succumbing before the end of the study was consistent with the diagnosed cause of death. Signs of acute congestion were often present but not clearly related to the degree of congestion found in the post-revascularization biopsies, which indicates a difference in etiology. The Kupffer cells invariably showed increased activity and loading with D/PAS + stained material. Polymorphonuclear cell infiltration the sinusoids was sometimes seen. The portal fields were usually quite well preserved, although some intensification of fibrosis was seen, especially around the bile ducts. Cellular infiltration of the portal fields was rare.

The over-all architecture of the liver, as seen in autopsy material from dogs still alive at the end of the study, was well preserved. Collapse of the connective framework was never observed, and necrosis of liver cells was rare. Signs of long-standing congestion were found in one transplanted and one control dog. A mixed polymorphonuclear- and mononuclear-cell infiltrate was often seen in the sinusoids, especially in the transplantation group. The portal areas were frequently enlarged by fibrosis, and this was more pronounced in the transplantation group. Many portal areas showed cellular infiltration, and this too affected the transplantation group more severely. The cellular infiltrate was often of a mixed poly- morphonuclear- and mononuclear-cell type, but in the transplantation group the mononuclear-cell type predominated.

Invasion of the bile-duct epithelium by mononuclear cells was seen in 6 transplanted dogs. In one control dog (C 6) an occasional lymphocyte had invaded the bile-duct epithelium as well. The cellular infiltrate found in the portal fields of this liver was, however, predominantly of the polymorphonuclear-cell type. Nonsuppurative destructive cholangitis is reported to occur frequently in human liver allografts and is considered to represent one component of allograft rejection.[5] Other histological features of chronic rejection such as cholestasis, foamy macrophages in the parenchyme, and vascular changes[1,7] were not seen. Many livers of the long-term survivors in both groups showed a polymorphonuclear-cell infiltrate in the portal fields, especially around the bile ducts. This phenomenon has also been observed in the livers of long-term survivors after experimental whole-liver autotransplantation, and has been ascribed to ascending cholangitis related to the presence of a cholecysto- duodenostomy.[1] In the present series of experiments, however, we performed choledocho- choledochostomies instead of cholecysto- duodenostomies. The beneficial

effect of a sphincter-retaining anastomosis on the prevention of ascending cholangitis seems to have been eliminated by the use of a transanastomotic splint. Microbiological investigation might have supported this assumption, but unfortunately was not part of the protocol.

11.4. Conclusions

The method of the peroperative sampling of liver tissue by the performance of wedge biopsies proved to be simple and free of complications, whereas the method of percutaneous sampling led to the death of one dog. Moreover, the samples obtained percutaneously were often fragmented and included only few portal fields. Conclusive interpretation regarding these fields was therefore not possible, which is unfortunate because the most important pathological changes in the liver of long-term survivors concerned the portal fields.

Signs of damage of the liver were seen in all post-revascularization specimens. At least some degree of congestion of the central veins and the pericentral sinusoids was always present, which indicates that some degree of outflow block had invariably occurred. The consistent finding of intrahepatocellular inclusion bodies raised questions about the preservation technique used.

Irreversible damage to the liver leading to early death, occurred in only one dog. In this dog the intrahepatocellular inclusion bodies had not disappeared at autopsy. The other two early deaths had other causes, and early resumption of liver function in these dogs was suggested histologically by the rapid disappearance of the inclusion bodies. By the 8th day the liver had made a remarkable recovery.

The histological findings in the samples taken at

autopsy of dogs whose death occurred before the 169th day, were consistent with the diagnosed cause of death. The most striking histological changes in the autopsy findings of long-term survivors concerned the bile ducts, especially the smaller ones, and the pericholangiolar portal tissue. Minor signs of rejection, defined as mononuclear-cell infiltration of the portal fields combined with invasion of the bile ducts, were present in 6 transplanted dogs. Signs of moderate or severe rejection were never seen. The portal fields in both groups of dogs, however, often showed an increased number of polymorphonuclear cells, indicating the presence of an infectious agent. The possibility of a relation between this phenomenon and the use of a transanastomotic biliary splint must be taken into consideration.

REFERENCES

1. Porter KA: Pathology of the orthotopic homograft and heterograft, in Starzl TE, Putnam CW (eds): Experience in Hepatic Transplantation. Philadelphia, Saunders, 1969, pp 422-471
2. Alican F, Hardy JD: Replantation of the liver in dogs. J Surg Res 7:368-375, 1967
3. Starzl TE, Kaupp HA, Brock DR, et al: Reconstructive problems in canine liver homotransplantation with special reference to the postoperative role of hepatic venous flow. Surg Gynecol Obstet 111:733-743, 1960
4. Krom RAF: Personal communication. January 1984
5. Fennell RH, Vierling JM: Non-suppurative destructive cholangitis in chronic rejection of transplantated livers. Gastroenterology 79:1016, 1980
6. Fennell RH, Shikes RH, Vierling JM: Relationship of pretransplant hepatobiliary disease to bile duct

damage, occurring in the liver allograft. Hepatology 3:84-89, 1983

7. Neuberger J, Portmann B, MacDougall BRD, et al: Recurrence of primary biliary cirrhosis after liver transplantation. N Engl J Med 306:1-4, 1982

CHAPTER 12. GENERAL DISCUSSION AND CONCLUSIONS

If one decides to attempt to cure children with end-stage hepatic disease, one must resort to liver transplantation (Section 2.1). On the basis of current indications for liver transplantation, about 15 to 20 children in The Netherlands will qualify for this procedure annually.[1,2] The majority of these children will have biliary atresia as underlying disease. Although the hepatic porto-enterostomy has undoubtedly improved the short-term prognosis in children with biliary atresia, the majority of these children still progress to end-stage hepatic disease (Section 2.2).

At present, it is logical to give preference to the orthotopic mode of liver transplantation, because this is the only mode which has given reasonably good results in man (Chapter 3). In children with biliary atresia, furthermore, it seems wise to remove the native liver at the time of transplantation, in view of the high incidence of recurrent cholangitis after hepatic porto-enterostomy,[3] and the fact that incidental carcinomas have been found in the native livers of children with biliary atresia.[4,5]

Until 1980, the over-all results of clinical orthotopic liver transplantation were not encouraging (Chapter 5). Since then, the one-year survival rate has doubled and is now about 70% in both children and adults.[6] Starzl et al. attribute this improvement mainly to the use of cyclosporin A and the concomitant reduction of the steroid dosage.[6] However, other factors such as better recipient selection and improved techniques, especially for the biliary anastomosis, must also be

taken into account.[7] The Groningen team, for example, obtains roughly the same results with classical immunosuppression.[7,8] Nevertheless, cyclosporin A may prove to have special value in pediatric liver transplantation by making it possible to avoid steroid-induced growth retardation.

There are still many problems to be solved in clinical orthotopic liver transplantation (Chapter 4). The shortage of donor livers and the limited safe organ-preservation time preclude donor-recipient selection on the basis of tissue typing. Due to the lack of an effective artificial liver and the limited supply and preservation time of donor livers, many patients are poor surgical risks. The operation itself is a formidable procedure, and interferes profoundly with hemodynamics and hemostasis. For many years the biliary anastomosis was the Achilles' heel of liver transplantation, but improvements in the techniques seem to have solved this problem to a great extent. Rejection was long believed to play a minor role in the poor results, but with the introduction of cyclosporin A the liver's status as immunoprivileged organ seems to have been weakened.

Besides the effects of immunosuppressive therapy on the growth of children other problems too are encountered in pediatric liver transplantation. For example, there is the problem of the smallness of the size of the blood vessels, but this can be overcome by the use of microsurgical techniques.[5] Moreover, children with biliary atresia may have associated anomalies of the vascular supply of the liver.[5,9] These anomalies will, however, be known beforehand, because at present all such children will have undergone hepatic porto-enterostomy.

The most important special problem in pediatric liver transplantation is the shortage of size-matched donor livers. The use of the left lateral segment of adult donor livers, the adult donor livers being more readily obtainable may offer a solution to this problem (Chapter 1).

In man, removal of all but the left lateral segment of the liver has proven to be feasible, and there can be little doubt that a right hepatic trisegmentectomy will be easier to perform on a cooled human donor liver on the bench than in vivo. The human liver is, however, much less lobulated than the canine liver and more parenchyme will have to be transected. Biliary leakage and loss of blood from the amputation site are therefore more likely to occur in man, but can be avoided by meticulous ligation of all intraparenchymal vessels to be transected.

From the results of the experimental study it can be concluded that it is technically feasible to transplant part of an adult liver orthotopically, at least under the experimental conditions applied. The preparation of the partial grafts on the bench proved simple and was not an important time-consumer. No mortality or morbidity could be attributed to the use of only part of the liver. Fifty percent of the dogs survived the 169-day period of the study, and most of these dogs did well clinically (Chapter 7). Bilirubin handling, albumin synthesis, and the clearance of both bromsulphthalein and antipyrine were reasonably well preserved (Chapter 10). Graft atrophy occurred in only one transplanted dog. In the transplanted dogs which survived the entire study period the weight of the graft had increased and equaled the weight of the removed native liver, despite the use of immunosuppressive drugs (Chapter 7). Comparison of the transplantation and control groups showed similar trends in the monitored items, although the changes were usually more pronounced in the transplantation group. No pronounced differences were found between immuno-suppressed and nonimmunosuppressed control dogs, but it must be acknowledged that these subgroups were small.

The experimental series was not free of difficulties and problems, but these are also known to occur when

total livers are transplanted orthotopically. The surgical risk appeared to be related more strongly to insufficient decompression of the infradiaphragmatic venous systems during the anhepatic phase than to the hour-long absence of the liver (Chapter 8). Of special interest is the observation that the bleeding disorder started at the end of the anhepatic phase and that the course of this disorder appeared to be closely related to the changes in the platelet count rather than to the changes in the concentrations of coagulation factors. The advantages of decompression of the infradiaphragmatic venous systems during clinical liver transplantation are slowly gaining recognition, but mainly in connection with the hemodynamic status of the patient rather than any coagulation disorder.[10] Better decompression might have prevented the coagulation disorder observed. The present study shows clearly that glucose consumption does not increase during a one-hour anhepatic peroid, at least in healthy dogs.

Although much attention was paid to the avoidance of damage to the liver and although early postoperative liver failure was rare, damage did occur, as seen biochemically and histologically (Chapter 10 and 11). Moreover, the finding of intrahepatocellular inclusion bodies in the post-revascularization biopsy specimens raises doubts concerning the technique used for the preservation of canine livers (Chapter 11).

As in clinical liver transplantation, anastomotic vascular problems were not a major cause of failure (Section 9.1). Pervenous angiography proved to be a very valuable tool for the diagnosis of such problems, as illustrated by the dog which developed hepatic artery thrombosis. The Achilles' heel of this study was the biliary reconstruction (Section 9.2). Especially worrying is the high incidence of splint-related complications. Moreover, the biliary splints may well have caused intermittent obstruction and recurrent ascending

cholangitis, which might explain the fluctuating liver enzyme profiles as well as the frequent finding of a polymorphonuclear-cell infiltrate and increased fibrosis around the bile ducts in the livers of the dogs autopsied at the end of the study period (Chapters 10 and 11).

No signs of rejection were found in the dogs which died before the end of the study and only minimal signs were found in the transplanted dogs autopsied at the end of the study (Chapter 11). It may therefore be concluded that the measures taken to prevent and control rejection were effective.

This experimental study has proven that it is technically feasible to transplant part of the donor liver orthotopically in dogs and that a partial liver graft can function adequately for a considerable period. The many problems encountered did not seem to be related to the use of only part of the donor liver or to rejection, but rather to defective preservation of the donor liver, insufficient decompression of the infradiaphragmatic venous systems during the anhepatic phase, and to an imperfect technique for the biliary reconstruction. Therefore, further experimental work is undoubtedly required.

REFERENCES

1. Wolff ED: Liver Transplantation in the Rat. Rotterdam, Doctoral Thesis, 1976
2. Bax NMA, Molenaar JC: The incidence and fatality of biliary atresia in The Netherlands; an indicator for the need for pediatric liver transplantation. J Jpn Soc Pediatr Surg 18:1023- 1027, 1982
3. Hays DM, Kimura K: Biliary Atresia. The Japanese Experience. Cambridge, Havard University Press, 1980, pp 107-126

4. Van Wijk J, Halgrimson CG, Giles G, et al: Liver transplantation in biliary atresia with concomitant hepatoma. S Afr Med J 46:885-889, 1972
5. Starzl TE, Koep LJ, Schröter GPJ, et al: Liver replacement for pediatric patients. Pediatrics 63:825-829, 1979
6. Starzl TE, Iwatsuki S, Van Thiel DH, et al: Evolution of liver transplantation. Hepatology 2:614-636, 1982
7. Calne RY: Liver grafting. Transplantation 35:109-111, 1983
8. Krom RAF: Personal communication. December 1983
9. Mihyamoto M, Nakamura Y, Kajimoto T: Biliary atresia and associated malformations. J Jpn Soc Ped Surg 17:885-894, 1981
10. Calne RY, McMaster P, Smith DP, et al: The use of partial cardiopulmonary bypass during the anhepatic phase of liver grafting. Lancet 2:612-614, 1979

SUMMARY

End-stage hepatic disease can only be cured by orthotopic liver transplantation. As pediatric surgeon, the writer of this thesis was interested in the problems that hamper the development of pediatric orthotopic liver transplantation. The shortage of size-matched donor livers for this purpose is a major problem. A possible solution would be the use of part of an adult donor liver, the adult organs being easier to obtain. The technical feasibility of this operation formed the basis of the experimental study described in the present thesis (Chapter 1).

Chapter 2 concerns the need for liver transplantation. At present, transplantation seems to be the only effective method available for the treatment of terminal liver failure (Section 2.1). The need for pediatric liver transplantation in The Netherlands and the most common indication for this treatment in the pediatric age group, i.e. biliary atresia, are discussed (Section 2.2).

A review of the various modes of liver transplantation led to the conclusion that only the orthotopic nonauxiliary mode has had clinical success (Chapter 3).

The many problems associated with clinical orthotopic liver transplantation are analysed in Chapter 4. The shortage of donor livers and the limited safe preservation time of the organ as well as the usually poor preoperative general condition of the recipient, preclude optimal donor recipient selection. The biliary anastomosis has also been a major cause of mortality and morbidity. Lastly, rejection seems to be an important determinant of the long-term prognosis of clinical

orthotopic liver transplantation, and the human liver seems to be a less immunopriviliged organ than has been thought.

The published results of clinical orthotopic liver transplantation in children are reviewed in Chapter 5. Up to 1980, the results were poor. The improvement achieved since 1983 is unquestionably related not only to the introduction of the new immunosuppressive agent, cyclosporine A, but also to the improved surgical techniques and to earlier recipient selection and transplantation.

Chapter 6 describes the experimental design, and discusses the choice of the experimental animal, the handling of rejection, the reasons for the design of a control model and for the choice of the orthotopic mode of partial liver transplantation, the age of the experimental animals, the relative size of the grafts, the duration of liver ischemia, and the duration of the follow-up period.

In Chapter 7 the surgical techniques are presented and the technical feasibility is described in terms of mortality, overt morbidity, and the autopsy findings.

Chapter 8 focuses on the peroperative complications and the technical feasibility is assessed, in terms of surgical risk in the absence of peroperative mortality.

Special attention was given to the postoperative monitoring of the patency of the various types of anastomosis. The methods used for and the results of monitoring are described and discussed (Chapter 9).

The postoperative functioning of the liver was evaluated in terms of not only mortality and overt morbidity, but also biochemical homeostasis and handling of exogenous loading (Chapter 10).

The quality of the liver after surgery was also followed histologically (Chapter 11).

Chapter 12 gives a general discussion and the ensuing conclusions.

The question as to whether it is feasible to transplant part of a donor liver in an orthotopic nonauxiliary position, at least in dogs, is answered positively.

SAMENVATTING

Terminale leverinsufficiëntie kan enkel door middel van een orthotoop levertransplantaat curatief behandeld worden. Als kinderchirurg was de schrijver van dit proefschrift geinteresseerd in de problemen, die de toepassing van deze behandeling bij kinderen, in de weg staan. Een zeer belangrijk probleem is het nijpend tekort aan donor levers van aangepaste afmetingen voor transplantatie bij kinderen. Een mogelijke oplossing voor dit probleem zou het gebruik van een deel van een volwassen donorlever kunnen zijn. Het aanbod van grote volwassen donorlevers is immers veel minder beperkt dan het aanbod van kleine kinderdonorlevers. Het experimenteel onderzoek naar de technische haalbaarheid van het transplanteren van een deel van een donorlever vormt de grondslag van dit proefschrift (Hoofdstuk 1).

Hoofdstuk 2 handelt over de behoefte aan levertransplantatie. Eerst wordt nagegaan of er geen alternatieve effectieve methoden voor de behandeling van terminale leverinsufficiëntie bestaan (2.1). Vervolgens wordt de behoefte aan levertransplantatie op de kinderleeftijd in Nederland onderzocht. Terminale leverinsufficiëntie ten gevolge van galgangatresie is verreweg de meest voorkomende indicatie voor levertransplantatie op de kinderleeftijd (2.2).

Uit het overzicht van de verschillende vormen van levertransplantatie, die werden toegepast, blijkt dat alleen de orthotope niet-auxiliaire vorm op dit ogenblik klinisch succes boekt (Hoofdstuk 3).

Hoofdstuk 4 gaat in op de problemen waarmee men bij klinische orthotope levertransplantaties geconfronteerd

wordt. Door het tekort aan donorlevers, de beperkte preservatieduur ervan en de meestal slechte preoperatieve conditie van de ontvanger, is geen optimale donor-ontvanger selectie mogelijk. Bovendien hebben de problemen rond de galweganastomose een belangrijke invloed op de mortaliteit en morbiditeit. Tenslotte blijkt rejectie van het transplantaat een belangrijkere rol te spelen bij de uiteindelijke prognose van klinische orthotope levertransplantatie dan voorheen gedacht werd.

De resultaten van het orthotoop transplanteren van de lever bij kinderen worden in Hoofdstuk 5 besproken. Tot 1980 waren deze resultaten bedroevend. De duidelijke verbetering, sedert 1983, is zeker niet alleen het gevolg van het gebruik van het nieuw immunosuppressivum, cyclosporine A, doch ook van de verbeterde chirurgische technieken en van de vroegtijdigere selectie en transplantatie van levertransplantatiekandidaten.

Hoofdstuk 6 beschrijft het algemeen experimentele ontwerp met daarin aandacht voor de keuze van het proefdier, de aanpak van rejectie, de reden voor het creëren van een controle model en voor het orthotoop niet-auxiliair transplanteren, de keuze van de leeftijd van het proefdier en van de relatieve grootte van de partiële donorlever, de keuze van de duur van leverischemie en van de duur van de postoperatieve vervolgperiode.

In Hoofdstuk 7 worden de chirurgische technieken beschreven en wordt technische haalbaarheid uitgedrukt in termen van mortaliteit, klinisch duidelijke morbiditeit en autopsiebevindingen.

In Hoofdstuk 8 wordt ingegaan op de peroperatieve complicaties en wordt technische haalbaarheid uitgedrukt in operatie-risico in afwezigheid van directe operatieve mortaliteit.

De verschillende anastomosen werden postoperatief zorgvuldig geëvalueerd (Hoofdstuk 9).

De functie van de lever na operatie werd niet alleen

nagegaan in termen van mortaliteit en morbiditeit doch ook in termen van biochemische homeostase en verwerking van exogeen toegediende substanties (Hoofdstuk 10).

Tenslotte werd de kwaliteit van de lever na operatie microscopisch vervolgd (Hoofdstuk 11).

Hoofdstuk 12 besluit het proefschrift met een algemene discussie en de uit het onderzoek te trekken conclusies.

De vraagstelling of het orthotoop niet-auxiliair transplanteren van een deel van een donorlever, althans bij honden, technisch haalbaar is wordt positief beantwoord.

ACKNOWLEDGMENTS

I wish to thank Professor J.C. Molenaar, head of the Department of Pediatric Surgery of the Sophia Children's Hospital in Rotterdam, very much for the generous support he gave me in the performance of the experiments and the writing of this thesis. Thanks are also extended to Dr. J. Jeekel, Professor of Surgery at the Erasmus University of Rotterdam, for his critical reading of the manuscript and his comments.

I am indebted to Dr. S.W. Schalm, hepatologist of the Dijkzigt Hospital in Rotterdam, who contributed greatly to the setting up of the experiments by sharing his experience with experimental liver transplantation.

An important financial contribution was provided by the Sophia Scientific Foundation, which also sponsored two study trips, one to visit Professor T.E. Starzl and his team in Denver, Colorado, which brought me into contact with clinical liver transplantation, and the other to visit Professor M. Kasai in Sendai and Dr. K. Kimura in Kobe, Japan, which gave me the opportunity to discuss the project with world experts in the treatment of patients with biliary atresia, and therefore to place the project in the clinical indication field it was designed for.

I am also indebted to two colleagues, Dr. F.W.J. Hazebroek, member of the staff of the Department of Pediatric Surgery of the Sophia Children's Hospital in Rotterdam, and Dr. M.H. Rövekamp, of the Department of Pediatric Surgery of the Wilhelmina Children's Hospital in Utrecht, for taking over many of my clinical duties during the periods of experimentation and preparation of the thesis.

The Surgery Laboratory of Erasmus University, headed

by Professor D.L. Westbroek, allowed me to consume an important part of the laboratory's budget, to use their superbly equipped operating theatres, and to draw on their tremendous know-how in canine organ transplantation.

Mrs. A.M. Dekkers-Byma carried out the histocompatibility testing. Mrs. J. de Kam provided skillful instrumental and manual assistance. Anesthesia was given by Mr. E.E.C. Colly and Mr. E. Ridderhof. Mr. J. Kasbergen, Mr. P. Kreeft, Mr. R.W.J. Meyer, and Mr. R. Spruyt cared for the dogs before and after surgery. Most of the hematological and biochemical analyses were performed by Mr. W.P. van Schalkwijk.

The radiological examinations were carried out by Mr. J. Fasotte, Mrs. B. Fasotte-van Leeuwen, and Miss W. van Leeuwen. Mrs. M.J. Kersten-Lagerman coped with the administrative side of the experimental study.

Dr. N. Dubois and Dr. G. Madern, trainees in general surgery at the time of the experimental study but fully qualified surgeons by now, kindly provided surgical assistance.

I am very grateful to my wife Dr. B.M.J. Vermeire, former pediatric anesthesist at the Sophia Children's Hospital, for her active participation in the project. By developing the anesthesiological protocol and supervising the anesthesiological management, she contributed considerably to the success of the experiments. She also assisted me in the writing of the section on the peroperative complications.

Mrs. A.C.P. Zondag, analyst-in-chief of the Hematology Section of the Department of Pediatrics, of the Sophia Children's Hospital and her team formed by Mrs. A.M. Kolb, Mr. E.H. Elk, and Mr. J.C.L. van der Palm, investigated the canine coagulation pattern during and after surgery and computerized all of the data of the experiments. I thank this enthousiastic and skillful team for their unfailing support.

I want to express my sincere thanks to the Department of Pathology of Erasmus University for their tremendous support, both quantitative and qualitative. Miss E. Buitenhuis and Mr. J. van Lier did the preparative work on the numerous biopsy specimens. Dr. P.E. Zondervan spent many hours on the microscopical analysis of the specimens and taught me how to look at them. He and Dr. F.W.J. ten Kate helped me in writing up the results and the discussion. I am deeply indebted to them for their expertise and for the time they spent on the project.

Dr. M. Meradji, pediatric radiologist of the Sophia Children's Hospital, made an essential contribution to the technique of the pervenous angiography and assisted me in preparing the written report.

The Laboratory of Internal Medicine II of Erasmus University, headed by Dr. J.W.O. van den Berg, kindly performed the antipyrine determinations.

I had many pleasant and valuable discussions with Dr. P.A. Provoost, biochemist and researcher of the Department of Pediatric Surgery of Erasmus University, who also encouraged me tremendously during the long and tedious pilot studies.

The Pharmacy of Dijkzigt Hospital prepared the Euro-Collins solution used for cooling and preservation of the livers. I especially wish to thank Dr. B.H. Graatsma for his collaboration.

Mrs. A. Kurvers, secretary of the Department of Pediatric Surgery of the Sophia Children's Hospital, kindly typed the experimental protocols and the many surgical and follow-up notes. Miss L.Th.M. Barge, secretary of the Department of Pediatric Surgery of the Wilhelmina Children's Hospital in Utrecht, typed the many revisions of the manuscript, and I am deeply indebted to her.

For the typing use was made of a word-processor. The assistance of Mr. J.P. Hundersmarck, head of the Computer Department of the Wilhelmina Children's Hospital in

Utrecht, to finalize the typed text of the manuscript is greatly appreciated.

The Figures and Tables were prepared by Mrs. C.C.M. Bandel-Schweinsberg in collaboration with the Audio-Visual Department of the Sophia Children's Hospital. Mrs. A.C. Ribbink-Goslinga and Mrs. I. Seeger, read most of the English text. For the remaining linguistic shortcomings, I take full responsibility. Mrs. E.A.D.M. Neleman-van den Broek, librarian of the Sophia Children's Hospital, checked the abbreviations used in the references.

I want to express my thanks here to the Directors of the Sophia Children's Hospital for the permission to do the experiments and for allowing me to use so many of the hospital facilities. I also express my thanks to the Directors of the Wilhelmina Children's Hospital in Utrecht for the moral and financial support required to finish the work started in Rotterdam.

The immunosuppressive drug azathioprine was generously supplied by the Wellcome Foundation. The Byk Company kindly provided the contrast medium ioxaglate used in the pervenous angiographic studies. Preoperative deworming of the animals was carried out with mebendazol, and fluanisone was used for sedation of the animals during the percutaneous liver biopsies; both drugs were gifts fom Janssen Pharmaceutica. I wish to express my thanks to these companies for their support.

Throughout the periods of experimentation and writing I have been the recipient of an enormous amount of good will for which I want to take this opportunity to express my feelings of gratitude.

I do like to apologize to my daughters Bianca and Hannelore for the many times that they had to be quiet and the many times that I was unable to share their joys and sorrows. Happily enough they have a mother who translates fathers's worries and he worries less because she understands.

CURRICULUM VITAE

De schrijver van dit proefschrift werd op 1 juni 1944 in Scherpenheuvel, België, geboren. In 1962 verliet hij het Sint Jan Berchmans College te Diest, België, waar hij de grieks latijnse humoniora richting had gevolgd.

Van 1962 tot 1969 was hij medisch student aan de Katholieke Universiteit van Leuven, België. Na het aldaar behalen van de graad van doctor in de genees-, heel- en verloskunde was hij tot 1972 arts-assistent kindergeneeskunde in het Academisch Ziekenhuis Sint Rafael te Leuven (hoofd Prof. Dr. R. Eeckels). In 1974 werd hij als kinderarts in het Belgisch specialistenregister kindergeneeskunde ingeschreven.

In 1972 begon hij aan zijn opleiding in de heelkunde. Tot 1974 was hij arts-assistent kinderheelkunde in het Universitäts Kinderspital in Zürich, Zwitserland (hoofd Prof. Dr. P.P. Rickham). Van 1974 tot 1977 was hij arts-assistent heelkunde in het Groote Schuur Ziekenhuis in Kaapstad, Zuid-Afrika (hoofd Prof. Dr. J.H. Louw). Tijdens deze Kaapse periode werkte hij gedurende zes maanden als arts-assistent kinderheelkunde in het Red Cross War Memorial Children's Hospital (hoofd Prof. Dr. S. Cywes). In 1976 werd hij als chirurg ingeschreven in het Belgisch en in 1977 in het Nederlands specialistenregister.

Van 1977 tot en met 1981 werkte hij als chef de clinique voor de Afdeling Kinderheelkunde van het Sophia Kinderziekenhuis te Rotterdam (hoofd Prof. Dr. J.C. Molenaar).

Sedert 1 januari 1982 is hij hoofd van de Afdeling Algemene Kinderheelkunde van het Wilhelmina Kinderziekenhuis te Utrecht.