ORTHOTOPIC AND HETEROTOPIC LIVER TRANSPLANTATION

Circulatory and hemostatic effects
of experimental long-term graft preservation

ORTHOTOPE EN HETEROTOPE LEVERTRANSPLANTATIE
Een dierexperimenteel onderzoek naar
de invloed van langdurige orgaanpreservatie
op de bloedsomloop en de haemostasis

PROEFSCHRIFT

Ter verkrijging van de graad van Doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof. dr C.J. Rijnvos
en volgens besluit van het College van Dekanen.
De openbare verdediging zal plaatsvinden op
woensdag 5 februari 1992 om 15:45 uur

door

Jan Dirk Blankensteijn

geboren te Oranjestad

Promotiecommissie:

Promotor:	Prof. dr O.T. Terpstra
Co-promotor:	dr J. Stibbe
Overige leden:	Prof. dr H.A. Bruining
	Prof. dr S.W. Schalm
	Prof. dr Th.J.M.V. van Vroonhoven

Aan mijn ouders
Aan Ariane

Financial support by:

E. MERCK NEDERLAND B.V.

B. BRAUN MEDICAL B.V.

UPJOHN NEDERLAND

Druk: I.C.G. Printing B.V., Dordrecht

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Blankensteijn, Jan Dirk

Orthotopic and heterotopic liver transplantation :
circulatory and hemostatic effects of experimental
long-term graft preservation / Jan Dirk Blankensteijn ;
[ill. of the author]. - [S.l. : s.n.]. - Ill.
Thesis Rotterdam. - With ref. - With summary in Dutch.
ISBN 90-9004826-X
Subject headings: organ preservation ; liver transplantation.

Even with the highest artistic ability in surgical techniques, one can do the wrong operation.

(J.H. Baron, 1974)

CONTENTS

CHAPTER 1.

GENERAL INTRODUCTION 1

1.1 Topics of the study 3
1.1.1 OLT versus HLT 3
1.1.2 Liver preservation 4
1.2 Questions to be answered 7
1.3 Objectives of the study 7
1.4 Overview of the contents 8
1.5 References 9

CHAPTER 2.

NEW ASPECTS OF HETEROTOPIC LIVER TRANSPLANTATION 15
In press, Transplant International.

2.1 Introduction 17
2.2 History 17
2.3 New aspects 22
2.3.1 Intraoperative fibrinolysis 23
2.3.2 Intraoperative hemodynamics 23
2.3.3 Correction of portal hypertension 24
2.3.4 Interactions between two livers 24
2.3.5 The role of portal blood flow in HLT 26
2.3.6 Temporary support in (sub)acute liver failure 27
2.3.7 Metabolic diseases of the liver 29
2.3.8 Modifications of HLT 29
2.4 Conclusions 30
2.5 References 31

CHAPTER 3.

LIVER PRESERVATION. The past and the future 37
Hepatology 1991;13:1235–1250.

3.1 Introduction 41
3.2 History of organ preservation 42
3.2.1 Continuous perfusion 44
3.2.2 Simple hypothermic storage 45
3.3 Mechanisms of harvest injury 46
3.3.1 Anoxic damage 47
3.3.2 Reperfusion damage 52
3.4 Cytoprotective compounds in liver preservation 59
3.4.1 Calcium– and cAMP– related mechanisms 61
3.4.2 Direct prevention of O_2^-. formation 64
3.4.3 Oxygen–derived free radicals scavengers 64
3.4.4 Attenuation of cytokine production 65
3.4.5 Unknown mechanisms of action 66
3.5 UW solution 66
3.6 Conclusions and future directions 69
3.7 References 71

CHAPTER 4.

EXPERIMENTAL DESIGN AND GENERAL RESULTS 87

4.1 Introduction 89
4.2 Materials and Methods 89
4.2.1 Animals and perioperative management 89
4.2.2 Surgical technique 91
4.2.2.1 Donor operation 91
4.2.2.2 Transplantation 91
4.2.2.3 Prostaglandin 93
4.2.3 Measurements 94
4.2.4 Morphology 95
4.2.5 Statistics 96
4.3 Results 97
4.3.1 General data 97

4.3.2 Hematological and biochemical data 101
4.3.3 Morphology 103
4.3.4 Mortality and survival 108
4.4 Discussion 111
4.5 Conclusions 114
4.6 References 115

CHAPTER 5.

HEMODYNAMIC CHANGES 119
part 1. *Transplantation 1990;49:665–668.*
part 2. *In press, Transplantation.*

5.1 Introduction 123
5.2 Methods 124
5.3 Results 125
5.4 Discussion 131
5.5 Conclusion 134
5.6 References 135

CHAPTER 6.

HEMOSTATIC CHANGES 139
part 1. *Transplant International 1991;4:12–17.*
part 2. *Submitted.*

6.1 Introduction 143
6.2 Methods 144
6.2.1 Blood samples 145
6.2.2 Hemostasis studies 145
6.3 Results 146
6.3.1 Type of transplantation 146
6.3.2 Graft storage time 149
6.3.3 Use of PGE_1 152
6.4 Discussion 152
6.5 Conclusion 155
6.6 References 156

CHAPTER 7.

GENERAL DISCUSSION AND CONCLUSIONS 161

7.1 Introduction 163
7.2 Preparation period 164
7.3 Portal flow interruption period 165
7.4 Early postreperfusion period 166
7.5 Late postreperfusion period 169
7.6 Prostaglandins 170
7.7 Conclusions 171
7.8 References 174

SUMMARY 179
SAMENVATTING 189
ABBREVIATIONS 199
DANKWOORD 200
CURRICULUM VITAE 203

Chapter 1

GENERAL INTRODUCTION

Almost a quarter of a century ago, Starzl accomplished the first successful liver transplantation in man.[1] Since then, hepatic allograft survival steadily increased as a result of progress in patient care, immunosuppressive treatment, surgical techniques, and organ preservation. So, orthotopic liver transplantation (OLT) has become an effective therapy for end–stage liver disease with one–year patient survival ranging from 70% to 90% at various centers.[2–5] Still, many patients with profound hemostatic disturbances or concomitant cardiovascular disease may not be able to tolerate such a major operation.

One aspect of the advances in surgical techniques is the evolution of the auxiliary heterotopic liver transplantation (HLT). This is an alternative to the orthotopic procedure and has some attractive potential advantages over OLT.

Another progress in liver preservation is the extension of the duration of graft preservation. As happened in kidney transplantation, this may change liver transplantation from an emergency procedure into a semielective one.

1.1 TOPICS OF THE STUDY

1.1.1 OLT versus HLT

The most obvious difference between OLT and HLT is that in HLT the recipient liver is left in place. This principle may give significant benefits, but may also create some problems.

First, in HLT the strenuous removal of the cirrhotic liver and the associated blood loss is evaded and second, there is no anhepatic phase. In OLT, these factors have serious hemodynamic and hemostatic implications for the critically ill patient with end–stage chronic liver disease. Furthermore, the recipient liver may act as functional reserve when the donor liver does not function well, immediately after reperfusion of the graft. In addition, in case of reversible liver disease when adequate regeneration and recovery of the patient's own liver has been established, the auxiliary graft can be removed or left to atrophy.[6,7]

Another important advantage of auxiliary transplantation is that matching to size is not necessary. This will result in a greater availability of donors. Finally, the procedure has the potential of being less time consuming,[8] although most surgeons who are experienced in the orthotopic procedure admit that heterotopic grafting is technically more demanding.

Despite these clear, theoretical merits, results after the first clinical auxiliary heterotopic liver transplantation by Absolon in 1964 were discouraging.[9] Though long-term survival was incidentally described,[10,11] most centers rejected this method in favor of OLT.[12] Problems encountered with HLT were related to lack of abdominal space for the additional liver and to the non-anatomical position of the graft vessels. Also, the possibility of leaving behind oncogenic tissue (or even an occult carcinoma) by not removing the recipient liver was a major concern.
Laboratory research aiming at improvement of the surgical procedure of HLT[13–15] led to more promising clinical outcome. In 1988, it was reported on six consecutive patients with end-stage chronic liver disease who underwent HLT.[8] Elsewhere, these patients were rejected for OLT because they had massive ascites, pronounced clotting deficiency, cachexia or poor pulmonary reserve. Following HLT, after a mean follow-up of 14 months, all patients were alive with good graft function. Not withstanding these hopeful facts, the theoretical advantages of leaving the host liver *in situ* have never been confirmed by comparative means.

1.1.2 Liver preservation

Having overcome the initial surgical difficulties of liver transplantation, the absence of a reliable method for extended organ preservation became a major dilemma. To keep the period of graft storage to a minimum the recipient operation was performed as an emergency procedure, often at night. Although improvements in preservation techniques have relaxed the emergency nature of liver transplantation, many problems remain.
One of the principal causes of loss of the graft and occasionally death of the patient is primary nonfunction of the liver. Usually, this is thought to be through an injury caused by inadequate harvest techniques or graft preservation. Primary nonfunction occurs in 2% to 12% of the transplanted livers.[16–19] In Europe, the primary nonfunction rate is about 6%.[20,21] The importance of good preservation methods was further emphasized by Howard et al.[22] who demonstrated significantly more rejection (71%) in a group of patients receiving grafts with severe storage damage compared to the group without storage damage (33%).
The pathophysiology of storage injury in liver transplantation is unknown, but oxygen is considered to play an important role.[23,24] After harvesting the liver, the organ becomes ischemic and oxygen is no longer provided for aerobic metabolism.

Accordingly, cell homeostasis is disturbed and the end products of anaerobic cellular processes accumulate.

On the other hand, several experiments with other organs suggest much of the harvest injury occurs during reperfusion.[23,25,26] In this theory, reperfusion of ischemic tissue results in the production of superoxide and other reactive oxygen free radical species. Oxygen radicals can damage endothelial cells by induction of changes in membrane lipids.[27]

The preferred method of preserving livers for brief periods is simple cold storage.[28] Although hypothermia remains the basis of all preservation techniques, simple cold storage has a specific time limit beyond which the organ is no longer viable. Hypothermia decreases the rate intracellular enzymes degrade essential cellular components necessary for organ viability, but it does not stop metabolism. Rather, it simply delays reaction rates and cell death until finally the organ ceases to function and loses viability.[29]

Until recently, cold storage of the graft in Collins' solution[30] provided acceptable graft function when the preservation time was 8 hr or less. In march 1988, Kalayoglu and co-workers from the University of Wisconsin reported the first clinical use of a new preservation fluid. This UW solution significantly increased the tolerance of cold ischemia by the human liver.[31]

Initial experimental results with UW solution held out hope for preservation times well over 24 hr.[32] However, within four years of the first clinical use of UW solution, it was suggested to reconsider the indifference to the duration of the cold ischemia time that followed the promising initial results. In a morphologic study, the safe cold ischemia time using UW solution in clinical liver transplantation was reduced to 20 hr,[33] and later, using graft function and outcome as parameters to 13 hr[34] and 12 hr.[35] Nevertheless, it is unknown if transplants are inevitably and completely lost after a cold ischemic period of more than 48 hr. One of the theoretical advantages of HLT is the assumption of the host liver to provide functional support for the patient during the period of establishment of graft function. If that assumption is true, long-term hypothermic storage will be better tolerated in HLT, compared to OLT. Therefore, the differences in susceptibility to long-term graft preservation need to be studied for OLT and HLT, separately.

A strategy to further improve preservation solutions is the use of cytoprotective drugs. Cytoprotection was originally defined as the property of certain agents (prostaglandins) to protect epithelial cells of hollow gastrointestinal viscera against various ulcerogenic agents. Now, this phenomenon is a well established, general concept.[36-38]

Soon after the cytoprotective quality of prostaglandins was described in the late seventies,[36] it became clear this effect was not limited to the gastrointestinal tract. In fact, cytoprotection could be demonstrated in the kidney,[39] pancreas[40,41] and liver.[39,42] The efficacy of hepatocytoprotection has been reproduced by several authors in a variety of models and proposed mechanisms of action.[38,39,42–46] It is an attractive approach to the management of damage by hypothermic storage of the graft in liver transplantation.

Two principle ways of testing the effects of prostaglandins are employed. One method is to assess the tolerance of the rat liver to different periods of warm ischemia/reperfusion *in vivo* or to cold ischemia/reperfusion in the isolated perfused rat liver model. On the other hand, the ability of prostaglandins to protect liver grafts from ischemia/reperfusion injury after simple cold storage can be evaluated by the results of subsequent transplantation. The latter method is the most suitable in the appraisal of prostaglandins in liver transplantation.

At present, few experiments with this model have been reported. Using prostaglandin I_2 as additive to the preservation fluids, a significant improvement of results was obtained after OLT with grafts stored for 24 hr in pigs[47] and for 24 and 48 hr in dogs.[48,49] In both canine[50] and human[51] liver transplantation, prostaglandin E_1 has been shown to improve graft function significantly and to decrease graft damage.

However, the preservation solutions applied in these experiments were those available at that time (Sacks', Collins' [C–2], and Eurocollins' solution). Successful liver transplants using simple cold storage with UW solution without prostaglandins have been reported also: for 24 hr and more in man[52] and for 48 hr in dogs.[32] It is not known if cytoprotective agents can give additional improvement of results of liver transplantation when grafts are preserved for 24–48 hr in UW solution. UW solution may provide enough and maximal cytoprotection by itself. In that case, there will be no significant extra benefit from prostaglandins.

All experiments using liver transplantation to assess organ viability were performed with orthotopic transplantations. The effect of prostaglandins on long–term preservation followed by HLT, is still to be investigated.

1.2 QUESTIONS TO BE ANSWERED

HLT and OLT are not competitive procedures. For most candidates for liver transplantation one technique will be most appropriate. Although some factors explicitly determine which procedure is preferable (for example in case of hepatic malignancy, HLT is contraindicated), other determinants will have to be evaluated for the individual patient. Many aspects of these determinants in HLT and OLT, however, are unknown.

The above mentioned considerations about HLT and OLT and about the possibility of long–term graft preservation elicited many questions. This thesis tries to answer the following:

1. Are intraoperative hemodynamic conditions during HLT better than during OLT?
2. Are hemostatic changes during HLT less pronounced than during OLT?
3. Are the hemodynamic and hemostatic effects of long–term preservation better tolerated by the recipient of an auxiliary graft?
4. Are prostaglandins effective in preventing liver injury after long–term preservation with UW solution?

1.3 OBJECTIVES OF THE STUDY

To answer the questions from the previous paragraph, the aims of the present study were outlined in three segments. These segments correspond with three experimental studies making up the main substance of this thesis.

1. To study the *hemodynamic* and *hemostatic* changes during OLT and HLT, when storage injury is kept to a minimum by short–term graft preservation.

2. To compare these findings with hemodynamic and hemostatic changes found after various periods of preservation, and to evaluate the possible benefits and drawbacks of HLT versus OLT in long–term graft preservation.

3. To study the effects of cytoprotective agents, in particular PGE_1, in the prevention of storage and reperfusion injury with long–term graft preservation and HLT or OLT.

1.4 OVERVIEW OF THE CONTENTS

The following two chapters review the evolution of the two basic issues of this thesis: heterotopic transplantation and graft preservation. In Chapter 2 the rationale for HLT is discussed and the clinical results in the eighties are presented. In Chapter 3 the history of liver preservation is summarized and prospects of long-term liver graft preservation are described.
In Chapter 4 the experimental design and general results are described. In Chapter 5 the intraoperative hemodynamic changes in OLT and HLT are described and the effects of long-term graft preservation and PGE_1 are analyzed. In Chapter 6 the same is done for the intraoperative coagulation and fibrinolysis parameters. In Chapter 7 the results obtained from the experiments are integrated into a general discussion according to four distinct intraoperative stages: the preparation period, the period of portal flow interruption, the early postreperfusion and the late postreperfusion period. References to the literature are given at the end of each chapter.

1.5 REFERENCES

1. Starzl TE, Groth CG, Brettschneider L, Penn I, Fulginiti VA, Moon JB, Blanchard H, Martin AJ,Jr., Porter KA. Orthotopic homotransplantation of the human liver. Ann Surg 1968; 168:392–415.

2. Calne RY. Liver transplantation: The recent Cambridge/King's college hospital experience. Transplant Proc 1988; 20:475–477.

3. Bismuth H. Liver transplantation: The Paul Brousse experience. Transplant Proc 1988; 20:486–489.

4. Kalayoglu M, Stratta RJ, Hoffmann RM, Sollinger HW, Belzer FO. Quadruple immunosuppressive therapy for liver transplantation. Transplant Proc 1988; 20:524–529.

5. Iwatsuki S, Starzl TE, Todo S, Gordon RD, Esquivel CO, Tzakis AG, Makowka L, Marsh JW, Koneru B, Stieber AC, Klintmalm GB, Husberg B. Experience in 1,000 liver transplants under cyclosporine–steroid therapy: a survival report. Transplant Proc 1988; 20:498–504.

6. Kuster GR, Woods JE. Auxiliary liver transplantation in the dog as temporary support in acute fulminating hepatic necrosis. Ann Surg 1972; 176:732–735.

7. Ricco JB, Diaz A, Franco D, et al. Traitement du coma hépatique experimental par transplantation de foie auxiliaire. Bordeaux Medical 1977; 10:1859.

8. Terpstra OT, Schalm SW, Weimar W, Willemse PJA, Baumgartner D, Groenland THN, ten Kate FJW, Porte RJ, de Rave S, Reuvers CB, Stibbe J, Terpstra JL. Auxiliary partial liver transplantation for end–stage chronic liver disease. N Engl J Med 1988; 319:1507–1511.

9. Terpstra OT, Schalm SW, Reuvers CB, Baumgartner D, Groenland THN, ten Kate FJW, Stibbe J, Terpstra JL, Weimar W, Willemse PJA. The role of auxiliary liver transplantation. Transplant Proc 1987; 19:4370–4372.

10. Fortner JG, Yeh SDJ, Kim DK, Shiu MH, Kinne DW. The case for and technique of heterotopic liver grafting. Transplant Proc 1979; 21:269–275.

11. Houssin D, Berthelot P, Franco D, Bismuth H. Heterotopic liver transplantation in end–stage HBsAg–positive cirrhosis. Lancet 1980; 1:990–993.

12. Starzl TE, Iwatsuki S, van Thiel DH, Gartner JC, Zitelli BJ, Malatack JJ, Schade RR, Shaw BW,Jr., Hakala TR, Rosenthal JT, Porter KA. Evolution of liver transplantation. Hepatology 1982; 2:614–636.

13. Reuvers CB, Terpstra OT, ten Kate FJW, Kooy PPM, Molenaar JC, Jeekel J. Long-term survival of auxiliary partial liver grafts in DLA-identical littermate beagles. Transplantation 1985; 39:113-118.

14. Reuvers CB, Terpstra OT, Boks AL, De Groot GH, Jeekel J, ten Kate FJW, Kooy PPM, Schalm SW. Auxiliary transplantation of part of the liver improves survival and provides metabolic support in pigs with acute liver failure. Surgery 1985; 98:914-921.

15. Terpstra OT, Reuvers CB, Schalm SW. Auxiliary heterotopic liver transplantation. Transplantation 1988; 45:1003-1007.

16. D'Alessandro AM, Kalayoglu M, Sollinger HW, Hoffmann RM, Pirsch JD, Lorentzen DF, Melzer JS, Belzer FO. Experience with Belzer UW cold storage solution in human liver transplantation. Transplant Proc 1990; 22:474-476.

17. Todo S, Nery JR, Yanaga K, Podesta L, Gordon RD, Starzl TE. Extended preservation of human liver grafts with UW solution. JAMA 1989; 261:711-714.

18. Cooper J, Rettke SR, Ludwig J, Ayalon A, Sterioff S, Wiesner RH, Krom RAF. UW solution improves duration and quality of clinical liver preservation. Transplant Proc 1990; 22:477-479.

19. Stratta RJ, Wood RP, Langnas AN, Duckworth RM, Markin RS, Marujo W, Grazi GL, Saito S, Dawidson I, Rikkers LF, Pillen TJ, Shaw BW,Jr.. The impact of extended preservation on clinical liver transplantation. Transplantation 1990; 50:438-443.

20. Bismuth H, Castaing D, Ericzon BG, Otte JB, Rolles K, Ringe B, Slooff M. Hepatic transplantation in Europe. First report of the European liver transplant registry. Lancet 1987; 2:674-676.

21. Reding R, Feyaerts A, Degoyet JD, de Hemptinne B, Otte JB. Early Graft Loss After Liver Transplantation - Etiology, Chronology, and Prognosis. Transplant Proc 1991; 23:1487-1488.

22. Howard TK, Klintmalm GB, Cofer JB, Husberg BS, Goldstein RM, Gonwa TA. The influence of preservation injury on rejection in the hepatic transplant recipient. Transplantation 1990; 49:103-107.

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

24. Farber JL, Young EE. Accelerated phospholipid degradation in anoxic rat hepatocytes. Arch Biochem Biophys 1981; 211:312-320.

25. Bulkley GB. The role of oxygen free radicals in human disease processes. Surgery 1983; 94:407–411.

26. Guarnieri C, Flamigni F, Caldarera CM. Role of oxygen in the cellular damage induced by re–oxygenation of hypoxic heart. J Mol Cell Cardiol 1980; 12:797–808.

27. Freeman BA, Crapo JD. Biology of disease. Free radicals and tissue injury. Lab Invest 1982; 47:412–426.

28. Tamaki T, Okouchi Y, Kozaki M, Kawamura A, Uchino J, Pegg DE. Hypothermic preservation of the rat liver assessed by orthotopic transplantation. III Improved functional recovery with isotonic citrate solution and a stable prostacyclin analogue. Transplantation 1988; 46:626–628.

29. Belzer FO, Southard JH. Principles of solid–organ preservation by cold storage. Transplantation 1988; 45:673–676.

30. Collins GM, Bravo–Shugarman M, Terasaki PI. Kidney preservation for transportation. Initial perfusion and 30 hours' ice storage. Lancet 1969; 2:1219–1222.

31. Kalayoglu M, Sollinger HW, Stratta RJ, D'Alessandro AM, Hoffmann RM, Pirsch JD, Belzer FO. Extended preservation of the liver for clinical transplantation. Lancet 1988; 1:617–619.

32. Jamieson NV, Sundberg R, Lindell S, Claesson K, Moen J, Vreugdenhil PK, Wight DGD, Southard JH, Belzer FO. Preservation of the canine liver for 24–48 hours using simple cold storage with UW solution. Transplantation 1988; 46:517–522.

33. Furukawa H, Todo S, Imventarza O, Wu YM, Scotti C, Day R, Starzl TE. Cold ischemia time vs outcome of human liver transplantation using UW–solution. Transplant Proc 1991; 23:1550–1551.

34. Sanchez–Urdazpal L, Gores G, Ward E, Maus T, Wahlstrom H, Wiesner RH, Krom RAF. Non–anastomotic biliary strictures after orthotopic liver transplantation. 5th Congres European Society for Organ Transplantation 1991; 73 (Abstract).

35. Adam R, Morino M, Diamond T, Astarcioglu I, Johann M, Azoulay D, Bao YM, Bismuth H. Influence of prolonged cold ischaemia using UW solution on graft function and outcome following liver transplantation. 5th Congres European Society for Organ Transplantation 1991; 140 (Abstract).

36. Robert A, Nezamis JE, Lancaster C, Hanchar J. Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury. Gastroenterology 1979; 77:433–443.

37. Robert A. Current history of cytoprotection. Prostaglandins 1981; 21:89–96.

38. Miyazaki M, Makowka L, Falk RE, Falk JA, McDonell M, Venturi D. Protection of thermochemotherapeutic-induced lethal acute hepatic necrosis in the rat by 16,16-dimethyl prostaglandin E_2. J Surg Res 1983; 34:415-426.

39. Ruwart MJ, Rush BD, Friedle NM, Piper RC, Kolaja GJ. Protective effects of 16,16-dimethyl PGE_2 on the liver and kidney. Prostaglandins 1981; 21:97-102.

40. Manabe T, Steer ML. Protective effects of PGE_2 on diet-induced acute pancreatitis in mice. Gastroenterology 1980; 78:777-781.

41. Reber HA, Tweedie JH, Mosley JG. The cytoprotective effect of 16,16 dimethyl prostaglandin E_2 (PG) on bile salt induced damage to the pancreas. Gastroenterology 1980; 78:1241.

42. Tarnawski A, Stachura J, Mach T, et al. Cytoprotective effect of 16,16-dimethyl prostaglandin E_2 ($dmPGE_2$) and some drugs on acute galactosamine induced liver damage in rat. Acta Hepatogastroenterol 1980; 1:236.

43. Araki H, Lefer AM. Cytoprotective actions of prostacyclin during hypoxia in the isolated perfused cat liver. Am J Physiol 1980; 238:H176-H181.

44. Ruwart MJ, Friedle NM, Rush BD. 16,16-dimethyl PGE_2 protects in vitro hepatocytes from carbon tetrachloride-induced damage. Gastroenterology 1982; 82:1166.

45. Ruwart MJ, Kolja GJ, Friedle NM, Rush BD, Tarnawski A, Stachura J, Mach T, Ivey KJ. Protection against CCl_4-induced liver damage by 16,16-dimethyl PGE_2. Gastroenterology 1981; 80:1266.

46. Stachura J, Tarnawski A, Ivey KJ, Ruwart M, Rush BD, Friedle NM, Szczudrawa J, Mach T. 16,16 dimethyl prostaglandin E_2 protection of the rat liver against acute injury by galactosamine, acetaminophen, ethanol and anit. Gastroenterology 1981; 80:1349.

47. Mora NP, Cienfuegos JA, Bernaldó de Quiros L, Tendillo FJ, Pereira F, Fores R, Alvarez I, Navidad R, Castillo Olivares JL. Successful liver allograft function after 24-hour preservation: cumulative effects of prostacyclin plus verapamil. Transplant Proc 1987; 19:3932-3936.

48. Toledo-Pereyra LH. Role of prostaglandins (PGI_2) in improving the survival of ischemically damaged liver allografts. Trans Am Soc Artif Intern Organs 1984; 30:390-394.

49. Monden M, Fortner JG. Twenty-four- and 48-hour canine liver preservation by simple hypothermia with prostacyclin. Ann Surg 1982; 196:38-42.

50. Ueda Y, Matsuo K, Kamei T, Kayashima K, Konomi K. Protective effect of prostaglandin E_1 (PGE_1) on energy metabolism and reticuloendothelial function in the ischemically damaged canine liver. Liver 1989; 9:6–13.

51. Gaber AO, Thistlethwaite JR,Jr., Busse-Henry S, Aboushloe M, Emond JC, Rouch D, Broelsch CE. Improved results of preservation of hepatic grafts preflushed with albumin and prostaglandins. Transplant Proc 1988; 20:992–993.

52. Todo S, Tzakis A, Starzl TE. Preservation of livers with UW or Eurocollins' solution. Transplantation 1988; 46:925–926.

Chapter 2

NEW ASPECTS OF HETEROTOPIC LIVER TRANSPLANTATION

This chapter has been accepted for publication in
TRANSPLANT INTERNATIONAL.

2.1 INTRODUCTION

Orthotopic liver transplantation (OLT) is a therapeutic option for patients with end-stage acute or chronic liver disease. In patients with advanced liver disease, however, the combination of portal hypertension, abundant venous collaterals, and severe clotting disturbances makes dissection and removal of the cirrhotic liver a demanding procedure. In the anhepatic phase, the hemodynamic condition of the patient is further compromised by decreased venous return unless a venovenous bypass is used.[1]
Heterotopic auxiliary liver transplantation (HLT) evades the surgical trauma of removal of the recipient liver and the need for a venovenous bypass system.[2] Furthermore, the host liver could provide synthetic and clearing liver function during the transplantation and in case of graft rejection or failure. Removal of the native liver also negates its potential recovery in patients with (sub)acute liver failure. Finally, when OLT is performed in patients with an inborn error of metabolism, it is hard to overcome a feeling of waste when an organ is disposed of that looks normal and functions virtually normal, except for one single enzyme system.
Consequently, for some patients HLT offers advantages over OLT. In this chapter the history and clinical results of HLT are reviewed and some special aspects of current research on HLT are highlighted.

2.2 HISTORY

The first laboratory experiments on liver transplantation were performed with auxiliary heterotopic grafts, carried out in 1955.[3,4] With regard to liver transplantation terminology, it is necessary to recognize a considerable confusion of tongues. In earlier days, all non-orthotopic transplantations were usually referred to as auxiliary. Since orthotopically positioned auxiliary grafts have been described,[5] the confusion is complete. To date, an international consensus exists on the nomenclature: as opposed to OLT, heterotopic (usually reduced-size) liver transplantation is recognized. Although the term auxiliary transplantation will still be used in describing the older transplantations, this thesis deals with reduced-size, auxiliary heterotopic liver transplantation (HLT).
The first auxiliary liver transplantation in man was performed in 1964.[6] From that moment until 1980, 47 patients had undergone heterotopic liver grafting but only 2 patients survived more than one year.[7] While OLT evolved to be the procedure of

choice, the potential advantages of leaving the diseased liver in place continued to inspire researchers to study various experimental auxiliary models.[8–12]

In the Laboratory for Experimental Surgery in Rotterdam, the problems associated with the auxiliary procedure were reviewed. With the definition of theoretical requirements for successful auxiliary heterotopic transplantation, a new concept of auxiliary partial liver transplantation was developed: a reduced–size liver, with both arterial and portal inflow and venous drainage through the suprahepatic vena cava of the graft into the recipient's infrahepatic vena cava, as close as possible to the diaphragm.[7,13–17]

The results of these experimental studies led to the initiation of a clinical program in October 1986. In 1988, the favorable outcome in the first six patients of this program was reported.[18] All patients had end–stage liver disease and were considered by another transplant center to be at high–risk for not surviving an OLT. After auxiliary partial liver transplantation, they were alive and well, with good graft function, after a mean follow–up period of 14 months.

By now it has become evident that either method, HLT and OLT, can give good results. In an open comparative study, HLT was demonstrated to give long–term metabolic support and adequate decompression of the portal system. Also, HLT was associated with a morbidity and mortality comparable to OLT in medium–risk patients with end–stage chronic liver disease.[19]

In the present survey, all heterotopic liver transplants that were performed from January 1980 through December 1990 are included. Data was collected from the European Liver Transplant Registry (ELTR), recent publications[18,20–26] and personal communications.

In the decade under study, 50 HLTs in 48 patients were performed in 11 centers (Figure 2.1). There were 27 men and 21 women with a median age (range) of 40.5 (20–69) and 47 (1–60) respectively. Three patients were 15 years or younger. Twenty–one patients underwent emergency transplantation. Details on indications are given in Table 2.1. In seven patients HLT was performed for (sub)acute liver disease (Table 2.2). The outcome of these transplantations is described below.

The main cause of death was sepsis, responsible for 12 of 32 deaths (Table 2.3). This is in accordance with the OLT experience. Four deaths were attributed to vascular complications. In contrast with OLT, where vascular complications are mainly arterial problems, in HLT the patency of the portal vein is most crucial. Two new cases of hepatocellular carcinoma in the recipient liver after HLT were found. The low incidence of rejection as a cause of graft failure is remarkable.

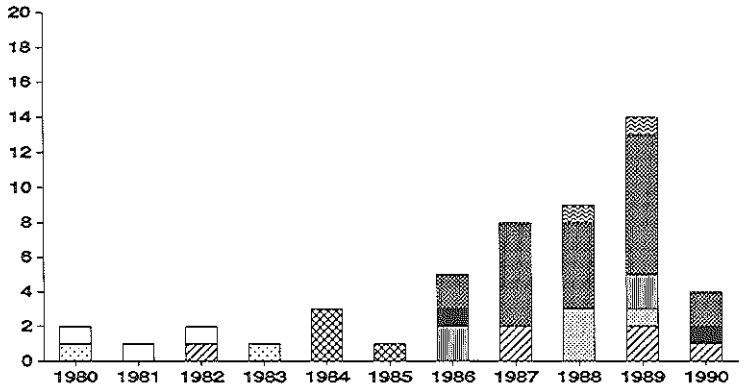


Figure 2.1. Annual number of heterotopic liver transplantations from 1980–1990, by transplantation center. ▨ *Brussels (Belgium);* ▩ *Tübingen (Germany);* □ *Nice (France);* ▤ *Paris (France);* ▥ *Toulouse (France);* ■ *Grenoble (France);* ▧ *Rotterdam (The Netherlands);* ▩ *Philadelphia (USA);* □ *Others: Innsbruck (Austria), Hannover (Germany), and Capetown (South-Africa).*

Survival was assessed by the life-table analysis according to Kaplan and Meier[27] and survival times were compared with the log-rank test. Only primary HLTs were included in the life-table analysis. The cumulative survival was compared for emergency versus elective operations (Figure 2.2) and year of transplantation (before versus after January 1987) (Figure 2.3).

When comparing the survival rates of HLT in the present study with the results of OLT, it should be noted that the majority of these HLTs were only occasionally performed at various centers, and for exceptional indications, or they were attempted in high-risk patients. Furthermore, as in HLT, results of OLT before and after 1986 are significantly different. In the first report of the ELTR, one-year survival – calculated in the cumulative series from 1968 through 1986– was 44% for emergency and 46% for elective transplantation.[28] To date, various centers have reported one-year survival ranging from around 70% to around 90% for elective transplantations.[29-32]

Table 2.1. Indications for heterotopic liver transplantation 1980–1990.

Chronic liver disease:	N	Acute liver disease: (developed within:)	N
Cirrhosis: – Post hepatitic	11	Fulminant hepatic failure	2
– Primary biliary	8	(0–2 weeks)	
– Alcoholic	4		
– Autoimmune	2	Acute hepatic failure	4
– Metabolic	1	(2–8 weeks)	
– Unknown	6		
Primary sclerosing cholangitis	2	Subacute hepatic failure	1
Biliary atresia	1	(8–26 weeks)	
Retransplantation	1		
Tumor: – Hepatocellular carcinoma	3		
– Secondary liver tumor	1		
– Benign liver tumor	1		
TOTAL	41	TOTAL	7

Table 2.2. Heterotopic liver transplantation for (sub) acute liver disease.

Center[a], year	Sex	Age	Etiology	Outcome
Paris, 1980	♀	17	Valproate	Sepsis, died 24 d.
Grenoble, 1986	♀	24	Unknown (viral ?)	Alive 55 mo.
Rotterdam, 1986	♂	31	Unknown	PNF[b], died 18 d.
Rotterdam, 1987	♀	18	Unknown	PNF, reHLT[c], died 15 d.
Rotterdam, 1989	♀	35	Autoimmune ?	Alive 31 mo.
Philadelphia, 1988	♀	19	Unknown (viral ?)	Alive 33 mo, no medication.
Philadelphia, 1989	♀	15	Wilson's disease	Rejection, OLT[d] 27 d.

[a] Centers: Faculty of Medicine Paris–Sud, Hôpital Paul Brousse, Villejuif, France; Centre Hospitalier Regional et Universitaire de Grenoble, France; University Hospital Dijkzigt, Erasmus University, Rotterdam, The Netherlands; Jefferson Medical College, Philadelphia, Pennsylvania, USA.
[b] Primary graft nonfunction.
[c] Retransplantation with heterotopic liver graft.
[d] Orthotopic liver transplantation.

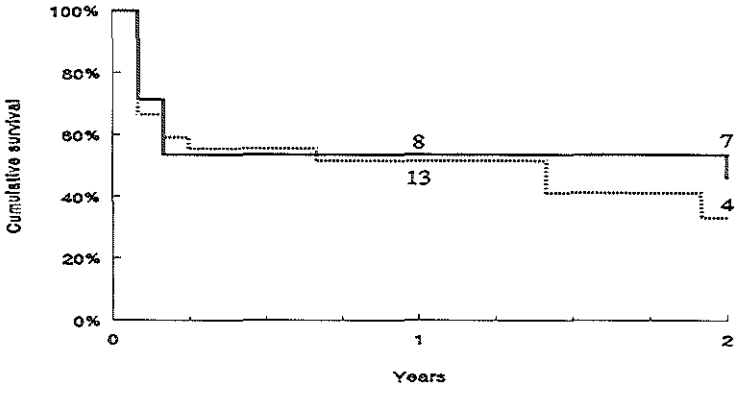


Figure 2.2. Cumulative survival of patients after primary heterotopic liver transplantation from 1980–1990, according to the circumstances of operation: (———) Emergency (N=21); (– – – –) Elective surgery (N=27).

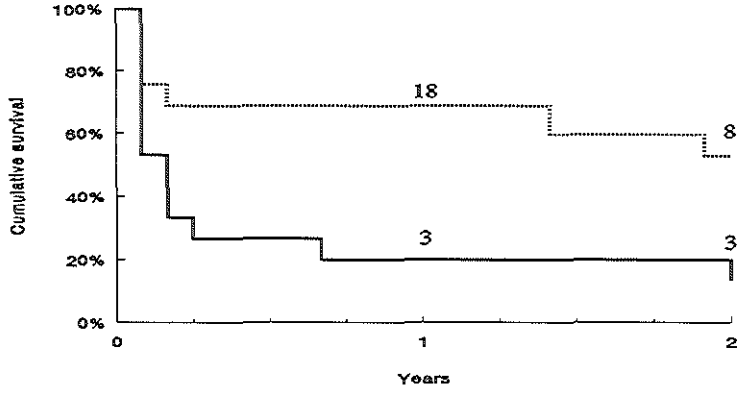


Figure 2.3. Cumulative survival of patients after primary heterotopic liver transplantation, compared by time of operation: (———) 1980–1986 (N=15); (– – – –) 1987–1990 (N=33) (P<0.005).

Table 2.3. Causes of deaths of heterotopic liver transplantation 1980–1990.

Cause of death	N
Bleeding in surgical field	4
Primary graft nonfunction	5
Vascular complication	4
Infection	12
Multiple organ failure	2
Rejection	2
Tumor (hepatocellular carcinoma in host liver)	2
Other	1
TOTAL	32

After January 1987, 14 emergency HLTs were performed with a one-year survival of 71%. In Rotterdam, 16 primary HLTs were performed for cirrhosis and sclerosing cholangitis with an one-year patient survival of 75%. Further improvement of results of HLT is to be expected when stringent indications are used and when others than the extreme high-risk patients will become candidates for heterotopic liver grafting.

2.3 New aspects

Among other countries, the concept of heterotopic liver transplantation continues to be the subject of various clinical and experimental studies in Argentina,[33] France,[22,34] Germany,[35] Japan,[36] Yugoslavia,[37] and the USA.[5,38] In the following section, some fascinating aspects of heterotopic liver transplantation will be described. These include: the absence of intraoperative fibrinolysis, the stability of hemodynamic parameters during the procedure of HLT, the effect on the portal pressure and hypersplenism, the interaction between the two livers *in situ*, the role of portal blood flow in HLT, the temporary support given by the heterotopic graft in acute liver failure, and the possible role of HLT in inborn errors of hepatic metabolism. Finally, two important modifications of HLT will be discussed.

2.3.1 Intraoperative fibrinolysis

The earliest reports on OLT already described increased fibrinolytic activity.[39] By comparing fibrinolytic activities, as measured by euglobulin clot lysis time and the formation of fibrin degradation products during both OLT and HLT in the pig, we demonstrated a more pronounced fibrinolytic activity during OLT.[40]
The origin of this hyperfibrinolysis is still controversial but there is strong evidence that tissue-type plasminogen activator (t-PA) is the key issue. Normally, t-PA is produced by endothelial cells and removed from the circulation by the liver. In OLT, t-PA can accumulate in the anhepatic phase, while additional release is also likely. t-PA levels have been demonstrated to increase in the anhepatic phase or after reperfusion.[41–43] Other investigators believed t-PA-release from the graft not to be a major determinant of hemostatic disorders in liver transplantation.[44,45]
In this thesis, it is reported on experimental porcine OLT and HLT in which t-PA levels are measured (Chapter 6).

2.3.2 Intraoperative hemodynamics

Cross-clamping the portal vein and the abdominal portion of the inferior vena cava causes major loss of venous return and congestion of the obstructed portal and systemic venous beds. These problems can be prevented by the use of a venovenous bypass system.[1] In clinical HLT for liver cirrhosis, portacaval collaterals can shunt the mesenteric blood flow. In addition the caval and portal anastomoses are performed with partially clamped recipient vessels. Indeed, our clinical experience with HLT is that the cardiac output hardly responds to partial clamping of the portal vein. It was demonstrated that in HLT a venovenous shunt with its concomitant hazards is expendable.[2]
It is likely that deleterious substances accumulate in the stagnant blood of the congested venous beds. When suddenly returned into the systemic circulation at revascularization, these factors may cause depression of cardiovascular function, in spite of the restoration of venous return. Many substances have been held responsible for this effect, including potassium, hydrogen ions, ionized calcium, and unidentified vasoactive hormones.[46–49] As long as the exact origin of this myocardial depression is unknown these substances can be designated myocardial depressant factors (MDF).

To study the role of the host liver in clearing MDF at reperfusion of a heterotopic graft, we compared intraoperative hemodynamics in the pig during HLT and OLT. These experiments will be described in Chapter 5.

2.3.3 Correction of portal hypertension

An auxiliary, heterotopic liver graft may be considered a functional side–to–side portacaval shunt. In this respect, HLT could alleviate portal hypertension. In 11 successful HLTs in chronic liver disease, the intraoperative pressure gradients between the portal vein and inferior vena cava decreased from a median value (mean, 95%–confidence limits) of 18 mm Hg (17.0, 13.8–20.2) to 6 mm Hg (6.4, 3.9–8.9) (Figure 2.4). In all 4 patients without a decrease in this portacaval pressure gradient, graft failure occurred, while only 2 of the remaining 13 patients developed portal vein thrombosis ($P<0.01$, Fisher exact test).
Hypersplenism is not only attributed to splenic congestion but also to gut–derived humoral factors causing splenic stimulation.[50,51] This theory explains why OLT can reverse hypersplenism,[52] while this effect is controversial for portasystemic shunt procedures.[53,54] In HLT, most collaterals are left intact and therefore, theoretically, hypersplenism might persist after HLT, corresponding the effect of a portasystemic shunt. In contradiction to this speculation, heterotopic, auxiliary partial liver transplantation was demonstrated to reverse hypersplenism.[55] A hypothesis that supports the reversal of hypersplenism by both OLT and HLT but not by a portasystemic shunt suggests the above mentioned splenotropic factors to be cleared from the blood, after successful liver transplantation.

2.3.4 Interactions between two livers

Theoretically, the presence of two livers may give rise to a "functional competition" as initially described between two liver lobes of which one is handicapped by bile duct ligation.[56] Hepatotropic factors could be responsible for portal blood flow being essential for the survival of an auxiliary graft in the presence of a healthy host liver.[17] With portal hypertension, the portal blood will be directed through the graft because of its lower vascular resistance compared the cirrhotic liver. Therefore, atrophy of the graft by means of "functional competition" is unlikely to occur.

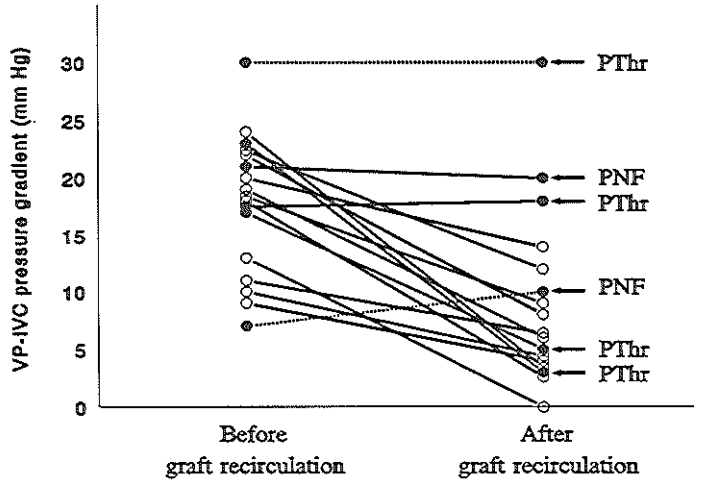


Figure 2.4. Portal–caval pressure gradient (mm Hg) before and after recirculation of the graft in human heterotopic liver transplantation in Rotterdam (including one reHLT). (●●) Graft failure (N=6); (oo) No graft failure (N=11); (———) Chronic liver disease (N=15); (– – – – –) (Sub)acute liver disease (N=2); (PThr) Portal vein thrombosis; (PNF) Primary graft nonfunction.

Indeed, in patients receiving an auxiliary, heterotopic partial liver transplantation, compensatory hyperplasia of the graft and atrophy of the native liver was observed.[57] Regeneration after partial liver resection is thought to be directed towards restoring the original liver cell mass. However, despite the apparently increased total liver cell mass after auxiliary transplantation, regeneration of the graft was demonstrated. Graft regeneration was therefore considered to be controlled by the amount of total *functional* liver cell mass. The graft, which had been reduced in size during the transplantation to approximately 80%, regained its original volume within 3 weeks after surgery. This is not different from the course after resection of the same liver volume for tumors. In contrast, the native liver decreased to ± 30% of its immediate postoperative size within 3–6 months.

The presence of an additional, allogenic reticulo–endothelial organ also implies immunological interactions between the two livers. Icard et al.[34] reported on an interesting study on class II major histocompatibility complex antigens on rat hepatocytes after transplantation. They suggested the rejection response to be more severe and the pattern of class II expression different in HLT compared to OLT. In case of graft rejection after OLT the inevitable liver failure would cause immunosuppression because of decreased lymphokine production, essential to hepatocytes class II induction. Additionally, hepatic phagocyte function –also related to graft rejection– was suggested to be decreased with rejection in progress after orthotopic grafting, but well maintained by the healthy host liver of the rat after auxiliary transplantation.
In contrast, in clinical HLT rejection problems were not encountered to a larger extent than in OLT.[19] This inconsistency with the experiments of Icard et al. could be explained by the already decreased function of the reticulo–endothelial system in cirrhotic livers. Although the number of patients is small, we have the impression heterotopic grafts are even less vulnerable to the immune attack than in the orthotopic position (Table 2.3). In OLT, rejection occurs in 40–60%,[58,59] while at present only 4 of 22 HLTs in Rotterdam were rejected. This is also in agreement with the observation in a rat model that an auxiliary liver graft yielded immunosuppression.[60]

2.3.5 The role of portal blood flow in HLT

When auxiliary transplantation is performed in the presence of normal hemodynamic conditions of the recipient liver, the distribution of the portal flow is a major concern.[5] In an animal study on correcting inborn errors of metabolism, best results were obtained with constriction or ligation of the recipient's own portal vein.[61] Clinical results of HLT in patients without portal hypertension were also affected by the interruption of the portal blood flow to the recipient liver. With constriction or ligation of the host portal vein good results were obtained, while otherwise primary graft nonfunction (PNF) or graft failure developed.
Constriction is theoretically attractive because the native liver still receives some portal blood. The preservation of portal flow to the host liver, however, increases the risk of thrombosis of the graft portal vein. Elevation of the vascular resistance of the graft by preservation injury or rejection will cause preferential flow to the native liver. Additionally, the innervated recipient liver is capable of regulating blood–flow, while the denervated graft is dependent on passive flow distribution. On the other hand,

complete ligation of the host portal vein assures graft portal flow, but may interfere with the potential recovery of the recipient liver.

2.3.6 Temporary support in (sub)acute liver failure

In acute hepatic failure caused by drug intoxication, hepatitis, or allergic drug reactions the liver might be expected to regenerate provided the patient survives the critical phase. In those cases, there is a need for a reliable means of temporary support. An auxiliary graft implanted during that phase could provide uninterrupted support until the host's own liver recovers of at least minimally effective function. Later the graft may be removed or left to atrophy. After recovery of the host liver, there is no need for life-long immunosuppression with its concomitant sequelae. Successful canine[11] and porcine[16,62] HLT for fulminant hepatic failure has been described.

Clinical experience with HLT for acute hepatic failure is scarce (Table 2.2). The first HLT for acute liver failure was performed by Bismuth in 1980.[23,63] A 17-year old female developed acute hepatic failure related to valproate sodium. A reduced-size liver graft was placed in the right hypochondrium. The portal vein, hepatic artery and infrahepatic vena cava were anastomosed end-to-side to the recipient vessels as initially described by Fortner.[64] The portal vein to the host liver was not interrupted. After ten days, septicemia, renal insufficiency, and possibly rejection occurred and she died on the 22nd postoperative day. At necropsy, the graft was hypertrophic and the recipient liver had further atrophied. Histologically, marked centrilobular parenchymal cell necrosis was noticed in the graft.

In Grenoble in 1986, a HLT was performed for a 24-year old female with acute hepatic failure, resembling non-A, non-B hepatitis.[24] The same technique was used as in the former patient, although no resection was performed. A reintervention for hemostasis was necessary after 24 hr and a rejection crisis on day 10 was suppressed with methylprednisolone. Because of intractable ascites the hepatic artery of the native liver was embolized on day 32, which successfully alleviated ascites in 5 days. After another rejection crisis and a revision of the biliary anastomosis, the patient was alive and well after 55 months. An angiography had confirmed occlusion of the native hepatic artery and portal vein: the patient's own liver became cirrhotic.

In the Rotterdam program, 3 patients have been heterotopically transplanted for (sub)acute liver failure. The technique differed from the previous two HLTs in that the suprahepatic inferior vena cava was used for the caval anastomosis.[18] A 18-year old man developed acute hepatic failure of unknown origin. A reduced-size HLT was

performed without interruption of the host portal vein. PNF occurred and when the necrotic graft was removed, the hepatic artery appeared to be occluded while the graft portal vein was patent. This patient died on day 18. The second patient, a 31-year old woman, also presented with acute liver failure of unknown origin. Due to lack of space a right hemihepatectomy of the graft was performed. She died on day 15 from PNF, despite re-HLT with ligation of the host portal vein.

The third patient was the most striking case of the Rotterdam experience. This case definitely proved the point that HLT is capable of giving temporary support until the host liver recovers. A 35-year old female was transplanted for subacute autoimmune hepatitis.[25] On day one, portal vein thrombosis necessitated thrombectomy of the graft portal vein and ligation of the portal vein to the native liver. On day 25, a second revision of the portal vein anastomosis was required. On day 45 scintigraphy showed good uptake and excretion of the radioisotope almost exclusively in the graft. Unexpectedly, at 6 months, the scintigraphic picture had completely reversed: the graft had diminished in size and function and uptake and excretion of the radioisotope was mainly found in the patient's own liver. Angiography showed preferential flow of portal blood to the recipient liver through venous collaterals. Immunosuppression was reduced and further atrophy is awaited.

Two patients with fulminant hepatic failure were heterotopically transplanted in Philadelphia. The first, a 19-year old female was treated for liver failure of possible viral origin.[20] As suprahepatic exposure increased the intracranial pressure, a HLT was performed. Because of minimal flow to the graft the portal vein to the native liver needed to be constricted about 80%. At 6 months, the graft was histologically normal and the native liver showed signs of severe resolving hepatitis. At about 2 years, the native liver had regained normal size and histological appearance. The heterograft had shrunken significantly and biopsy showed no hepatocytes. Immunosuppression was stopped. The second patient, a 15-year old girl presented with fulminant Wilson's disease. A HLT with an ABO-incompatible graft was performed because she could only be operated in a half-seated position due to severe intracranial hypertension. Again, the host portal vein was constricted about 80%. She recovered neurologically from coma to full alertness within 10 days. Severe rejection necessitated retransplantation on the 27th postoperative day and a OLT was performed.

Taken together, of seven HLTs for acute liver failure, three patients died and one patient survived on graft function (after embolization of the native hepatic artery). The remaining three patients received temporary support from the heterotopic graft, until the native liver recovered in two patients and until an OLT was possible in one.

One of the most difficult problems in the management of patients with acute liver failure is the assessment of the need to and the timing of liver grafting. OLT in an early phase of the disease negates the possibility of spontaneous recovery; delay of the decision to transplant may lead to further deterioration of the patient's clinical condition. As the procedure of HLT is reversible, the decision to transplant can be made quicker. In addition, HLT may have a salutary effect beyond that of simply providing life sustaining hepatic support. The resection of the grafted liver may enhance native liver regeneration.

2.3.7 Metabolic diseases of the liver

Alpha–1–antitrypsin deficiency, glycogen storage disease, tyrosinemia, Wilson's disease and many other inborn errors of metabolism are gratifying indications for liver transplantation. Most of the characteristic metabolic perturbations of these disorders are corrected after liver transplantation. Since liver cirrhosis develops in the course of many of these diseases, adult liver transplantation is a frequent consequence.
The timing of OLT for metabolic disturbances in children is a dilemma. On one hand the recipient in question might not have deteriorated sufficiently to demand transplant at the time one of the scarce, paediatric donors becomes available. On the other hand, postponement of transplantation will almost inevitably lead to a further decline of the general condition of the recipient. Much of this reluctance can be overcome by leaving the recipient liver *in situ*. In this respect, the most attractive treatment for metabolic disease of the liver is hepatocyte transplantation,[65] but as long as this treatment modality is not clinically successful, auxiliary transplantation appears to be the procedure of choice.
There is no clinical experience with HLT in children with inborn errors of hepatic metabolism. Data of experimental research suggest portal inflow to the graft to be essential and when this is achieved long–term substitution of the lacking enzyme occurs.[61]

2.3.8 Modifications of HLT

Fourtanier et al.[22] reported a new technique of heterotopic liver transplantation in a patient with portal vein thrombosis. OLT and the standard subhepatic HLT were therefore technically impossible. The graft was positioned in the left subphrenic space after splenectomy, with a cavorenal anastomosis, splenoportal venous anastomosis and

splenohepatic arterial anastomosis. The presence of a large splenic vein, splenomegaly and distended abdominal cavity in the recipient, made this type of heterotopic transplantation particularly suited for this patient. This case report showed modified heterotopic transplantation to be an alternative in patients who are otherwise unsuited for liver transplantation.

Another modification of HLT is the auxiliary transplantation of liver segments in the orthotopic position after resection of the left liver lobe of the recipient, as originally described by Bismuth and Houssin in 1985.[23] In this way the preferable localization under the diaphragm is combined with leaving the recipient liver (partially) *in situ*. This may provide temporary support in case of acute liver failure, allowing the recipient liver to regenerate.[5] One patient treated with orthotopic auxiliary liver transplantation is reported by the Hannover group. Her own liver recovered and she was taken off immunosuppressive therapy.[66]

2.4 CONCLUSIONS

For the majority of patients with chronic liver disease and for patients with malignant liver disease OLT is the method of choice. For patients who have very advanced disease with severely disturbed hemostasis, for patients with pre-existing cardiovascular or pulmonary impediment, and for patients with acute hepatic failure and critical intracranial hypertension, HLT might be a better solution. The remaining synthetic and clearing function of the recipient liver during the transplantation provides greater hemostatic and hemodynamic stability.

It is argued that oncogenic tissue (and maybe an occult carcinoma) is left *in situ* when an auxiliary procedure is performed. This is especially true for patients with hepatitis B and they should therefore not be considered candidates for HLT. Whether the risk of carcinoma in the recipient liver is a contraindication for transplantation in other patients with cirrhotic livers is a matter of discussion.

The most exciting application of HLT is in patients with (sub)acute hepatic failure. Because HLT is a reversible procedure it can provide temporary support, awaiting recovery of the host liver. However, difficulties concerning portal blood flow distribution should be addressed. This also accounts for HLT in treating patients with metabolic liver disease. Nevertheless, as long as hepatocyte transplantation is not clinically available, HLT should be considered a potential treatment modality for these indications.

2.5 REFERENCES

1. Shaw BW,Jr., Martin DJ, Marquez JM, Kang YG, Bugbee AC,Jr., Iwatsuki S, Griffith BP, Hardesty RL, Bahnson HT, Starzl TE. Venous bypass in clinical liver transplantation. Ann Surg 1984; 200:524–534.

2. Groenland THN, Visser L, Terpstra OT, Terpstra JL, Reuvers CB, Baumgartner D, Schalm SW. Stable hemodynamics during heterotopic auxiliary partial liver transplantation for end–stage liver cirrhosis. Transplant Proc 1988; 20:538–540.

3. Goodrich EO,Jr., Welch HF, Nelson JA, Beecher TS, Welch CS. Homotransplantation of the canine liver. Surgery 1956; 39:244–251.

4. Welch CS. A note on the transplantation of the whole liver in dogs. Transpl Bull 1955; 2:54–56.

5. Then PK, Feldman L, Broelsch CE. Flow and vascular resistance measurements in auxiliary liver segments transplanted in orthotopic position. Transplant Proc 1989; 21:2378–2380.

6. Absolon KB, Hagihari PF, Griffen WO, Lillehei RC. Experimental and clinical heterotopic liver homotransplantation. Rev Intern Hepatol 1965; 15:1481–1487.

7. Terpstra OT, Schalm SW, Reuvers CB, Baumgartner D, Groenland THN, ten Kate FJW, Stibbe J, Terpstra JL, Weimar W, Willemse PJA. The role of auxiliary liver transplantation. Transplant Proc 1987; 19:4370–4372.

8. Malt RA, Seigne TD, Corry RJ, Chávez–Peón F, Schauble JF, Miyakuni T. Auxiliary partial liver transplantation in Macaca Mulatta. Ann Surg 1970; 171:575–582.

9. Sheil AG, Rogers JH, Halliday JP, Storey BG, Kelly GE, Mason R. Auxiliary canine liver transplantation from cadaver donors. Arch Surg 1970; 100:290–294.

10. Slapak M, Beaudoin JG, Lee HM, Hume DM. Auxiliary homotransplantation. A new technique and an evaluation of current techniques. Arch Surg 1970; 100:31–41.

11. Kuster GR, Woods JE. Auxiliary liver transplantation in the dog as temporary support in acute fulminating hepatic necrosis. Ann Surg 1972; 176:732–735.

12. Lavarello RJ, Kinne DW, Kin DK, Huvos AG, Fortner JG. Life–sustaining canine hepatic autotransplants. Arch Surg 1973; 107:878–882.

13. Reuvers CB, Terpstra OT, ten Kate FJW, Kooy PPM, Molenaar JC, Jeekel J. Long-term survival of auxiliary partial liver grafts in DLA-identical littermate beagles. Transplantation 1985; 39:113–118.

14. Reuvers CB, Terpstra OT, Boks AL, De Groot GH, Jeekel J, ten Kate FJW, Kooy PPM, Schalm SW. Auxiliary transplantation of part of the liver improves survival and provides metabolic support in pigs with acute liver failure. Surgery 1985; 98:914–921.

15. Reuvers CB, Terpstra OT, ten Kate FJW, Kooy PPM, Provoost AP, Molenaar JC, Jeekel J. Rejection and survival of auxiliary partial liver grafts in non-tissue-typed pigs. Eur Surg Res 1986; 18:86–95.

16. Reuvers CB, Terpstra OT, Groenland THN, Boks AL, Faithfull NS, ten Kate FJW. Hemodynamics and coagulation in experimental auxiliary liver transplantation during fulminant hepatic failure. Ann Surg 1986; 204:552–558.

17. Terpstra OT, Reuvers CB, Schalm SW. Auxiliary heterotopic liver transplantation. Transplantation 1988; 45:1003–1007.

18. Terpstra OT, Schalm SW, Weimar W, Willemse PJA, Baumgartner D, Groenland THN, ten Kate FJW, Porte RJ, de Rave S, Reuvers CB, Stibbe J, Terpstra JL. Auxiliary partial liver transplantation for end-stage chronic liver disease. N Engl J Med 1988; 319:1507–1511.

19. Metselaar HJ, Hesselink EJ, de Rave S, Groenland THN, Bakker CM, Weimar W, Schalm SW, Terpstra OT. A comparison between heterotopic and orthotopic liver transplantation in patients with end-stage chronic liver disease. Transplant Proc 1991; 23:1531–1532.

20. Moritz MJ, Jarrell BE, Armenti V, Radomski J, Carabasi RA, Zeitoun G, Columbus K, Rubin R, Muñoz SJ, Maddrey W. Heterotopic liver transplantation for fulminant hepatic failure—a bridge to recovery. Transplantation 1990; 50:524–526.

21. Houssin D, Berthelot P, Franco D, Bismuth H. Heterotopic liver transplantation in end-stage HBsAg-positive cirrhosis. Lancet 1980; 1:990–993.

22. Fourtanier G, Lloveras JJ, Roos S, Pradere B, Ohayon E, Rumeau JL, Durand D, Escat J. Heterotopic liver transplantation in a case of cirrhosis with portal vein thrombosis. Transplant Proc 1990; 22:1572–1573.

23. Bismuth H, Houssin D. Partial resection of liver grafts for orthotopic or heterotopic liver transplantation. Transplant Proc 1985; 17:279–283.

24. Létoublon C, Guignier M, Barnoud D, Magne J-L, Martin-Barbaz F, Zarski J-P, Faure H, Carpentier F, Guidicelli H. Transplantation hépatique hétérotopique pour hépatite fulminante. Chirurgie 1989; 115:30–35.

25. Metselaar HJ, Hesselink EJ, de Rave S, ten Kate FJW, Laméris JS, Groenland THN, Reuvers CB, Weimar W, Terpstra OT, Schalm SW. Recovery of failing liver after auxiliary heterotopic transplantation. Lancet 1990; 1:1156–1157.

26. Stampfl DA, Muñoz SJ, Moritz MJ, Rubin R, Armenti VT, Jarrell BE, Maddrey WC. Heterotopic liver transplantation for fulminant Wilson's disease. Gastroenterology 1990; 99:1834–1836.

27. Kaplan EL, Meier EA. Nonparametric estimation from incomplete observations. J Am Statistical Assoc 1958; 53:457–481.

28. Bismuth H, Castaing D, Ericzon BG, Otte JB, Rolles K, Ringe B, Slooff M. Hepatic transplantation in Europe. First report of the European liver transplant registry. Lancet 1987; 2:674–676.

29. Calne RY. Liver transplantation: The recent Cambridge/King's college hospital experience. Transplant Proc 1988; 20:475–477.

30. Bismuth H. Liver transplantation: The Paul Brousse experience. Transplant Proc 1988; 20:486–489.

31. Kalayoglu M, Stratta RJ, Hoffmann RM, Sollinger HW, Belzer FO. Quadruple immunosuppressive therapy for liver transplantation. Transplant Proc 1988; 20:524–529.

32. Iwatsuki S, Starzl TE, Todo S, Gordon RD, Esquivel CO, Tzakis AG, Makowka L, Marsh JW, Koneru B, Stieber AC, Klintmalm GB, Husberg B. Experience in 1,000 liver transplants under cyclosporine–steroid therapy: a survival report. Transplant Proc 1988; 20:498–504.

33. Ruggieri JP, Ferrer A, Abrego J, Tuci N, Scolari G, Muino JC, Cejas H, de Artega E. Experimental liver heterotopic transplant in pigs. Transplant Proc 1988; 20:716–718.

34. Icard P, Sawyer GJ, Houssin D, Fabre JW. Marked differences between orthotopic and heterotopic auxiliary liver allografts in the induction of class II MHC antigens on hepatocytes. Transplantation 1990; 49:1005–1007.

35. Schmiedel T, Lauschke G, Franke WG, Schuster R, Weber K, Hliscs R. Demonstration of dual liver systems after experimental auxiliary partial liver transplantation using the alternative of sequential hepatobiliary scintigraphy. Z Exp Chir Transplant Kunstliche Organe 1988; 21:206–212.

36. Ku Y, Nishiyama H, Fujiwara S, Tanaka Y, Saitoh M, Ohyanagi H, Saitoh Y. Rejection and blood flow in auxiliary partial canine liver homografts. Transplant Proc 1989; 21:2228–2229.

37. Simic M, Vukovic R, Fabri M. Damage in the hemato–enteral barrier in experimental liver transplantation. Acta Chir Iugosl 1990; 37 Suppl 1:75–78.

38. D'Silva M, Pirenne J, Glassford E, Mayer D, Bai S, Gittes RF, Lee S. Arterialization of the liver. III. Influence of systemic and portal pressure gradients following heterotopic partial liver transplantation. Microsurgery 1990; 11:184–187.

39. Starzl TE, Marchioro TL, von Kaulla KN, Herman G. Homotransplantation of the liver in humans. Surg Gynecol Obstet 1963; 117:659–676.

40. Porte RJ, Blankensteijn JD, Knot EAR, de Maat MPM, Groenland THN, Terpstra OT. A comparative study on changes in hemostasis in orthotopic and auxiliary liver transplantation in pigs. Transplant Int 1991; 4:12–17.

41. Porte RJ, Knot EAR, de Maat MPM, Willemse PJA, Schalm SW, Stibbe J, Groenland THN, Terpstra OT. Fibrinolysis detected by thrombelastography in heterotopic, auxiliary liver transplantation: effect of tissue–type plasminogen activator. Fibrinolysis 1988; 2 (Suppl 3):67–73.

42. Bakker CM, Porte RJ, Knot EAR, de Maat MPM, Stibbe J, Terpstra OT. Fibrinolysis in auxiliary partial liver transplantation. Transplant Proc 1990; 22:2305.

43. Dzik WH, Arkin CF, Jenkins RL, Stump DC. Fibrinolysis during liver transplantation in humans: role of tissue–type plasminogen activator. Blood 1988; 71:1090–1095.

44. Arnoux D, Boutiere B, Houvenaeghel M, Rousset–Rouviere A, Le Treut P, Sampol J. Intraoperative evolution of coagulation parameters and t–PA/PAI balance in orthotopic liver transplantation. Thromb Res 1989; 55:319–328.

45. Suzumura N. Coagulation disorders during orthotopic liver transplantation. Nippon Geka Gakkai Zasshi 1989; 90:847–854.

46. Kalpokas M, Bookallil M, Sheil AG, Rickard KA. Physiological changes during liver transplantation. Anaesth Intensive Care 1989; 17:24–30.

47. Kost GJ, Jammal MA, Ward RE, Safwat AM. Monitoring of ionized calcium during human hepatic transplantation. Critical values and their relevance to cardiac and hemodynamic management. Am J Clin Pathol 1986; 86:61–70.

48. Aggarwal S, Kang YG, Freeman JA, Fortunato FL, Pinsky MR. Postreperfusion syndrome: cardiovascular collapse following hepatic reperfusion during liver transplantation. Transplant Proc 1987; 19(Suppl 3):54–55.

49. Carmichael FJ, Lindop MJ, Farman JV. Anaesthesia for hepatic transplantation: cardiovascular and metabolic alterations and their management. Anesth Analg 1985; 64:108–116.

50. Thomas HC, McSween RNM, White RG. Role of the liver in controlling the immunogenicity of commensal bacteria in the gut. Lancet 1973; 1:1288–1291.

51. Triger DR, Wright R. Hyperglobulinemia in liver disease. Lancet 1973; 1:1494–1496.

52. Yanaga K, Tzakis AG, Shimada M, Campbell WE, Marsh JW, Stieber AC, Makowka L, Todo S, Gordon RD, Iwatsuki S, Starzl TE. Reversal of hypersplenism following orthotopic liver transplantation. Ann Surg 1989; 210:180–183.

53. Ferrara J, Ellison C, Martin EW, Cooperman M. Correction of hypersplenism following distal splenorenal shunt. Surgery 1979; 86:570–573.

54. Soper NJ, Rikkers LF. Effect of operations for variceal hemorrhage on hypersplenism. Am J Surg 1982; 144:700–703.

55. Borel Rinkes IHM, Vanderhoop AG, Hesselink EJ, Metselaar HJ, de Rave S, Zonderland HM, Schalm SW, Terpstra OT. Does auxiliary heterotopic liver transplantation reverse hypersplenism and portal hypertension? Gastroenterology 1991; 100:1126–1128.

56. Schalm L, Bax HR, Mansens BJ. Atrophy of the liver after occlusion of the bile ducts or portal vein and compensatory hypertrophy of the unoccluded portion and its clinical importance. Gastroenterology 1956; 31:131–155.

57. Willemse PJA, Ausema L, Terpstra OT, Krenning EP, ten Kate FJW, Schalm SW. Regeneration of the graft and host liver atrophy after auxiliary partial liver transplantation for chronic liver failure. Hepatology 1991; (In Press)

58. Klintmalm GB, Nery JR, Husberg BS, Gonwa TA, Tillery GW. Rejection in liver transplantation. Hepatology 1991; 10:978–985.

59. Grant D, Wall W, Ghent C, Duff J, Kutt J, Stiller C, Frei J. Liver transplantation: the problem of rejection. Transplant Proc 1986; 18 suppl 4:163–166.

60. Astarcioglu I, Gugenheim J, Gigou M, Amorosa L, Fabiani B, Reynes M, Bismuth H. Immunosuppressive properties of auxiliary liver allografts into sensitized rats. Transplantation 1990; 49:1186–1188.

61. Madern GC, Terpstra OT, Sinaasappel M, Provoost AP, Rothuizen J, Molenaar JC. Heterotopic liver transplantation corrects the inborn error of hepatic metabolism in a dog model. Transplant Proc 1991; 23:716–717.

62. Rauhs R, Steininger R, Roth E. Eine erfolgversprechende Therapie des akuten Coma hepaticum durch heterotope auxiliare Leberlappentransplantation. Experimentelle Studie am Schwein. Acta Chir Austriaca 1981; 5:133–136.

63. Le Bihan G, Coquerel A, Houssin D, Bourreille J, Szekely A–M, Bismuth H, Hémèt J, Samson M. Insuffisance hépatique aiguë mortelle au cours d'un traitement par le valproate de sodium. Gastroenterol Clin Biol 1982; 6:477–481.

64. Fortner JG, Yeh SDJ, Kim DK, Shiu MH, Kinne DW. The case for and technique of heterotopic liver grafting. Transplant Proc 1979; 21:269–275.

65. Bumgardner GL, Fasola C, Sutherland DER. Prospects for hepatocyte transplantation. Hepatology 1988; 8:1158–1161.

66. Gubernatis G, Pichlmayr R, Kemnitz J, Gratz K. Auxiliary partial orthotopic liver transplantation (APOLT) for fulminant hepatic failure: first successful case report. World J Surg 1991; 15:660–666.

Chapter 3

LIVER PRESERVATION

The past and the future

This is an updated version (December 1991)
of an earlier publication in
HEPATOLOGY 1991;13:1235–1250

LIVER PRESERVATION

The past and the future

Jan D. Blankensteijn and Onno T. Terpstra

Department of Surgery, University hospital
Dijkzigt, Erasmus University, Rotterdam, The Netherlands

3.1 INTRODUCTION

The ultimate objective of organ preservation is unrestricted and immediate graft function after a transplant under optimal conditions in the most suitable recipient. This means time and efficacy are the basic features of the search for the ideal preservation method. Theoretically, freezing and continuous aerobic perfusion are the only means of obtaining truly long–term preservation (from one month to years). From a clinical point of view, however, the preferred method of preserving livers for brief periods prior to transplantation is simple cold storage.[1]

In the first report of the European Liver Transplant Registry,[2] reviewing 1,315 liver transplantations performed at 32 different centers from 1968–1987, one of the principal causes of loss of the graft was primary nonfunction of the liver. In most cases this was thought to be through an injury caused by inadequate preservation. Moreover, grafts with severe storage damage are rejected more frequently.[3]

Until recently, cold storage of the graft in Collins' solution provided acceptable graft function when the preservation time was 8 hr or less.[4] To keep the period of cold storage to a minimum, close coordination of two full surgical teams –one for the donor and one for the recipient– was necessary, and the recipient operation had to be performed as an emergency procedure, often at night. Only a very narrow margin of safety existed in the storage time in case of unforeseen delays or unanticipated, time consuming difficulties in the recipient operation.

To evaluate the possibility of liver transplantation on a (semi–) elective basis, Belzer[5] looked at the clinical needs for preservation times and concluded that 12 to 18 hr was necessary for liver transplantation to be performed electively.

Similar to kidney transplantation, (semi–) elective liver transplantation will yield substantial benefits: because simultaneous donor and recipient operation are not required, an unnecessary recipient laparotomy may be prevented if the donor organ appears unsuitable for transplantation. Furthermore, if laparotomy findings indicate the recipient to be unsuitable for transplantation, extended preservation gives time for another patient to be prepared. Because organs can be procured before final arrangements are made for the recipient, timing of the donor operation will be less critical; this will have particular merits when the donor's condition is unstable. This implies an increase in efficiency of organ utilization and the number of donor organs. Extended preservation will also allow for more preparation time and well–rested support in anaesthesia, nursing, operating room, blood bank, and intensive care unit.

The same surgeon performing the donor operation and –after a resting period– also the recipient operation, will allow recipient hepatectomy to be tailored to the donor organ. With the development of the University of Wisconsin (UW) solution[6] it is claimed that the goal of semielective liver transplantation has been achieved.[7,8] Without a doubt, however, the search for improving the available preservation methods will continue. Preservation times of more than 12 to 18 hr will be necessary to enable assessment of the viability of the harvested organ, to apply immunomodulation of the recipient and to perform cross–matching based on analysis of tissue antigens. Although the importance of cross–matching in liver transplantation remains to be determined, repeat–transplant candidates and highly sensitized patients may benefit from tissue typing.

The main objective of this study was to summarize the history of organ preservation and to integrate the reported potential mechanisms of storage damage to a generally applicable hypothesis on liver harvest injury. This hypothesis was then used to discuss the rationale of the basic components of the UW solution and its current and future additives.

3.2 HISTORY OF ORGAN PRESERVATION

The first experiments in transplantation of the whole liver were hampered by the extreme sensitivity of this organ to anoxia.[9,10] In 1956, Goodrich et al.[10] showed normothermic complete anoxia for a period of 30 min to render the liver unsuitable for transplantation.

The earliest device developed to protect the harvested liver was based on hypothermia: Starzl et al.[11] induced hypothermia by whole–body cooling of the donor to 30 °C and then perfused the excised liver with cold Ringer's lactate solution. In this way the hepatic core temperature fell to approximately 15°C. These organs then appeared to be able to sustain life in recipient dogs if transplanted as orthotopic homografts within 2 hr. Sicular and Moore[12] demonstrated canine liver cells *in vitro*, stored at hypothermia, to remain functionally intact for about 4 hr after death. Longer anoxic times in Starzl's experiments, however, resulted in a high rate of acute failure due to outflow block of the transplants, hemorrhagic diathesis and acute liver failure. Whereas the problem of outflow block was likely to be caused by spasm of the intrahepatic veins of the dog, hemorrhagic diathesis and acute liver failure, as would become evident later, were mainly the result of poor liver preservation.

In the history of organ preservation, experiences in kidney and liver preservation frequently supplemented one another. Since the previous mentioned technique of infusion of cold electrolyte solution was the first effective method in liver preservation, it immediately became the standard for the quick cooling of the kidney.[13] In 1963, Calne and co-workers[14] concluded their report on renal preservation by ice cooling as follows: "The cooling and rewarming periods can be reduced by perfusion techniques, which may be especially pertinent to the clinical application of cadaver transplants, since there is an inevitable delay in removing the kidney after death, and the relative large mass of the human kidney takes longer to cool by surface methods." Obviously, this remark was even more appropriate for the large mass of the human liver. Consequently, most of the initial efforts to extend the storage time were based on the concept of continuous hypothermic perfusion of the graft. In 1963, Marchioro and associates[15] reported a method of hypothermic cadaveric perfusion with the use of an extracorporeal heart–lung machine. In 1965, Mikaeloff et al.[16] described an application of a method, reported in 1961 by Kestens and McDermott,[17] in which hypothermic perfusion *in situ* was confined to the liver. Long-term recipient survival after homotransplantation of canine livers removed as long as 6 hr after death was achieved applying this technique.

Other methods not based on continuous perfusion were also assessed. Brown et al.[18] and Moss et al.[19] evaluated preservation by means of freezing to –6°C and –20°C to –60°C, respectively. Organs preserved for 1 to 14 days in these experiments, however, were incapable of supporting life as orthotopic transplants.

In 1967, a few years after Calne et al.[14] had stressed the importance of hypothermia in kidney preservation, Belzer et al.[20] demonstrated 24-hr and 72-hr preservation of canine kidney to be feasible by means of extracorporeal hypothermic pulsatile perfusion. In the same year, Slapak et al.[21] reported 24-hr preservation of the canine liver with a hypothermic, hyperbaric pulsatile perfusion technique. The latter results, however, were achieved by transplanting the liver in the neck of the recipient animal; therefore it could not be established whether this method would be adequate to maintain the life of an hepatectomized animal. In fact, it soon became clear that compared with kidney transplantation, progress in preservation techniques of the liver was far less prevalent.

On March 1, 1963, the first human liver transplantation was performed by Starzl in Denver.[22] Reviewing 15 cases of clinical liver transplantation performed between 1963 and 1965,[23] Schalm et al.[24] noted that all homografts, except in one case, were revascularized within 3–3½ hr of the death of the donor. Therefore they designed a

preservation method of simple cooling and flushing of the liver *in situ* with a nonphysiological perfusion fluid, which was then replaced by infusion of a plasma–based preservation fluid. The latter was intended to keep the vascular bed of the liver as intact as possible. This method provided a reliable preservation of about 3½ hr of the liver homograft, as confirmed by successful heterotopic non–auxiliary transplantation in the dog. This technique was then adapted and successfully used in the Cambridge–King's College Hospital liver transplant programme.[25] Simultaneously, Collins and associates[4] reported successful canine kidney preservation with a similar technique of initial perfusion and 30 hr ice storage. Again, as with continuous perfusion techniques, progress in kidney preservation was far ahead of liver preservation. The Denver/Pittsburgh group subsequently started using the "Collins C2" formulation in liver transplantation.[26] From that time on, two different pathways of liver preservation were continuously explored: the complex method of continuous or intermittent perfusion and the method of initial perfusion followed by simple hypothermic storage.

3.2.1 Continuous perfusion

In 1968, Brettschneider et al.[27] demonstrated that homografts could be effectively preserved for 8 hr with a combination of hypothermia, hyperbaric oxygen and continuous perfusion with diluted homologous blood through the portal vein and the hepatic artery. Another technique using continuous, hypothermic, asanguinous perfusion with oxygenated supernatant of cryoprecipitated plasma, which appeared to be consistently effective up to 10 hr,[28] was not capable of successfully preserving porcine livers for 24 hr.[29]

Although the Cambridge group continued to use the previously described modified plasma protein solution clinically, the limitation of 3 to 4 hr for hypothermic storage in many cases resulted in a transplantation operation outside their own institution, with transfer of medical and nursing personnel and the recipient to the hospital were the donor had died. This caused many logistic problems and encouraged the Cambridge group to search for an improvement of the complicated continuous perfusion apparatus with hyperbaric oxygen, which was available at that time. They developed a single passage, hypothermic, intermittent "squirt" perfusion technique allowing preservation of the porcine liver up to 17 hr.[30]

In the following 15 years, relative little progress was made, and although a few reports showed successful 24–hr preservation[31,32] and even incidental graft survival after 48

hr[33] preservation by perfusion techniques, the diverse results merely reflected the problems in establishing a consistent technique. Among other factors, the type and length of preservation, the chemical characteristics of the perfusate, the actual technique of perfusion (intermittent/continuous, pulsatile/nonpulsatile) and the individual response of the liver to hypothermic preservation were responsible for variations in the results. Moreover, continuous perfusion techniques have the disadvantages of being expensive, technically complex, bulky and not easily portable. Therefore these methods found little favor in clinical practice.

Just before the breakthrough in liver preservation discussed below, continuous hypothermic perfusion with a modified isotonic citrate solution was described as providing reliable 48–hr preservation of rat liver.[34] In addition, promising results were recently reported by Nakajima et al.,[35] who used an artificial blood substitute based on a stabilized hemoglobin solution. Successful 48–hr preservation of the canine liver was achieved by continuous hypothermic perfusion, with viability assessed by orthotopic transplantation.

3.2.2 Simple hypothermic storage

In 1971, Abouna et al.[36] and Spilg et al.[37] reported successful liver transplantation after simple cold storage of the graft for 6 to 8 hr, using balanced salt solutions to which dextrose was added. After the initial perfusion in the experiments of Spilg et al., livers were stored in a second solution mainly composed of fresh frozen plasma. The solution Abouna used for graft storage was almost the same as the flush solution. Except in the report of Mieny and Myburgh,[38] who successfully transplanted four baboon livers after 20–hr preservation with a chilled dextran–electrolyte–sorbitol solution, until 1979 reliable preservation time remained well under the 12 hr: In 1977 Benichou et al.[26] reported a comparison between Ringer's lactate, Schalm's solution as modified by Wall et al.[25] and Collins' solution.[4] Surprisingly, all three fluids appeared effective in preserving liver grafts for 9 hr; this was much longer than one would be led to expect by the previous reports on simple cold storage, particularly with regard to Ringer's lactate. Benichou et al. therefore concluded that hypothermia is the most important determinant for success in short–term preservation, no matter the solution. However, the extra value of the special composition of a solution became clear in 18 hrs cold storage: Ringer's lactate yielded significantly worse results than did the other two solutions. In the same report these authors described clinical preservation with a modified Collins' solution in seven patients, with preservation times varying from 6½–

10 hr. Subsequently, most centers started to use the modified Collins' solution or its more recent derivative, Euro–Collins for human liver preservation[39] and continued to do so until 1988. In that year UW solution dramatically improved liver preservation by simple cold storage; this will be described below.

With respect to this thesis, it is interesting that the only report of successful preservation by simple cold storage, preceding the UW solution era, was in a model with auxiliary liver transplantation.[40] Toledo–Pereyra and associates showed that it is possible to store canine livers hypothermically for 24 hr after flushing with crystalloid or colloid hyperosmolar solutions. They used Sacks' solution[41], which is comparable to Collins' solution, as crystalloid hyperosmolar solution, and as colloid hyperosmolar solution they used the modified silica gel fraction of plasma (developed by the same group[31]), which is comparable to the Cambridge modification of Schalm's solution.[25] These favorable results with auxiliary transplantation are even more conspicuous when the results of a similar experiment conducted by Tamaki et al. in 1985[42] are taken into account: they were able to preserve liver grafts for a cold ischemic period of only 6 hr, but completely failed to do so for 18 hr, using Collins' solution and the modified Schalm's plasma protein fraction solution in orthotopic transplantation in the rat.

Recently, Belzer and co–workers of the University of Wisconsin (Madison, USA), have succeeded in producing a remarkably effective solution for liver preservation, the so–called UW solution.[6] It was conceived on the basis of a thorough understanding of organ damage and protection mechanisms. This knowledge was gathered by extensive analysis of the basic principles of anaerobic hypothermic ischemia and organ–specific metabolism. Therefore, before the development of the UW solution is explained, various basic aspects of the harvest injury to the liver are elucidated in the next section.

3.3 MECHANISMS OF HARVEST INJURY

Literature provides numerous reports merely focussing on one or two of the determinants of the multifactorial pathogenesis of harvest injury. This may lead to seemingly inconsistent results. Apart from the fact that various experimental designs are used in different animal models, the most prominent determinants of these contradictions are anoxic versus reperfusion damage, parenchymal versus nonparenchymal damage and warm and cold ischemia.

After removal of an organ from the circulation of the donor, the most logical way of keeping the harvested organ viable is perfusion by oxygenated whole blood with use

of a heart–lung machine. The results and dilemmas of this approach are already described above. Any practical preservation method should consist of the (temporary) withdrawal of blood flow from the organ, which necessitates a period of ischemia after organ procurement and a moment of reperfusion during the recipient procedure. Injury may be induced by ischemia (literally, absence of blood) *per se* because it necessitates flushing of the donor organ, which in its turn may result in interstitial and cellular swelling when inappropriate fluids are used. This is, however, usually not the type of organ damage denoted by the expression "ischemic damage". In fact, it is a misnomer because hypoxia or anoxia is what is generally referred to, and "anoxic damage" would be a better term.[43] Anoxic and reperfusion damage are the two principal mechanisms of harvest injury. Accordingly, for these mechanisms, differences between parenchymal and nonparenchymal damage and between warm and cold ischemia are discussed separately.

3.3.1 Anoxic damage

In aerobic cells the energy necessary to maintain cell integrity is supplied by the mitochondrial cytochrome system through complete (tetravalent) reduction of oxygen to water. This involves the generation of ATP, by which means the energy is stored for later consumption (oxidative phosphorylation). These terminal reactions in aerobic glycolysis need a continuous supply of oxygen. As cells become anoxic, oxidative phosphorylation ceases and –without precautions– the stored ATP is consumed very rapidly. Since practically all energy–dependent functions use ATP, this molecule plays a central role in the viability of cells. Lack of ATP leads to cell injury by impairment of energy–dependent intracellular homeostatic functions. Subsequent to ATP depletion, diverse sequelae culminate in lethal cell injury. Therefore ATP can be considered the *initial* common pathway of cell death in organ preservation.
The pathway from ATP deficit to irreversible cell damage is obscure. However, some microscopic aspects of the cell surface (i.e., loss of microvilli and "blebbing") seem to be fairly constant. Cell surface blebbing is described by Lemasters et al.[44] in a sequence of events in which the sudden progression from reversible to irreversible injury is associated with and considered to be caused by rupture of a terminal cell surface bleb. Blebbing is thought to be a direct result of disruption of the cytoskeleton. Since cellular microfilaments and microtubules, of which this cytoskeleton is composed, are in an energy–requiring, dynamic state of continuous formation and

disassembly, ATP deficiency may be directly related to this phenomenon. Other mechanisms, however, are suggested also and will be discussed below.

ATP can be saved by cooling the organ, which reduces the tissue's metabolic demands for nutrients and oxygen. Therefore, hypothermia plays an essential role in the preservation of the anoxic cell. Alone, it is effective in prolonging the time during which anoxic tissue will remain viable.[7] However, this advantage is gained at a price because the intracellular homeostatic functions are decelerated equally. All measures necessary to prevent damage from loss of cellular homeostatic capacity constitute the toll for the hypothermia–induced reduction of the metabolic rate. Although theoretically the cell will ultimately be confronted with the same dangers as in warm ischemia, viability will be jeopardized earlier through changes induced by the loss of homeostatic capacity. Obviously, in the process of extending the cold ischemic period, when the most important obstacle is overcome, progress will go just as far as the second, most important metabolic disturbance allows. In addition, every measure in itself has the potential of introducing new threats for the already compromised cells.

Disturbance of the electrolyte balance is a major consequence of slowing the metabolism by hypothermia. Normally, the cells are bathed in an interstitial fluid high in sodium (Na^+) and calcium (Ca^{2+}) and low in potassium (K^+), compared with the intracellular electrolyte concentrations.[45] The intracellular concentrations of Na^+ and K^+ are maintained by an energy–dependent cation transport system in the cell membrane.[46,47] This enzyme system, Na^+/K^+–activated ATPase uses ATP to make Na^+ impermeable outside the cell, counteracting the colloidal osmotic pressure derived from the intracellular proteins and other impermeable anions, and cause K^+ to accumulate in the cell. Hypothermic preservation suppresses the activity of this Na^+ pump and decreases the membrane potential of the plasma membrane. Consequently, chloride (Cl^-) enters the cell down a concentration gradient with a cation and water to cause cell oedema.[5] Because this process leads to progressive cellular destruction, retention of intracellular electrolytes is a primary requirement for successful organ preservation.[48]

The extracellular fluid is very rich in Ca^{2+} (10^{-3} M) compared with the intracellular Ca^{2+} concentration (10^{-6} M). In addition, the electrical potential across the plasma membrane tends to drive Ca^{2+} in the cells. Such a large electrochemical gradient is maintained by the relative impermeability of the plasma membrane to Ca^{2+} and by active extrusion from the cell.[49]

Some considerations must be given at this point to the matter of the Ca^{2+} being a biological messenger. It has been more than a century ago since Ringer[50] noticed that

isolated hearts did not beat in the absence of Ca^{2+}. Although 99% of Ca^{2+} in the human body is present in the bones it is nowadays clear that Ca^{2+} plays a unique role in transmitting "signals" generated at the plasma membrane to a large number of target functions inside the cell.[51] The intracellular Ca^{2+} modulated (enzyme) functions are adjusted by variations in Ca^{2+} activity in a narrow range, around μM/L. Therefore the messenger function requires intracellular Ca^{2+} to be maintained at a very low activity level to switch targets on and off. This is achieved in two ways: by complexing the Ca^{2+} that has entered the cell down the concentration and electrical gradient from the extracellular space and by transporting the Ca^{2+} reversibly across various membrane systems. Three membrane systems have been shown able to transport Ca^{2+}: the plasma membrane, the sarcoplasmatic and endoplasmatic reticulum and the mitochondrion. The intracellular Ca^{2+}–transporting membranous network probably predominates over the plasma membrane in the *rapid* regulation of Ca^{2+}.[51]

The type of interaction between Ca^{2+} and the target molecule is, in most cases, not known. A small–molecular–weight protein has been discovered to be an important factor mediating the transmission of the Ca^{2+} signal and its interaction with the target function (Table 3.1). This ubiquous molecule, discovered by Cheung in 1967,[52] is termed "calmodulin." It is inactive in itself but the active form, the Ca^{2+}–calmodulin complex, binds reversibly to the target enzyme.[51]

Besides Ca^{2+}, another important biological messenger has been identified: cyclic AMP (CAMP).[53] However, complications are added to the issue by the fact that the Ca^{2+}–calmodulin complex activates two key enzymes with opposite functions in the metabolism of cAMP: adenylate cyclase (generation) and phosphodiesterase (breakdown).

Obviously, at the current stage it is impossible to present a reasonable generalized model of the exact role of Ca^{2+} in cell death. However, because a large number of Ca^{2+} membrane transport systems appear to have been invoked by evolutionary pressure, the importance of the messenger function of Ca^{2+} and the resulting necessity of regulating its activity inside cells with utmost efficiency and precision cannot be overlooked.

Several ways exist by which warm and cold ischemia may lead to altered Ca^{2+} homeostasis.[54] Depletion of ATP stores will inhibit the ATP–dependent pumps to extrude Ca^{2+} from the cell and the energy–dependent shuttles of the organelle membranes. In addition, the decrease in pH will induce a release of normally complexed Ca^{2+} as free ions. Other ions may also be related to pathologic fluxes of Ca^{2+} –for instance, the Na^+/K^+–homeostasis, which is also seriously changed during

Table 3.1. Calmodulin-regulated enzymes or cellular processes.

Phospholipase A_2	Adenylate cyclase
Phosphodiesterase	Guanylate cyclase
Phosphorylase B kinase	Myosin light chain kinase
Membrane phosphorylation	Neurotransmitter release
Ca-pumping ATPase (red cells) (sarcoplasmatic reticulum)	Microtubules disassembly

ischemia, as described above. Cold ischemia in particular, leads to decreased membrane fluidity, which in itself –or in combination with cellular swelling– may be related to abnormal Ca^{2+} movements. There is considerable evidence that cytosolic changes in Ca^{2+} levels are evoked both by release from intracellular depots and pathologic influx through the plasma membrane.[55–57] Unless the extrusion of Ca^{2+} from the cell is also influenced, only a transient increase in cytosolic Ca^{2+} level would be expected after the release of Ca^{2+} from intracellular stores.[58]

Many studies have associated these elevated Ca^{2+} levels with biochemical changes and histologic damage and cell death.[54,58,59] In an interesting report, Schanne and associates[49] described how accumulating intracellular Ca^{2+} can induce cell death. They found that rat hepatocytes could be destroyed with the cytotoxic compound A23187, whose only known specific biological activity is to create Ca^{2+} channels that overcome the permeability barrier of the plasma membrane. Furthermore, these investigators showed an absolute requirement for extracellular Ca^{2+} in the killing of primary cultures of adult rat hepatocytes by nine other toxins. Because all the toxins they used are capable of interacting with cell membranes, an interpretation of this Ca^{2+} dependence of cell death is that each agent requires extracellular Ca^{2+} to produce membrane injury. Besides the fact that it seems very unlikely that all ten toxins would be dependent on Ca^{2+}, some of these agents have been shown capable of producing membrane disruption without the presence of Ca^{2+}. Therefore the most plausible explanation is that in each case, the toxin causes disruption of the permeability barrier function of the plasma membrane, allowing a lethal influx of Ca^{2+} down the steep electrochemical gradient between the outside and the inside of the cell.

The exact way in which Ca^{2+} brings about cell death is open to speculation. Ca^{2+} plays a critical role in the maintenance of the structure and function of platelet cytoskeleton: Bellomo et al.[58] described menadione–induced oxidative stress on the platelet cytoskeleton to be Ca^{2+}–dependent, either as a direct consequence of the increase of cytosolic Ca^{2+} or mediated through the activation of Ca^{2+}–dependent proteases. Blebbing as described above is therefore frequently associated with elevated Ca^{2+} levels. However, Lemasters et al.[44] demonstrated blebbing of hepatocytes in a model of chemical hypoxia without a rise in cytosolic Ca^{2+}. This controversy may be due to the variety of mechanisms by which Ca^{2+} homeostasis is thought to be altered and to dissimilar relative importance of these mechanisms in both cold and warm ischemia. On the other hand, it means that disturbances in the cytoskeleton and cell surface blebbing are not exclusively caused by Ca^{2+} increase.

Another possible mechanism of Ca^{2+}–induced damage is activation of phospholipases.[60] These enzymes remove fatty acids from membranes without the aid of oxygen. Since catabolism of fatty acids does require oxygen, fatty acids accumulate and the bilayer membrane configuration is disturbed.[54]

In addition, Ca^{2+} is a regulator of a number of proteases, of which the conversion of xanthine dehydrogenase (XD) to xanthine oxidase (XO) is thought to play a significant role in the origin of storage damage.[61] However, the deleterious effects of phospholipase and protease activation together with the possible potentiation of the effect of oxygen–derived free radicals,[62] may be of more importance during reperfusion. This will be discussed below.

Although the rate of metabolism is greatly reduced under cold–storage conditions, the cell still needs some energy. In the absence of oxygen, the cell is able to draw this energy from anaerobic glycolysis and glycogenolysis, which entail increased production of lactic acid and hydrogen ions. The resulting tissue acidosis can damage cells and induce lysosomal instability, activate lysosomal enzymes and alter mitochondrial properties.[6] On the other hand, mild extracellular acidosis has been shown to be protective against the onset of irreversible injury to hepatocytes during ischemic stress.[63,64] In hepatocytes depleted of ATP by chemical hypoxia, Gores et al.[65] showed this protective effect of cellular acidosis to be mediated by intracellular pH. In that report, because anaerobic glycolysis is also inhibited in chemical hypoxia, the most important source of intracellular acidosis was considered hydrolysis of ATP. The mechanism of this protection by intracellular acidosis is unknown, but it is hypothesized that acidic pH suppresses autolytic degradative processes by proteases and phospholipases activated by ATP depletion as described above.

Most of the above-mentioned experiments focus on the hepatocyte. On the other hand, it is well established that during ischemia endothelial lesions appear earlier than do parenchymal lesions.[66] This concerns both warm[67] and cold[67–69] ischemic damage. Although the parenchymal cells may be viable after a certain period of preservation, graft survival will not be achieved when endothelial cells are lethally injured. This may explain the dissociation between functional tests of preserved hepatocytes and graft survival.

Focussing on cold ischemic damage only, it appears that microcirculatory damage will always be the critical factor. The correlation between the duration and the amount of anoxic damage (i.e., injury without reperfusion) to parenchymal and nonparenchymal cells and of reperfusion injury, as measured by several biochemical[70,71] and structural[67–69] studies, is depicted in Figure 3.1.

Although Holloway et al.[68] demonstrated UW solution and isotonic citrate solution to provide significant protection against injury to the hepatic microcirculation by cold preservation, they could not identify the responsible mechanisms. In addition, evidence has accumulated showing that microcirculation suffers mostly at and after reperfusion. Anoxic damage through basis of warm ischemia may be partially caused by ATP depletion in itself and by the ensuing disturbances in the cytoskeleton. Because Ca^{2+} accumulation needs some time and because low temperatures render organelles especially susceptible to cytosolic Ca^{2+} changes,[72] elevated Ca^{2+} levels may be the most important pathway through which cold ischemic damage is induced, the decisive factor being the sinusoidal lining cells. This damage, however, will probably not be manifest until the moment of reperfusion.

3.3.2 Reperfusion damage

It is well established that many organs suffer from considerable damage induced immediately after a period of either warm or cold ischemia at reperfusion.[73] The ischemic intestine[74,75] and lung[76,77] have been shown to be particularly susceptible to this reperfusion injury, and involvement of oxygen-derived free radicals in the pathophysiology of the cell damage has been suggested. Postischemic injury as a consequence of generation of cytotoxic metabolites of oxygen may also be significant in other organs, including the kidney,[78–80] heart,[81–83] pancreas,[84] liver,[85] and skin.[86] Because oxygen-derived free radicals appear to play such an important role in the mechanism of reperfusion injury, their backgrounds will be reviewed first.

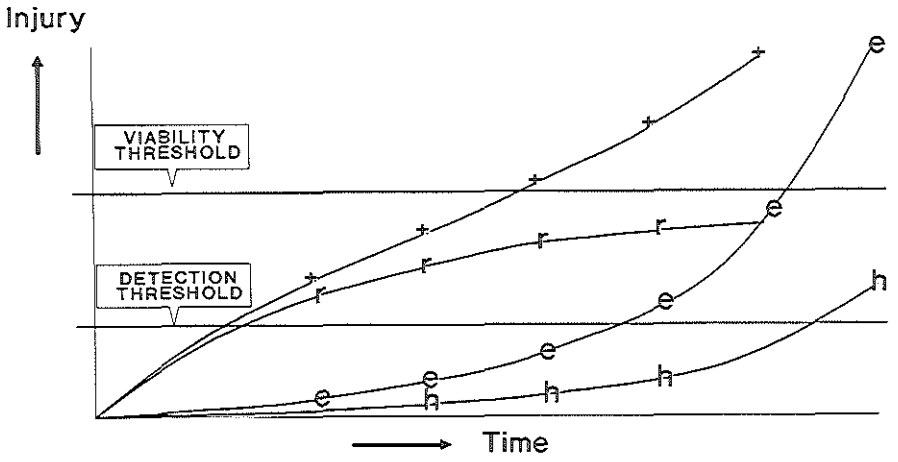


Figure 3.1. Harvesting injury from cold ischemia as measured by several biochemical and morphological studies. The total amount of damage (+–+) *is split up into its components: anoxic damage to hepatocytes* (h–h)*, anoxic damage to endothelial cells* (e–e) *and reperfusion damage* (r–r)*. This graph shows that endothelial damage always exceeds hepatocyte damage. In addition it is shown that reperfusion damage will be the most important factor, initially (detection threshold). With extended ischemic periods, however, measures to prevent reperfusion damage will no longer be able to inhibit the total amount of injury to progress beyond the viability threshold.*

Oxygen-derived free radicals.

Definition. A normal chemical bond consists of a pair of electrons sharing a single molecular orbit. A free radical is any atom, group of atoms or molecule that has one unpaired electron occupying an outer orbit.[87] This should not be confused with ions whose positive (Na^+) or negative charge (Cl^-) depends on the relationship of the number of electrons to the number of protons. Free radicals may be considered to contain an open bond or half a bond, rendering them chemically highly reactive and, therefore, transient. In chemical formulas the odd electron is symbolized by a dot. Free radicals can occur in both organic (e.g., quinones) and inorganic molecules (e.g., $O_2^{-\cdot}$) and are critical in the normal operation of a wide spectrum of biologic processes.[88]

Reactions. Biological reactions concerning free radicals can be classified as initiation reactions, propagation reactions and termination reactions.[87] The formation of a free radical, for instance, by radiolysis, photolysis or during oxidation–reduction reactions, is termed initiation. Free radicals can then proceed further by free radical intermediates, termed propagation reactions, which may be thousands of events long. If two radicals react, both radicals are eliminated from the propagating pool: this is termination. Virtually all cell components are capable of reacting with free radicals. Chemical modification of these molecules leads to metabolic and structural modifications of cells that can ultimately cause cell death. Notable cellular components at risk from free radical damage include proteins, membrane lipids, nucleic acids and DNA (Table 3.2).

Evolutionary considerations. Because of the ubiquity of molecular oxygen in aerobic organisms and its ability to readily accept electrons, oxygen–centered free radicals are often mediators of cellular free radical reactions.
The toxic potential of oxygen and its byproducts may be so important that it has influenced the basic biochemical structure of the organisms on this planet.[89] The first appearance of simple amino acids might have been a result of the combination of a reducing chemical atmosphere composed of hydrogen, ammonia, methane, water vapor, with no molecular oxygen, high levels of irradiation (absent ozone layer), high temperature and abundant surface water.[87] Later more complex biochemical communities evolved into primitive anaerobic organisms. The subsequent evolution of blue–green algae, approximately $2*10^9$ years ago, which could harness light energy to split H_2O into free O_2 and a hydrogen pool (e.g. $C_6H_{12}O_6$), allowed the release of O_2 into the biosphere. As the presence of O_2 tends to change compounds composed of carbon, hydrogen, oxygen and nitrogen into CO_2, N_2 and H_2O, this ingenious photosynthetic mechanism which could convert the electromagnetic energy of light into useful chemical energy, involved an unavoidable toxic byproduct, O_2, harmful for all living matter. This evolutionary pressure on the existing biological organisms resulted in a wide variety of defense mechanisms which ensured continuous survival of life, with an eventual death of the individual organism due to the accumulated oxidative injury.[90]

Physiologic defense mechanisms. Today, most cells use the oxidation potential of O_2 to enormous advantage in the cytochrome system, were reduction to water is used to generate ATP.[89] However, the cytochrome system may have evolved primarily as a means for the detoxification of oxygen, a mechanism that was only secondarily

Table 3.2. Cellular free radical targets.

TARGET	CONSEQUENCE
"SMALL" MOLECULES:	
Unsaturated and thiol–containing amino acids	Protein denaturation and cross–linking, enzyme inhibition Organelle and cell permeability changes
Nucleic acid bases	Cell cycle changes, mutations
Carbohydrates	Cell surface receptor changes
Unsaturated lipids	Cholesterol and fatty acid oxidation Lipid cross–linking Organelle and cell permeability changes
Cofactors	Decreased nicotinamide and flavin–containing cofactor availability and activity, ascorbate oxidation, porphyrin oxidation
Neurotransmitters	Decreased neurotransmitter availability and activity, including serotonin and epinephrine
Antioxidants	Decreased availability, including α–tocopherol and β–carotene
MACROMOLECULES:	
Protein	Peptide chain scission, denaturation
DNA	Strand scission, base modification
Hyaluronic acid	Change in synovial fluid viscosity

exploited for its energy–producing capacity through selective pressures for more efficient metabolism of scarce organic carbon substrates.

Under normal condition, most O_2 in biologic systems undergoes tetravalent reduction by efficient intracellular systems such as the cytochrome complex.[91] However, 1% to 2% of O_2 "leaks" from this pathway to undergo univalent reduction, as illustrated in Figure 3.2.[90] As can be seen in this figure, the univalent pathway results in the

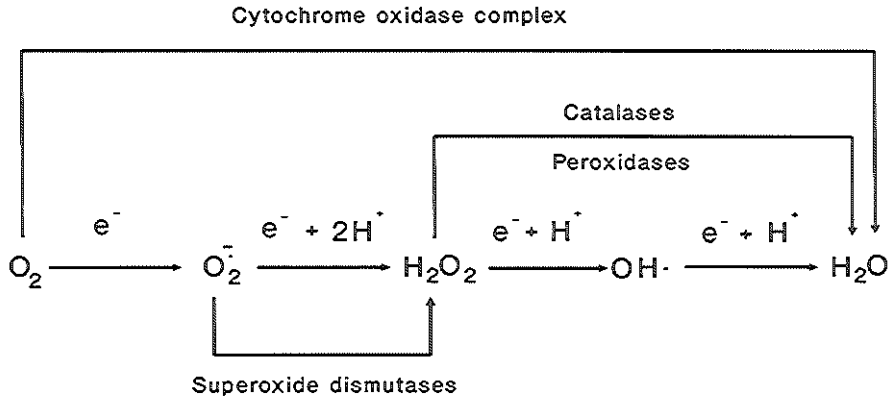


Figure 3.2. The univalent pathway for reduction of molecular oxygen. In addition the enzymatic defense mechanisms to bypass and prevent the accumulation of reactive intermediates are shown.

---

formation of $O_2^{-\cdot}$ (superoxide anion or superoxide radical), H_2O_2 (hydrogen peroxide), and OH· (hydroxyl radical), as the intermediates. These intermediates are too reactive to be tolerated in living tissue and their removal and control has been the challenge of all aerobic organisms since the first appearance of O_2 in the atmosphere.
The protective and controlling mechanisms can be divided into enzymatic, hydrophobic, hydrophilic and structural groups, as shown in Table 3.3.[87] In Figure 3.2, the enzymatic bypass mechanisms are depicted in relation to the univalent pathway.

Oxygen–derived free radicals and hepatic reperfusion injury.
Various sources of oxygen–derived free radicals are suggested. According to McCord,[61] the major source of the oxygen–derived free radicals is XO.[92] This enzyme –of which rich sources are present in the intestine, lung and liver– is synthesized in

Table 3.3. Classification of protective controlling mechanisms of oxygen derived free radicals.

MAIN GROUP	EXAMPLE
Enzymatic	Superoxide dismutase, Catalase and Peroxidase (cytochrome oxidase)
Hydrophobic	α–Tocopherol (vitamin E) and β–carotenes in cellular membranes Glutathione peroxidase
Hydrophilic	Ascorbic acid and Cysteine Reduced glutathione Ceruloplasmin and transferrin in plasma
Structural	Cholesterol in biomembranes Localization of certain reactions to peroxisomes and mitochondria

the form of XD. This form accounts for about 90% of the total activity in healthy tissue. XD does not produce oxygen–derived free radicals, but can reduce NAD^+ to form uric acid from xanthine:

$$\text{xanthine} + H_2O + NAD^+ \text{–(XD)} \rightarrow \text{uric acid} + NADH + H^+$$

XO can use O_2 instead of NAD^+, producing $O_2^{\cdot-}$ (or H_2O_2 or both) as follows:

$$\text{xanthine} + H_2O + 2O_2 \text{ –(XO)} \rightarrow \text{uric acid} + 2O_2^{\cdot-} + 2H^+$$

As hypothesized by McCord,[61] the elevated cytosolic Ca^{2+} concentration, which results from ischemia as described above, activates a protease capable of converting XD to XO. Concomitantly, the consumption of the residual amounts of ATP results in an elevated concentration of AMP, which is further catabolized to adenosine, inosine, and then hypoxanthine. This hypoxanthine, as well as xanthine, serves as an oxidizable substrate for XD or XO. Thus, during anoxia a new enzyme activity appears along with

one of its two required substrates. No oxygen–derived free radicals are produced because the remaining substrate required for XO activity $-O_2-$ is absent. During reperfusion of the tissue, O_2 is suddenly abundant. This results in a burst of oxygen–derived free radicals and H_2O_2 production. If the tissue is anoxic for even a short period, a rapid increase in the amount of XO activity occurs. In the liver, XO content doubles after about 30 min (which is about the same in spleen, lung and kidney).[61] In the heart, the same increase requires only 8 min of nonperfusion. Skeletal muscle is unique in this respect because XD does not convert to XO during nonperfusion; this correlates well with the clinically observable resistance of skeletal muscle to ischemic injury.

In contradiction to this mechanism of generation of oxygen–derived free radicals, Metzger et al.[93] and de Groot and Brecht[94] found no evidence for a role of XO in hepatic reperfusion injury. In a model of reperfusion after a period of warm ischemia of the rat liver, they found no effect of allopurinol (an inhibitor of xanthine oxidase) and demonstrated the oxidant stress not to be maximal when the available concentration of substrates for XO is highest (i.e., at the onset of reperfusion). These studies used a model of warm ischemia in which the initiation of oxygen–derived free radicals may be quite dissimilar to the cold ischemic situation. Indeed, Marzi et al.[95] observed a rapid accumulation of xanthine and hypoxanthine after simple cold storage of the rat liver, which is consistent with the hypothesis that XO generates free radicals during reperfusion.

Another possible source of oxygen–derived free radicals is the activation of leukocytes and resident macrophages.[66,93,96] When activated, Kupffer cells release a wide variety of toxic substances, including oxygen–derived free radicals, tumor necrosis factor, leukotrienes, cytokines, platelet activation factor, prostanoids and proteases.[97] This activation may be induced by surface receptors of endothelial cells, exposed by anoxic damage (as has been demonstrated in other models).[95,98] In this case, oxygen–derived free radicals are probably released by membrane associated enzymes like lipoxygenase and cyclooxygenase, which are active in arachidonic acid metabolism.[88]

The involvement of the reticuloendothelial system in the origin of ischemia/reperfusion damage is in agreement with the observation that cyclosporine ameliorates this damage in the rat and in the pig.[99] Cyclosporine is a powerful inhibitor of the function of macrophages to produce cytokines. In fact, increased leukocyte endothelial interactions have been observed by intravital microscopy in liver transplantation.[100]

What has not been clarified is why the naturally occurring scavenging mechanisms –in particular superoxide dismutase (SOD)– are unable to dismutate the oxygen–derived

free radicals at reperfusion. However, because no need exists for these natural scavengers during anoxia, they may be chemically altered during this period. Corresponding with this assumption, decreased SOD activity has been measured during hypoxia.[101,102] The simplest explanation is a relative overload of oxygen–derived free radicals arising at reperfusion. The combination of high oxygen tensions due to inadequately functioning mitochondria, damaged during the ischemic period, with decreased scavenging mechanisms may result in a high free radical load, culminating in further membrane damage and cell death.[87]

On the other hand, oxygen–derived free radicals may be less important than other products of the activated Kupffer cells. Tumor necrosis factor (TNF) and interleukin–6 (IL–6) are two cytokines recognized as critical mediators of ischemia/reperfusion injury.[103] Agents which block TNF production (e.g. prostaglandins and dexamethasone, see below) or anti–TNF antibodies have been shown to block or attenuate certain models of liver injury.[103,104]

On the basis of currently available data it may be hypothesized that –at reperfusion following *warm* ischemia– the main source of oxygen–derived free radicals and other toxic mediators is the activation of leukocytes, macrophages or Kupffer cells. This is in agreement with the assumption that direct injury to the cytoskeleton and the resulting alteration of surface receptors are the most important consequences of anoxic damage.

Reperfusion after *cold* ischemia probably induces an overload of oxygen–derived free radicals mainly produced by XO. In the latter mechanism, increased cytosolic Ca^{2+} levels during anoxia may play an important role.

The potential mechanisms of anoxic and reperfusion damage are depicted in Figure 3.3.

3.4 CYTOPROTECTIVE COMPOUNDS IN LIVER PRESERVATION

The term "cytoprotection" stems from the observation that prostaglandins could protect epithelial cells of hollow gastrointestinal viscera against various ulcerogenic agents that would otherwise produce cell damage and necrosis.[105] This advantageous effect appeared not to be limited to the digestive tract.[106] To date, the concept of hepatocytoprotection represents the beneficial property of any agent preventing harvest injury. In the next section, some of the various drugs (Table 3.4) are described and considerations of the working mechanism are given, arranged as to the composition of warm and cold ischemic and anoxic and reperfusion damage to hepatocytes and endothelial cells, as described above and illustrated in Figure 3.3.

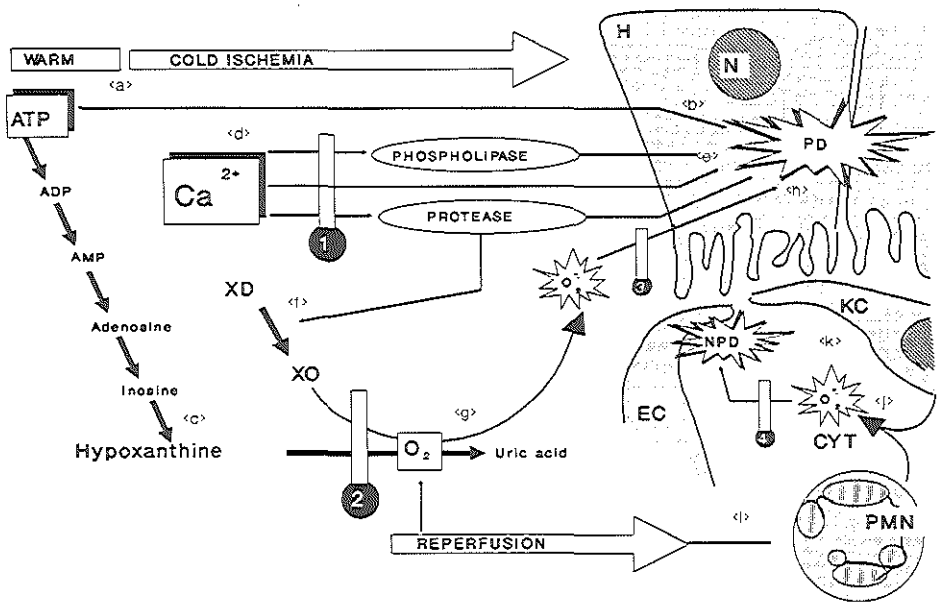


Figure 3.3. Hypothesized mechanism of harvesting injury. Warm ischemia (and cold ischemia at a lower rate) causes depletion of ATP **<a>**. *This induces damage to the energy-requiring cytoskelet of the hepatocyte* **<b>** *(and to the nonparenchymal cells; not shown). ATP is broken down stepwise leading to an accumulation of hypoxanthine* **<c>**. *With longer lasting cold ischemia, Ca^{2+} accumulates* **<d>**. *Directly, but also by the activation of various phospholipases and proteases this also leads to cytoskelet damage of the hepatocyte* **<e>** *(and to the nonparenchymal cells; not shown). Simultaneously, this activation of proteases induces a rapid conversion of xanthine dehydrogenase to xanthine oxidase* **<f>**. *Consequently, both activated enzyme (xanthine oxidase) and its substrate (hypoxanthine) are abundant. At reperfusion, oxygen is supplied in excess, leading to a burst of intracellular oxygen-derived free radicals* **<g>**, *also adding to the parenchymal damage* **<h>**. *At reperfusion, polymorphonuclear leukocytes* **<i>** *and Kupffer cells are activated by tissue antigens of endothelial cells (which have come to expression by the preceding anoxic injury) to produce extracellular oxygen-derived free radicals and other toxic mediators* **<j>**. *This causes an escalation of nonparenchymal damage* **<k>**.

(H) *hepatocyte;* (N) *nucleus;* (EC) *endothelial cell;* (KC) *Kupffer cell;* (PMN) *polymorphonuclear leukocyte;* (XD) *xanthine dehydrogenase;* (XO) *xanthine oxidase;* (PD) *parenchymal damage;* (NPD) *nonparenchymal damage;* (ADP) *adenosine diphosphate;* (AMP) *adenosine monophosphate; and* (CYT) *cytokines.*

(1) *Ca^{2+} entry blockers and cAMP related mechanisms;* (2) *direct prevention of oxygen-derived free radical generation;* (3) *scavengers of intracellular oxygen-derived free radicals; and* (4) *scavengers of extracellular oxygen-derived free radicals and inhibitors of cytokine production.*

Table 3.4. Agents used to prevent oxygen–derived free radical induced harvesting injury.

Related to calcium or cAMP:	**Attenuation of cytokine production:**
Calcium entry blockers	Adrenocorticoids
Isoproterenol	Prostaglandins
Chlorpromazine	
Dibucaine	
Aprotinin	**Unknown:**
Prostaglandins	
	Chloroquine
Direct prevention of O_2^- formation:	Coenzyme Q_{10}
	Tris–Hydroxymethyl–Amino–Methane
Allopurinol	Phenoxybenzamin
Oxygen–derived free radical scavengers:	
Chelators	
Mannitol and DMSO	
Superoxide dismutase (SOD)	

3.4.1 Calcium– and cAMP– related mechanisms

Agents in this category act by reducing the intracellular Ca^{2+} accumulation induced by anoxia as delineated above. As already pointed out, the interrelationship of Ca^{2+} and cAMP is not understood. However, cAMP has been reported to produce an immediate and transient efflux of Ca^{2+} in perfused livers of rats.[107] Hypothetically, this is caused by the inhibition of the generation of the Ca^{2+}–calmodulin complex (Figure 3.4). This results in free calmodulin, which facilitates the extrusion of the simultaneous released Ca^{2+}. On the other hand, cAMP may also directly promote Ca^{2+} efflux. Calmodulin and cAMP are believed to act by the Ca^{2+}–specific ATPase.[108] Therefore, drugs believed to invoke cAMP production are included in this category also.

Ca^{2+} entry blockers such as *verapamil* bind to membrane structures responsible for the "slow channels." The presumed efficacy of these drugs, however, may be questioned because no potential gated "slow Ca^{2+} channels" have been described in non–excitable tissues.[51] Still, *diltiazem* has been demonstrated to have a protective effect in liver

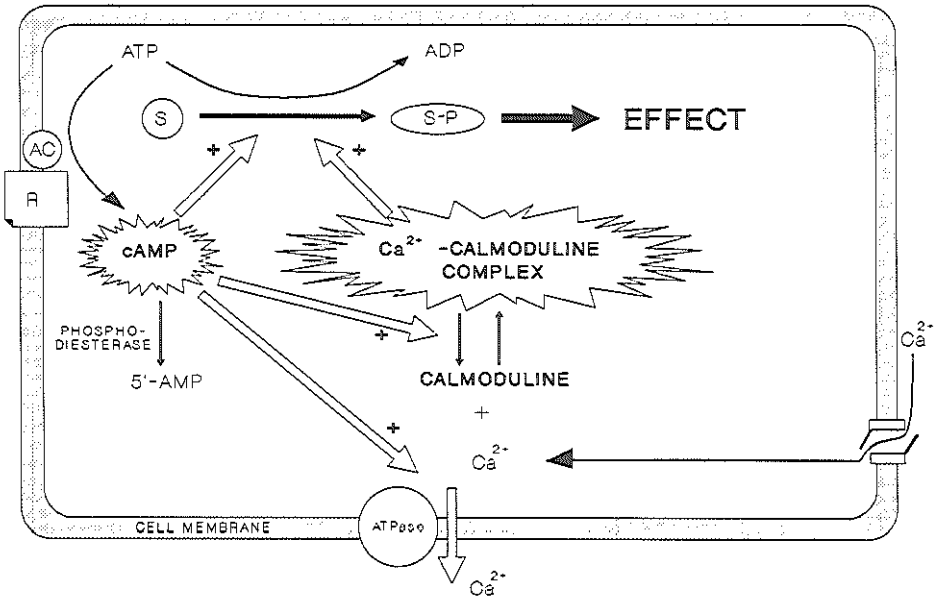


Figure 3.4. Schematic diagram depicting the hypothesized role of cyclic AMP (cAMP) *and the* Ca^{2+}*–Calmoduline complex as intracellular second messengers. When a hormone binds to its membrane receptor* (R)*, this activates adenylate cyclase* (AC)*. cAMP synthesized by adenylate cyclase diffuses through the cytoplasm and activates the phosphorylation of specific substrate proteins* (S)*, usually enzymes. Phosphorylation activates these enzymes* (S–P)*, which in turn produces the cell–specific effects. The* Ca^{2+}*–Calmoduline complex has a similar pathway of inducing certain effects. Furthermore cAMP inhibits the formation of the* Ca^{2+}*–Calmoduline complex and it probably facilitates* Ca^{2+} *extrusion by* Ca^{2+}*–ATPase in the cell membrane.*

---

ischemia and reperfusion in pigs.[109] Verapamil has been shown to be useful alone[110–112] and in combination with prostaglandin I_2.[113] This seemingly contradiction may be explained by the fact that calcium blockers not only change plasma membrane Ca^{2+} transport but may also protect cells by inhibiting Ca^{2+} mobilization from intracellular sources.[114]

Nisoldepine, also a Ca^{2+} entry blocker, was proposed to interfere at the level of the Kupffer and endothelial cells, preventing the release of proteases and other toxic peptides in a rat liver transplantation model.[115]

Isoproterenol has a β_2–mimetic effect and activates adenylate cyclase, increasing cAMP.[116] Lambotte et al. have demonstrated the protective effect of isoproterenol in orthotopic canine liver transplantation.[117]

Chlorpromazine is an antagonist of histamine, α–adrenergic and dopamine receptors. It is, however, also suggested to act by Ca^{2+}–calmodulin antagonism,[118] and it has been shown to suppress the activity of phospholipases[119] and to stabilize lysosomes.[120] Nonetheless, the efficacy of chlorpromazine is controversial.[117,121,122]

Dibucaine inhibits phospholipase A_2. Theoretically, it may therefore prevent injury by inhibiting the Ca^{2+}–related activation of phospholipase A_2. Its cytoprotective effects are a matter of controversy.[123,124]

Aprotinin is a naturally occurring inhibitor of proteolytic enzymes; it therefore may possess cytoprotective effects by inhibiting Ca^{2+}–related activation of proteases.[125,126]

Prostaglandins. In the 1930s, three laboratories independently described a uterine smooth muscle–contracting activity deriving from semen. It was termed prostaglandin because it was assumed to originate in the prostate gland. The first two were called prostaglandin E and F, with respect to their solvent partitioning into Ether and phosphate (Fosfat in Swedish). A prostaglandin (PG) is a twenty–carbon fatty acid containing a five–carbon ring. Nine groups of PGs have been identified and assigned the letter designations A through I, followed by a subscript denoting the number of carbon–carbon double bonds outside the ring. The latest–discovered PG, PGI_2, has been shown to posses a variety of interesting biological activities, including inhibition of platelet aggregation, vasodilatation, stabilization of lysosomal membranes and increase of blood flow to the splanchnic region.[127–129] All these effects, which are also attributed to PGE_1, may play a role in the hepatocytoprotective properties of PGs. Araki and Lefer, however, convincingly showed the stabilization of lysosomal membranes by PGI_2 to be the most prominent mechanism.[130] Especially Kupffer cells, which are rich in lysosomes, could benefit from these stabilizing effects. In the absence of properly functioning Kupffer cells, clearance of endotoxin will be impaired. This could also be responsible for damage to hepatocytes.[97]

This stabilization of lysosomal membranes, however, is no more than a description of what can be demonstrated when cells are protected by mechanisms –still to be elucidated– resulting in diminished cell death as measured by the reduced appearance of lysosomal enzymes. Therefore other theories of the mechanism of action of PGs are required. In this respect the cytoprotective action of PGs is thought to be related to the well–established mechanism of platelet aggregation inhibition: PGI_2 and PGE_1 stimulate platelet adenyl cyclase, leading to an increase in platelet cAMP.[128] As shown above, cAMP may play a key role in preventing liver harvest injury in an intricate interaction with Ca^{2+} and calmodulin.[131] The fact that PGE_1 has been shown to inhibit adenyl cyclase in fat cells, demonstrates that PGs may have opposite effects in

different tissues.[132] Moreover, Ueda et al.[133] were not able to demonstrate significant differences in hepatic tissue cAMP concentrations between non-treated and PGE_1-pretreated ischemic canine livers.

The hepatocytoprotective properties of the PGI_2 and PGE_1 have been reported by various authors, and their efficacy is reproducible in a variety of models.[1,109,133-138] Of particular interest in this respect is the report of Araki and Lefer, who demonstrated protection of hypoxic-induced liver damage in an isolated perfused cat liver model using PGI_2.[130] Using PGI_2 as additive to the preservation fluids, a significant improvement of animal and graft survival and graft function was obtained after orthotopic liver transplantation in dogs and pigs with liver grafts stored for 24 and 48 hr. [113,139,140] In both canine[133] and human[141] liver transplantation, prostaglandin E_1 has been shown to improve graft function significantly and to decrease graft damage.In addition, PGE_1 has been useful in treating fulminant hepatic failure[142] and in treating primary graft nonfunction.[143,144]

3.4.2 Direct prevention of $O_2^{-\cdot}$ formation

Allopurinol is known to be an XO inhibitor because it decreases the breakdown of xanthine and hypoxanthine to urate in gouty arthritis. Several studies have shown a protective effect of allopurinol against ischemia-induced renal injury.[145] Initially, these beneficial effects were thought to be established by preventing the loss of purine bases from the anoxic cell. Once degradation of a nucleotide has proceeded beyond the xanthine-hypoxanthine level to uric acid, there is no way back to generate ATP. At present, however, it is generally accepted that allopurinol owes its beneficial effect in ischemia to prevention of generation of oxygen-derived free radicals.[146] Furthermore, while micromolar concentrations of allopurinol should be sufficient for complete inhibition of XO, it is considered a radical scavenger in millimolar concentrations.[147] Allopurinol has been used in liver ischemia with contradictory results.[31,93,148,149]

3.4.3 Oxygen-derived free radicals scavengers

As already mentioned, physiologically occurring oxygen-derived free radical scavengers are abundant in a variety of human and animal tissues; this suggests that this enzyme plays a significant, even vital, role in protecting the organism against the damage of $O_2^{-\cdot}$.

In particular, *SOD*[150] has been studied extensively.[85] With respect to liver preservation, it has been shown experimentally that SOD is effective in diminishing the extent of harvest and reperfusion injury.[85,94,151] These results, however, are still subject of discussion: Southard et al.[152,153] questioned the potential effect of oxygen–derived free radical scavengers in liver preservation in a study in which they measured XO and SOD activity of liver and kidney tissue of rats, dogs, and humans. In man, the highest concentrations of SOD were found in the intestine and liver. Nevertheless, no data exist on the activity of endogenous scavenger–enzymes, after long–term cold storage of these organs. Conceivably, these enzymes are chemically altered since there is no need for these scavenging mechanisms during anoxia.[87] Others suggest exogenous SOD is not likely to enter cells to detoxify $O_2^{\cdot-}$ generated in hepatocytes.[154] For that reason, the effects of exogenous SOD may be confined to prevention of damage caused by extracellularly generated $O_2^{\cdot-}$ (leukocytes). As the usefulness of SOD could be limited by its short half–life of 5 min, a new slow delivery type of SOD, SMA–SOD, was developed. It appeared to prevent reperfusion injury after warm ischemia in pigs.[155] In addition, various nonphysiologic agents have been shown to have scavenging properties. *Chelators* block Fe^{2+}–related propagation reactions of oxygen–derived free radicals, and *Mannitol* and *Dimethylnitrosamine* are known hydroxyl radical scavengers. Their potential beneficial effect in liver preservation are open to speculation.

3.4.4 Attenuation of cytokine production

With the recognition of TNF as a terminal mediator of liver injury, a wide range of agents will gain renewed interest for their presumed TNF–suppressing properties in the near future.

Corticosteroids. Conventionally, the cytoprotective effects of corticosteroids are attributed to their membrane–stabilizing properties.[156] However, "lysosomal stabilization" is not what can be called a biochemical mechanism of action. An enormous variety of mechanisms of action are ascribed to these drugs, many theoretical. The antiphlogistic effect, for which corticosteroids are famous, is probably caused by inhibition of arachidonic acid production required to activate the enzymatic pathway of inflammation (prostaglandins and leukotrienes). Corticosteroids stimulate the synthesis of a protein called lipomodulin, which in its turn inhibits the activity of phospholipases (note the resemblance with calmodulin), thus preventing the initial

release of arachidonic acid.[157] This mechanism may be involved in the property of corticosteroids to suppress cytokine production.
The role of corticosteroids in preventing harvest injury remains to be established. *Dexamethasone* but also *prostaglandin* E_2 have been shown to be able to inhibit TNF production in vivo.[103] This immunosuppresive effect is an important new aspect of the cytoprotective property of corticosteroids and prostaglandins.[158]

3.4.5 Unknown mechanisms of action

Because the mechanisms of the previously mentioned agents are mainly hypothetical, no more than descriptions of the chemical properties are available of the following drugs, without an understanding of the relationship with the suggested efficacy in preventing harvest injury.
Coenzyme Q_{10} is a quinone derivate (with 10 isoprene units) and is a highly mobile carrier of electrons between the flavoproteins and the cytochromes (b–c1–c–a/a3) of the electron–transport chain in mitochondria. It may act as an antioxidant in the protection of oxygen–derived free radicals–induced damage.[159–161] Interestingly, it is suggested that calcium potentiates the deleterious effects of oxygen–derived free radicals on the electron–transport chain due to impairment of NADH–coenzyme Q–reductase activity.[62]
Phenoxybenzamin blocks receptors for histamine(1), acetylcholine and serotonin. It has been shown to be useful in kidney preservation, but its value in liver preservation is a matter of controversy.[4,121,123]

3.5 UW SOLUTION

By reconsidering the components of cold–storage solutions for organ preservation, a new solution was developed at the University of Wisconsin by a pioneer in the field of organ transplantation, Folkert Belzer. In 1987, Wahlberg et al.[162] reported the first results of 72–hr preservation of the canine pancreas with the so called UW solution. Soon, successful 24– to 48–hr simple cold storage of the canine liver was described.[7] In march 1988, Kalayoglu and co–workers reported the first clinical use of this new preservation solution in liver transplantation.[163] Tolerance of cold ischemia by the human liver appeared to be significantly improved from 8 hr or less with Collins' solution to more than 10 hr (range is 11–20 hr) in nine cases. Soon, larger series were reported with similar results.[8,164]

On the other hand, within four years of the first clinical use of UW solution, the initial enthusiasm has been repeatedly tempered. In a retrospective study, human livers were considered to enter the danger zone of storage injury once 20 hr of simple cold storage had passed.[165] In another morphologic study in man, liver preservation with UW solution beyond 13 hr was followed by an alarmingly high incidence of non-anastomotic biliary strictures, most likely as a result of ischemic damage.[166] Also, graft function was demonstrated to be significantly affected when the cold ischemia time exceeded 12 hr in another clinical retrospective study.[167] Nevertheless, the clinical use of UW solution has added considerably to the extension of the safe cold storage time and to satisfactory graft function and outcome of liver transplantation.

The key to the success of this advance lay in the approach of these investigators to organ preservation because they recognized the different metabolic aspects of the various organs. Because the success of cold storage preservation is primarily related to the prevention of tissue oedema, the selection of an organ-specific impermeant was the most important aspect.[163]

Besides the reduction of cellular swelling, the following requirements for the new solution were defined: prevention of intracellular acidosis, prevention of expansion of the interstitial space during the flush-out period, prevention of injury from oxygen-derived free radicals and availability of substrates for regenerating high-energy phosphate compounds during reperfusion. Table 3.5 shows the composition of the UW solution. The solution is based on lactobionate and raffinose as impermeants to suppress hypothermia-induced tissue swelling, replacing glucose and mannitol in Collins' solution and hypertonic citrate, respectively. The latter sugars are effective as impermeants in the kidney, but the liver shows free permeability to these small carbohydrates, rendering them unsuitable as impermeants. Phosphate is included in the solution as a buffer to prevent tissue acidosis, induced by the increased rate of anaerobic metabolism in the ischemic cells. Hydroxyethyl starch is added as a colloid for oncotic support during the flush-out period. Allopurinol and reduced glutathione are included as free radical scavengers and magnesium sulfate is used for its presumed membrane stabilizing properties. Adenosine is added as a precursor for ATP resynthesis.

Obviously some agents are added for theoretical reasons only. Although results with UW solution are better than any obtained to date with any technique of liver preservation, the composition is somewhat ambiguous with respect to the role of oxygen-derived free radicals in harvest injury. Adhering to the hypothesis of oxygen-derived free radicals being responsible for a major portion of harvest injury, it is

Table 3.5. Composition and rationale of ingredients of UW-solution.

SUBSTANCE	AMOUNT IN ONE LITER		RATIONALE
Basic components:			
K^+-lactobionate	100	mmol	Impermeant
Na KH_2PO4	25	mmol	Buffer
$MgSO_4$	5	mmol	Membrane stabilization
Raffinose	30	mmol	Oncotic support
Additives:			
Hydroxyethyl starch	50	g	Colloid
Allopurinol	1	mmol	Xanthine oxidase inhibitor
Glutathione	3	mmol	Scavenger
Adenosine	5	mmol	ATP precursor

The solution is brought to a pH 7.4 at room temperature with NaOH. The final osmolarity is 320 mOsm/L. Prior to use the following agents are added: insulin (40 U/L), dexamethasone (16 mg/L), and Penicillin G (200.000 U/L).

apparant that adenosine can serve as a substrate for XO. In that way it may stimulate the formation of free radicals. Belzer and Southard[6] do recognize this problem in stating that omitting adenosine from the UW solution may be necessary in lung or intestine preservation. The fact that they do include adenosine in the liver storage solution is based on earlier studies on the XO and SOD activity in the liver and kidney.[152] These observations suggested that oxygen-derived free radicals may be of little significance in human livers and kidneys. This was confirmed in a study on pig liver preservation, finding no support for a pivotal role of oxygen-derived free radical induced lipid peroxidation.[168] Still, allopurinol is added as a free radical scavenger and indeed, in another study,[169] UW solution did show protection against reperfusion injury by inhibiting lipid peroxidation, which corresponds with an oxygen radical initiated process.

Another important consideration in this multifactorial approach is that no attempt was made to gain an increase in cellular cAMP. This ignores theoretical propositions and experimental evidence that this might be an important, early step in the prevention of

intracellular Ca^{2+} accumulation, as shown above.[131] Therefore, prostaglandins may theoretically be a valuable additive to the UW solution.

It is remarkable how little is known about the exact role of the various additives of the UW solution in preventing harvest injury. Therefore, in trying to define the relevance of the various components of the UW solution empirically, the omission of several additives has been investigated. Recently, Jamieson et al.[170] demonstrated that raffinose, lactobionate and glutathione could not be omitted. However, in their experiment, after 48 hr of ice storage, rabbit livers were reperfused *ex vivo* and organ transplantation was not performed to confirm graft viability. In another study, lactobionate was also considered essential in UW solution, while raffinose could be replaced by glucose without deleterious effect.[171] Except for the finding that colloid improved the rheological properties of the UW solution, none of the additives besides the basic components improved preservation in kidney transplantation.[172] Even the relevance of hydroxyethyl starch is controversial.[7,173,174]

Another interesting subject is the development of variants of the UW solution in which high K^+ concentration is replaced by high Na^+ concentration. This has a number of potential advantages, one related to the fact that solutions containing high K^+ levels (UW solution) can induce serious endothelial damage. Indeed, the low K^+ UW version appeared to be equally or slightly more effective.[175,176] Also, in the rat model, adenosine, starch, insulin and dexamethasone could be omitted in a new lactobionate–based preservation solution, with higher sodium content and a high buffering capacity by the addition of histidine.[177]

3.6 Conclusions and future directions

With improving technical proficiency and the introduction of cyclosporine, liver transplantation has evolved to a well–established treatment of end–stage cirrhosis. Extensive research resulted in a substantial advance in the most prominent feature of liver transplantation at the moment: organ preservation. Because the liver is threatened by multiple important hazards at organ procurement and during subsequent storage and reperfusion, a multifactorial approach showed to be essential in the development of an effective cold storage solution and safely preserving liver grafts over a period long enough to perform liver transplantation as an semielective procedure.

Many interesting aspects of organ preservation will be the subject of further investigation in the future. The combination of cold storage –to harvest and to transport the donor organ– and subsequent placement on a perfusion machine once it arrives in

the actual transplantation center –to enable selection of the most suitable recipient– is one of the promising modifications. It has already been reported to provide successful 5–day preservation of the canine kidney.[178]

As Francavilla et al.[179] demonstrated rapid organ cooling to be detrimental, Pienaar et al.[180] studied the possibility of preserving livers without flushing or perfusion. After 6 hr storage, liver grafts were able to sustain life in subsequent liver transplantation in pigs. In another study in pigs,[181] liver function appeared to be markedly reduced when omitting cold perfusion, even with immediate transplantation.

Another major topic in the near future will be the assessment of graft viability and donor selection criteria. Nuclear magnetic resonance imaging[182] and other methods to measure the loss of liver nucleotides and energy charge[183–186] and biochemical essays of the liver perfusate[70] and structural examinations[187] may provide sensitive markers to predict graft viability before transplantation. Furthermore, function tests of the donor liver may prove to be useful in predicting early graft function.[188]

Finally, the next logical step was to improve the solution used to rinse the liver graft before implantation. A new specific rinsing solution (Carolina solution) was composed and used in rats.[189–191] It was shown to protect endothelial cells from reperfusion injury and to improve survival compared to Ringer's solution.

Future studies concerning other mechanisms of harvest injury, incorporating the above–mentioned and/or other additives to the preservation solution, will be necessary to further prolong the safe storage period. This could permit donor–recipient tissue–matching and reduce the emergency aspects of liver transplantation.

3.7 REFERENCES

1. Tamaki T, Okouchi Y, Kozaki M, Kawamura A, Uchino J, Pegg DE. Hypothermic preservation of the rat liver assessed by orthotopic transplantation. III Improved functional recovery with isotonic citrate solution and a stable prostacyclin analogue. Transplantation 1988; 46:626–628.

2. Bismuth H, Castaing D, Ericzon BG, Otte JB, Rolles K, Ringe B, Slooff M. Hepatic transplantation in Europe. First report of the European liver transplant registry. Lancet 1987; 2:674–676.

3. Howard TK, Klintmalm GB, Cofer JB, Husberg BS, Goldstein RM, Gonwa TA. The influence of preservation injury on rejection in the hepatic transplant recipient. Transplantation 1990; 49:103–107.

4. Collins GM, Bravo–Shugarman M, Terasaki PI. Kidney preservation for transportation. Initial perfusion and 30 hours' ice storage. Lancet 1969; 2:1219–1222.

5. Belzer FO. Principles of organ preservation. Transplant Proc 1988; 20:925–927.

6. Belzer FO, Southard JH. Principles of solid–organ preservation by cold storage. Transplantation 1988; 45:673–676.

7. Jamieson NV, Sundberg R, Lindell S, Claesson K, Moen J, Vreugdenhil PK, Wight DGD, Southard JH, Belzer FO. Preservation of the canine liver for 24–48 hours using simple cold storage with UW solution. Transplantation 1988; 46:517–522.

8. Todo S, Nery JR, Yanaga K, Podesta L, Gordon RD, Starzl TE. Extended preservation of human liver grafts with UW solution. JAMA 1989; 261:711–714.

9. Welch CS. A note on the transplantation of the whole liver in dogs. Transpl Bull 1955; 2:54–56.

10. Goodrich EO,Jr., Welch HF, Nelson JA, Beecher TS, Welch CS. Homotransplantation of the canine liver. Surgery 1956; 39:244–251.

11. Starzl TE, Kaupp HA, Brock DR, Lazarus RE, Johnson RV. Reconstructive problems in canine liver homotransplantation with special reference to the postoperative role of hepatic venous flow. Surg Gynecol Obstet 1960; 111:733–743.

12. Sicular A, Moore FD. A study of the post mortem survival of tissues. J Surg Res 1961; 1:16–21.

13. Starzl TE. Experience in renal transplantation. Philadelphia: WB Saunders, 1964:

14. Calne RY, Pegg DE, Pryse-Davies J, Brown FL. Renal preservation by ice-cooling. An experimental study relating to kidney transplantation from cadavers. Br Med J 1963; 2:651–655.

15. Marchioro TL, Huntley RT, Waddell WR, Starzl TE. The use of extracorporeal perfusion for obtaining post mortem grafts. Surgery 1963; 54:900–903.

16. Mikaeloff P, Kestens PJ, Dureau G, Rassat JP. Transplantation orthotopique du foie chez le chien après conservation de l'organe par perfusion. Mem Acad Chir Par 1965; 91:711–724.

17. Kestens PJ, McDermott WVJr. Perfusion and replacement of the canine liver. Surgery 1961; 50:196–199.

18. Brown H, Patel J, Blair DW, Brown ME. Biochemical studies with preserved transplanted canine liver. J Am M Ass 1966; 196:775–777.

19. Moss GS, Reed P, Riddell AG. Observations on the effects of glycerol on the cold storage of the canine liver. J Surg Res 1966; 6:147.

20. Belzer FO, Ashby BS, Dunphy JE. 24-Hour and 72-hour preservation of canine kidneys. Lancet 1967; 2:536–539.

21. Slapak M, Wigmore RA, MacLean LD. Twenty-four hour liver preservation by the use of continuous pulsatile perfusion and hyperbaric oxygen. Transplantation 1967; 5:1154–1158.

22. Starzl TE, Marchioro TL, von Kaulla KN, Herman G. Homotransplantation of the liver in humans. Surg Gynecol Obstet 1963; 117:659–676.

23. Starzl TE, Marchioro TL, Porter KA. Advances in surgery. Year Book Medical Publishers. Chicago: Medical Publishers, 1966:295–312.

24. Schalm SW, Terpstra JL, Drayer B, van den Berg C, Veltkamp JJ. A simple method for short-term preservation of a liver homograft. Transplantation 1969; 8:877–881.

25. Wall WJ, Calne RY, Herbertson BM, Baker PG, Smith DP, Underwood J, Kostakis A, Williams R. Simple hypothermic preservation for transporting human livers long distances for transplantation. Report of 12 cases. Transplantation 1977; 23:210–216.

26. Benichou J, Halgrimson CG, Weil R, Koep LJ, Starzl TE. Canine and human liver preservation for 6 to 18 hr by cold infusion. Transplantation 1977; 24:407–411.

27. Brettschneider L, Daloze PM, Huguet C, Porter KA, Groth CG, Kashiwagi N, Hutchison DE, Starzl TE. The use of combined preservation techniques for extended storage of orthotopic liver homografts. Surg Gynecol Obstet 1968; 126:263–274.

28. Belzer FO, May R, Berry MN, Lee JC. Short term preservation of porcine livers. J Surg Res 1970; 10:55–61.

29. Perkins HA, May RE, Belzer FO. Cause of abnormal bleeding after transplantation of pig liver stored by a perfusion technique. Arch Surg 1970; 101:62–68.

30. Calne RY, Dunn DC, Herbertson BM, Gordon EM, Bitter–Suermann H, Robson AJ, MacDonald AS, Davis DR, Smith DP, Reitter FH, Webster LM. Liver preservation by single passage hypothermic "squirt" perfusion. Br Med J 1972; 4:142–144.

31. Toledo–Pereyra LH, Simmons RL, Najarian JS. Factors determining successful liver preservation for transplantation. Ann Surg 1975; 181:289–298.

32. Petrie CR, Woods JE. Successful 24–hour preservation of the canine liver. Arch Surg 1973; 107:461–464.

33. Sung DTW, Woods JE. Forty–eight–hour preservation of the canine liver. Ann Surg 1974; 179:422–426.

34. Tamaki T, Kamada N, Wight DG, Pegg DE. Successful 48–hour preservation of the rat liver by continuous hypothermic perfusion with haemaccel–isotonic citrate solution. Transplantation 1987; 43:468–471.

35. Nakajima I, Fuchinoue S, Teraoka S, Tojinbara T, Fujikawa H, Kawai T, Honda H, Agishi T, Ota K. Long–term liver preservation using artificial blood substitute. Transplant Proc 1989; 21:1314–1315.

36. Abouna GM, Koo CG, Howanitz LF, Ancarani E, Porter KA. Successful orthotopic liver transplantation after preservation by simple hypothermia. Transplant Proc 1971; 3:650–653.

37. Spilg H, Uys CJ, Hickman R, Saunders SJ, Terblanche J. Successful liver transplantation after storage for 6–8 hours, using a simple hypothermic immersion technique. Transplantation 1971; 11:457–460.

38. Mieny CJ, Myburgh JA. Successful 20–hr preservation of the primate liver by simple cooling. Transplantation 1971; 11:495–496.

39. Dreikorn K, Horsch R, Rohl L. 48 to 96 hour preservation of canine kidneys by initial perfusion and hypothermic storage using Euro Collins solution. Eur Urol 1980; 6:221–224.

40. Toledo–Pereyra LH, Chee M, Lillehei C, Condie RM. Liver presevation by cold storage with hyperosmolar solutions for twenty–four hours. Cryobiology 1979; 16:43–49.

41. Sacks SA, Petritsch PH, Kaufman JJ. Canine kidney preservation using a new perfusate. Lancet 1973; 1:1024–1028.

42. Tamaki T, Kamada N, Pegg DE. Hypothermic preservation of the rat liver assessed by orthotopic transplantation. A comparison of flush solutions. Transplantation 1986; 41:396–397.

43. Farber JL, Young EE. Accelerated phospholipid degradation in anoxic rat hepatocytes. Arch Biochem Biophys 1981; 211:312–320.

44. Lemasters JJ, DiGuiseppi J, Nieminen AL, Herman B. Blebbing, free Ca^{2+} and mitochondrial membrane potential preceding cell death in hepatocytes. Nature 1987; 325:78–81.

45. MacKnight ADC, Leaf A. Regulation of cellular volume. Physiol Rev 1977; 57:510–573.

46. Glynn IM. Membrane adenosine triphosphatase and cation transport. Brit Med Bull 1968; 24:165.

47. Skou JC. Enzymatic basis for active transport of Na^+ and K^+ across cell membrane. Physiol Rev 1965; 45:596.

48. Martin DR, Scott DF, Downes GL, Belzer FO. Primary cause of unsuccessful liver and heart transplantation: cold sensitivity of the ATPase system. Ann Surg 1972; 175:111–117.

49. Schanne FAX, Kane AB, Young EE, Farber JL. Calcium dependence of toxic cell death: A final common pathway. Science 1979; 206:700–702.

50. Ringer S. Regarding the action of hydrate of soda, hydrate of ammonia, and hydrate of potassium on the ventricle of the frog's heart. J Physiol 1883; 3:195–202.

51. Carafoli E. Membrane transport and the regulation of the cell calcium levels. In: Cowley RA, Trump BF, eds. Pathophysiology of shock, anoxia and ischemia. Baltimore:Williams & Wilkins, 1982:95–111.

52. Cheung WY. Phosphodiesterase: Pronounced stimulation by snake venom. Biochem Biophys Res Commun 1967; 29:478–482.

53. Levine RA. The role of cyclic AMP in hepatic and gastrointestinal function. Gastroenterology 1970; 59:280–300.

54. Cotteril LA, Gower JD, Fuller BJ, Green CJ. Oxidative damage to kidney membranes during cold ischemia. Evidence of a role for calcium. Transplantation 1989; 48:745–751.

55. Snowdowne KW, Freudenrich CC, Borle AB. The effect of anoxia on cytosolic free calcium, calcium fluxes and cellular ATP levels in cultured kidney cells. J Biol Chem 1985; 260:11619–11622.

56. Arnold PE, Lumlertgul D, Burke TJ, Schrier RW. In vitro versus in vivo mitochondrial calcium loading in ischemic acute renal failure. Am J Physiol 1985; 248:845–848.

57. Humes HD. Role of calcium in pathogenesis of acute renal failure. Am J Physiol 1986; 250:579–583.

58. Bellomo G, Orrenius S. Altered thiol and calcium homeostasis in oxidative hepatocellular injury. Hepatology 1985; 5:876–882.

59. Mirabelli F, Salis A, Vairetti M, Bellomo G, Thor H, Orrenius S. Cytoskeletal alterations in human platelets exposed to oxidative stress are mediated by oxidative and Ca^{2+}-dependent mechanisms. Arch Biochem Biophys 1989; 270:478–488.

60. Wong PVK, Cheung WY. Calmodulin stimulates human platelet phospholipase A_2. Biochem Biophys Res Commun 1979; 90:473–476.

61. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med 1985; 312:159–163.

62. Nauta RJ, Tsimoyiannis E, Uribe M, Walsh DB, Miller D, Butterfield A. The role of calcium ions and calcium channel entry blockers in experimental ischemia–reperfusion–induced liver injury. Ann Surg 1991; 213:137–142.

63. Bonventre JV, Cheung JC. Effects of metabolic acidosis on viability of cells exposed to anoxia. Am J Physiol 1985; 249:C149–C159.

64. Gores GJ, Nieminen AL, Dawson TL, Herman B, Lemasters JJ. Extracellular acidosis delays the onset of cell death in ATP-depleted hepatocytes. Am J Physiol 1988; 255:C315–C322.

65. Gores GJ, Nieminen AL, Wray BE, Herman B, Lemasters JJ. Intracellular pH during "chemical hypoxia" in cultured rat hepatocytes. Protection by intracellular acidosis against the onset of cell death. J Clin Invest 1989; 83:386–396.

66. Caldwell-Kenkel JC, Currin RT, Tanaka Y, Thurman RG, Lemasters JJ. Reperfusion injury to endothelial cells following cold ischemic storage of rat livers. Hepatology 1989; 10:292–299.

67. Myagkaya G, van Veen H, James J. Ultrastructural changes in rat liver sinusoids during prolonged normothermic and hypothermic ischemia in vitro. Virchows Arch [Cell Pathol] 1984; 47:361–373.

68. Holloway CM, Harvey PR, Mullen JB, Strasberg SM. Evidence that cold preservation-induced microcirculatory injury in liver allografts is not mediated by oxygen-free radicals or cell swelling in the rat. Transplantation 1989; 48:179–188.

69. Koizumi M, Ohkohchi N, Katoh H, Koyamada N, Fujimori K, Sakurada M, Andoh T, Satomi S, Sasaki T, Taguchi Y, Mori S, Kataoka S, Yamamato TY. Preservation and reflow damage in liver transplantation in the pig. Transplant Proc 1989; 21:1323–1326.

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

71. McKeown CMB, Edwards V, Philips MJ, Harvey PRC, Petrunka CN, Strasberg SM. Sinusoidal lining cell damage: the critical injury in cold preservation of liver allografts in the rat. Transplantation 1988; 46:178–182.

72. Fuller B. Storage of cells and tissues at hypothermia for clinical use. In: Bowler K, Fuller B, eds. Temperature and animal cells. Cambridge:Company of Biologists, 1987:341–342.

73. Bulkley GB. Free radical-mediated reperfusion injury: a selective review. Br J Cancer 1987; 55:66–73.

74. Parks DA, Bulkley GB, Granger DN, Hamilton SR, McCord JM. Ischemic injury in the cat small intestine: Role of superoxide radicals. Gastroenterology 1982; 82:9–15.

75. Parks DA, Granger DN, Bulkley GB, Shah AK. Soybean trypsin inhibitor attenuates ischemic injury to the feline small intestine. Gastroenterology 1985; 89:6–12.

76. Stuart RS, Baumgartner WA, Borkon AM, Hall TS, Breda MA, Brawn JD, Hutchins GM. Five hour hypothermic lung preservation with oxygen free-radical scavengers. Transplant Proc 1985; 17:1454–1456.

77. Taylor AE, Martin D, Parker JC. The effects of oxygen radicals on pulmonary edema formation. Surgery 1983; 94:433–438.

78. Baker GL, Corry RJ, Autor AP. Oxygen free radical induced damage in kidneys subjected to warm ischemia and reperfusion. Protective effect of superoxide dismutase. Ann Surg 1985; 202:628–641.

79. Hoshino T, Maley WR, Bulkley GB, Williams GM. Ablation of free radical–mediated reperfusion injury for the salvage of kidneys taken from non–heartbeating donors. Transplantation 1988; 45:284–289.

80. Green CJ, Healing G, Lunec J. Evidence of free radical-induced damage in rabbit kidneys after simple hypothermic preservation and auto-transplantation. Transplantation 1986; 41:161.

81. Gardner TJ, Stewart JR, Casale AS, Downey JM, Chambers DE. Reduction of myocardiacal injury with oxygen-derived free radical scavengers. Surgery 1983; 94:423-427.

82. Stewart JR, Blackwell WH, Crute SL, Loughlin V, Greenfield LJ, Hess ML. Inhibition of surgically induced ischemia-reperfusion injury by oxygen free radical scavengers. J Thorac Cardiovasc Surg 1983; 86:262.

83. Stewart JR, Gerhardt EB, Wehr CJ, Shuman T, Merril WH, Hammon JW,Jr., Bender HW,Jr.. Free radical scavengers and myocardial preservation during transplantation. Ann Thorac Surg 1986; 42:390-393.

84. Sanfey H, Bulkley GB, Cameron JL. The pathogenesis of acute pancreatitis. The source and role of oxygen-derived free radicals in three different experimental models. Ann Surg 1985; 201:633-638.

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

86. Manson PN, Anthenelli RM, Im MJ. The role of oxygen free radicals in ischemic tissue in island skin flaps. Ann Surg 1983; 198:87-90.

87. Del Maestro RF. An approach to free radicals in medicine and biology. Acta Physiol Scand 1980; 492:153-168.

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

89. Bulkley GB. The role of oxygen free radicals in human disease processes. Surgery 1983; 94:407-411.

90. Fridowich I. The Biology of oxygen radicals. The superoxide radical is an agent of oxygen toxicity; superoxide dismutases provide an important defense. Science 1978; 201:875-880.

91. McCord JM. The superoxide free radical: its biochemistry and pathophysiology. Surgery 1983; 94:412-414.

92. McCord JM, Fridowich I. The reduction of cytochrome c by milk xanthine oxidase. J Biol Chem 1968; 243:5753-5760.

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

94. De Groot H, Brecht M. Reoxygenation injury in rat hepatocytes – mediation by O_2^- /H_2O_2 liberated by sources other than xanthine oxidase. Biol Chem Hoppeseyler 1991; 372:35–41.

95. Marzi I, Zhong Z, Lemasters JJ, Thurman RG. Evidence that graft survival is not related to parenchymal cell viability in rat liver transplantation. The importance of nonparenchymal cells. Transplantation 1989; 48:463–468.

96. Jaeschke H, Smith CV, Mitchell JR. Reactive oxygen species during ischemia–reflow injury in isolated perfused rat liver. J Clin Invest 1988; 81:1240–1246.

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

98. Warren JS, Ward PA. Review: oxidative injury to the vascular endothelium. Am J Med Sci 1986; 29:97–102.

99. Kim YI, Kawano K, Nakashima K, Goto S, Kobayshi M. Alleviation of 3.5–hour warm ischemic injury of the liver in pigs by cyclosporine pretherapy. Transplantation 1991; 51:731–733.

100. Bühren V, Marzi I, Walcher F, Menger M, Hower R. Effects of Euro–Collins, University–of–Wisconsin, and Histidine–Tryphtophane–Ketoglutarate Solution on Hepatic Microcirculation Following Liver Transplantation in the Rat. Transplant Proc 1991; 23:643–644.

101. Guarnieri C, Flamigni F, Caldarera CM. Role of oxygen in the cellular damage induced by re–oxygenation of hypoxic heart. J Mol Cell Cardiol 1980; 12:797–808.

102. Marubayashi S, Dohi K, Sumimoto K, Oku J, Ochi K, Kawasaki T. Changes in activity of oxygen free radical scavengers and in levels of endogenous antioxydants during hepatic ischemia and subsequent reperfusion. Transplant Proc 1989; 21:1317–1318.

103. Nagakawa J, Hishinuma I, Hirota K, Miyamoto K, Yamanaka T, Tsukidate K, Katayama K, Yamatsu I. Involvement of tumor necrosis factor–α in the pathogenesis of activated macrophage–mediated hepatitis in mice. Gastroenterology 1990; 99:758–765.

104. Colletti LM, Remick DG, Burtch GD, Kunkel SL, Strieter RM, Campbell DA,Jr.. Role of tumor necrosis factor–α in the pathophysiologic alterations after hepatic ischemia/reperfusion injury in the rat. J Clin Invest 1990; 85:1936–1943.

105. Robert A, Nezamis JE, Lancaster C, Hanchar J. Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury. Gastroenterology 1979; 77:433–443.

106. Robert A. Current history of cytoprotection. Prostaglandins 1981; 21:89–96.

107. Friedman N, Park CR. Early effects of 3',5'–adenosine monophosphate on the fluxes of calcium and potassium in the perfused liver of normal and adrenalectomized rats. Proc Natl Acad Sci USA 1968; 61:504–508.

108. Katzung BG, Chatterjee K. Vasodilators & the treatment of angina pectoris. In: Katzung BG, ed. Basic & clinical pharmacology. Los Altos:Lange Medical Publication, 1984:133.

109. Steininger R, Mühlbacher F, Rauhs R, Roth E, Bursch W. Protective effect of PGI_2 and diltiazem on liver ischemia and reperfusion in pigs. Transplant Proc 1988; 20:999–1002.

110. Lambotte L, de Hemptinne B, Alvarez–Lopez A, Besse T. Effects of calcium blocking agents and prostaglandins I_2 or E_2 on the tolerance of the rat liver to ischemia. Transplant Proc 1988; 20:986.

111. Ar'Rajab A, Ahrén B, Bengmark S. Improved liver preservation for transplantation due to calcium channel blockade. Transplantation 1991; 51:965–967.

112. Cheng S, Ragsdale JR, Sasaki AW, Lee RG, Devency CW, Pinson CW. Verapamil improves rat hepatic preservation with UW solution. J Surg Res 1991; 50:560–564.

113. Mora NP, Cienfuegos JA, Bernaldó de Quiros L, Tendillo FJ, Pereira F, Fores R, Alvarez I, Navidad R, Castillo Olivares JL. Successful liver allograft function after 24–hour preservation: cumulative effects of prostacyclin plus verapamil. Transplant Proc 1987; 19:3932–3936.

114. Umeshita K, Monden M, Ukei T, Gotoh M, Nakano Y, Endoh W, Okamura R, Mori T. Different cytoprotective effects of calcium blockers in hypothermic liver preservation. Transplant Proc 1989; 21:1290–1291.

115. Takei Y, Marzi I, Kauffman FC, Currin RT, Lemasters JJ, Thurman RG. Increase in survival time of liver transplants by protease inhibitors and a calcium channel blocker, nisoldipine. Transplantation 1990; 50:14–20.

116. Murad F. Regulation and the role of cyclic metabolites. In: Cowley RA, Trump BF, eds. Pathophysiology of shock,anoxia and ischemia. Baltimore:Williams & Wilkins, 1982:147–153.

117. Lambotte L, Pontegnie-Istace S, Otte JB, Kestens PJ. The effect of isoproterenol and Collins' solution on the preservation of canine livers with simple cooling. Transplant Proc 1974; 6:301-303.

118. Thomas GE, Levitsky S, Feinberg H. Chlorpromazine inhibits loss of contractile function, compliance and ATP in ischemic rabbit heart. J Mol Cell Cardiol 1983; 15:621-628.

119. Mittnacht SJr, Farber JL. Reversal of ischemic mitochondrial dysfunction. J Biol Chem 1981; 256:3199-3201.

120. Chien KR, Abrams J, Serroni A, Martin JT, Farber JL. Accelerated phospholipid degradation and associated membrane dysfunction in irreversible ischemic, liver cell injury. J Biol Chem 1978; 253:4809-4813.

121. Rangel DM, Bruckner WL, Byfield JE, Dinbar A, Yakeishi Y, Stevens GH, Fonkalsrud EW. Enzymatic evaluation of hepatic preservation using cell-stabilizing drugs. Surg Gynecol Obstet 1969; 129:963-972.

122. Sundberg R, Ar'Rajab A, Ahrén B, Bengmark S. Improvement of liver preservation quality with UW solution by chlorpromazine pretreatment of the donor in an experimental model. Transplantation 1989; 48:742-744.

123. Ozaki N, Tokunage Y, Wakashiro S, Ikai I, Morimoto T, Shimahara Y, Kamiyama Y, Yamaoka Y, Ozawa K, Nakase Y. Evaluation of cytoprotective drugs for liver preservation by pyridine nucleotide fluorometry. Surgery 1988; 104:98-103.

124. Malis CD, Bonventre JV. Mechanism of calcium potentiation of oxygen free radical injury to renal mitochondria. J Biol Chem 1986; 261:14201-14208.

125. Lie TS, Seger R, Hong GS, Preissinger H, Ogawa K. Protective effect of aprotinin on ischemic hepatocellular damage. Transplantation 1989; 48:396-399.

126. Hunt BJ, Cottam S, Segal H, Ginsburg R, Potter D. Inhibition by aprotinin of tPA-mediated fibrinolysis during orthotopic liver transplantation letter. Lancet 1990; 336:381.

127. Moncada S, Gryglewski RJ, Bunting S, Vane JR. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature 1976; 263:663-665.

128. Moncada S, Vane JR. Prostacyclin: its biosynthesis and clinical potential. Phil Trans R Soc Lond [Biol] 1981; 294:305-329.

129. Lefer AM, Ogletree ML, Smith JB, Silver MJ, Nicolaou KC, Barnette WE, Gasic GP. Prostacyclin: a potentially valuable agent for preserving myocardial tissue in acute myocardial ischemia. Science 1978; 200:52–54.

130. Araki H, Lefer AM. Cytoprotective actions of prostacyclin during hypoxia in the isolated perfused cat liver. Am J Physiol 1980; 238:H176–H181.

131. Sikujara O, Monden M, Toyoshima K, Okamura J, Kosaki G. Cytoprotective effect of prostaglandin I_2 on ischemia–induced hepatic cell injury. Transplantation 1983; 36:238–243.

132. Stryer L. Hormone action. In: Stryer L, ed. Biochemistry. New York:WH Freeman and company, 1981:853–854.

133. Ueda Y, Matsuo K, Kamei T, Kayashima K, Konomi K. Protective effect of prostaglandin E_1 (PGE_1) on energy metabolism and reticuloendothelial function in the ischemically damaged canine liver. Liver 1989; 9:6–13.

134. Ueda Y, Matsuo K, Kamei T, Ono H, Kayashima K, Tobimatsu M, Konomi K. Prostaglandin E_1 but not E_2 is cytoprotective of energy metabolism and reticuloendothelial function in the ischemic canine liver. Transplant Proc 1987; 19:1329–1330.

135. Rush B, Merritt MV, Kaluzny M, van Schoick T, Brunden MN, Ruwart M. Studies on the mechanism of the protective action of 16,16–dimethyl PGE_2 in carbon tetrachloride induced acute hepatic injury in the rat. Prostaglandins 1986; 32:439–454.

136. Todo S, Yokoi H, Podesta L, ChapChap P, Pan C, Okuda K, Kamiyama Y, Demitris J, Makowka L, Iwatsuki S, Starzl TE. Amelioration of normothermic canine liver ischemia with prostacyclin. Transplant Proc 1988; 20:965–968.

137. Ontell SJ, Makowka L, Mazzaferro V, Trager J, Ove P, Starzl TE. The protective effect of SRI 63–441 on ischemic liver injury using the isolated perfused rat liver:combined protocol with superoxide dismutase. Transplant Proc 1988; 20:972–973.

138. Olthoff KM, Wasef E, Seu P, Imagawa DK, Freischlag JA, Hart J, Busuttil RW. PGE_1 reduces injury in hepatic allografts following preservation. J Surg Res 1991; 50:595–601.

139. Monden M, Fortner JG. Twenty–four– and 48–hour canine liver preservation by simple hypothermia with prostacyclin. Ann Surg 1982; 196:38–42.

140. Toledo–Pereyra LH. Role of prostaglandins (PGI_2) in improving the survival of ischemically damaged liver allografts. Trans Am Soc Artif Intern Organs 1984; 30:390–394.

141. Gaber AO, Thistlethwaite JR,Jr., Busse-Henry S, Aboushloe M, Emond JC, Rouch D, Broelsch CE. Improved results of preservation of hepatic grafts preflushed with albumin and prostaglandins. Transplant Proc 1988; 20:992–993.

142. Abecassis M, Falk RE, Dindzans V, Lopatin W, Makowka L, Levy GA, Falk R. Prostaglandin E_2 prevents fulminant hepatitis and the induction of procoagulant activity in susceptible animals. Hepatology 1987; 7:1104–1106.

143. Greig PD, Woolf GM, Sinclair SB, Abecassis M, Strasberg SM, Taylor BR, Blendis LM, Superina RA, Glynn MFX, Langer B, Levy GA. Treatment of primary liver graft nonfunction with prostaglandin E_1. Transplantation 1989; 48:447–453.

144. Grazi GL, Mazziotti A, Sama C, Stefanini GF, Gozzetti G. Reversal of primary liver graft non-function using prostaglandins. Hepato-Gastroenterol 1991; 38:254–256.

145. Toledo-Pereyra LH, Simmons RL, Najarian JS. Effect of allopurinol on the preservation of ischemic kidneys perfused with plasma or plasma substitutes. Ann Surg 1974; 180:780–782.

146. Parks DA, Bulkley GB, Granger DN. Role of oxygen free radicals in shock, ischemia, and organ preservation. Surgery 1983; 94:428–432.

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

148. Ohhori I, Izumi R, Yabushita K, Watanabe T, Hashimoto T, Takamori M, Hirowawa H, Shimizu K, Konishi K, Miyazaki I. Prevention of liver damage by using free radical scavengers and changes in plasma PG levels during liver ischemia. Transplant Proc 1989; 21:1309–1311.

149. Nordström G, Seeman T, Hasselgren P-O. Beneficial effect of allopurinol in liver ischemia. Surgery 1985; 97:679–683.

150. McCord JM, Fridowich I. Superoxide Dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969; 244:6049–6055.

151. Olson LM, Klintmalm GB, Husberg BS, Nery JR, Whitten CW, Paulsen AW, McClure R. Superoxide dismutase improves organ preservation in liver transplantation. Transplant Proc 1988; 20:961–964.

152. Southard JH, Marsh DC, McAnulty JF, Belzer FO. Oxygen-derived free radical damage in organ preservation: Activity of superoxide dismutase and xanthine oxidase. Surgery 1987; 101:566-570.

153. Southard JH, Marsh DC, McAnulty JF, Belzer FO. The importance of O_2-derived free radical injury to organ preservation and transplantation. Transplant Proc 1987; 19:1380-1381.

154. Freeman BA, Turrens JF, Mirza Z, Crapo JD, Young SL. Modulation of oxidant lung injury by using liposome-entrapped superoxide dismutase and catalase. Fed Proc 1985; 44:2591-2595.

155. Hasuoka H, Sakagami K, Orita K. A New Slow Delivery Type of Superoxide Dismutase Prevents Warm Ischemia Damage in Swine Orthotopic Liver Transplantation. Transplant Proc 1991; 23:693-696.

156. Santiago-Delpin EA, Figueroa I, Lopez R, Vazques J. Protective effect of steroids on liver ischemia. Am Surg 1975; 41:683-695.

157. Goldyne ME. Prostaglandins & other eicosanoids. In: Katzung BG, ed. Basic & clinical pharmacology. Los Altos:Lange medical publications, 1984:217-226.

158. Moran M, Mozes MF, Maddux MS, Veremis S, Bartkus C, Ketel B, Pollak R, Wallemark C, Jonasson O. Prevention of acute graft rejection by the prostaglandin E_1 analogue misoprostol in renal-transplant recipients treated with cyclosporine and prednisone. N Engl J Med 1990; 322:1183-1188.

159. Marubayashi S, Dohi K, Sumimoto K, Sugino K, Ochi K, Kawasaki T. Role of free radicals in ischemic rat liver preservation: prevention of damage by Vitamin E, CoQ_{10}, or reduced gluthatione admininistration. Transplant Proc 1988; 20:974-975.

160. Sumimoto K, Inagaki K, Marubayashi S, Kawasaki T, Dohi K. Energy metabolism in warm ischemically damaged liver grafts after transplantation and protection by coenzyme Q_{10} pretreatment. Transplant Proc 1988; 20:958-960.

161. Stryer L. Oxidative Phosphorylation. In: Stryer L, ed. Biochemistry. New York:Freeman and company, 1981:312-3.

162. Wahlberg JA, Love R, Landegaard L, Southard JH, Belzer FO. 72-hour preservation of the canine pancreas. Transplantation 1987; 43:5-8.

163. Kalayoglu M, Sollinger HW, Stratta RJ, D'Alessandro AM, Hoffmann RM, Pirsch JD, Belzer FO. Extended preservation of the liver for clinical transplantation. Lancet 1988; 1:617–619.

164. Stratta RJ, Wood RP, Langnas AN, Duckworth RM, Markin RS, Marujo W, Grazi GL, Saito S, Dawidson I, Rikkers LF, Pillen TJ, Shaw BW,Jr.. The impact of extended preservation on clinical liver transplantation. Transplantation 1990; 50:438–443.

165. Furukawa H, Todo S, Imventarza O, Wu YM, Scotti C, Day R, Starzl TE. Cold ischemia time vs outcome of human liver transplantation using UW-solution. Transplant Proc 1991; 23:1550–1551.

166. Sanchez-Urdazpal L, Gores G, Ward E, Maus T, Wahlstrom H, Wiesner RH, Krom RAF. Non-anastomotic biliary strictures after orthotopic liver transplantation. 5th Congres European Society for Organ Transplantation 1991; 73 (Abstract).

167. Adam R, Morino M, Diamond T, Astarcioglu I, Johann M, Azoulay D, Bao YM, Bismuth H. Influence of prolonged cold ischaemia using UW solution on graft function and outcome following liver transplantation. 5th Congres European Society for Organ Transplantation 1991; 140 (Abstract).

168. Steininger R, Rauhs R, Bursch W, Tuchy G, Hackl W, Simmel A, Enk M, Muhlbacher F. Effect of free radical scavengers in UW solution on pig liver preservation--a comparison with EC solution. Transplant Proc 1990; 22:506–507.

169. Ferguson DM, Gores GJ, Ludwig J, Krom RAF. UW solution protects against reperfusion injury by inhibiting lipid peroxidation. Transplant Proc 1991; 23:1552–1553.

170. Jamieson NV, Lindell S, Sundberg R, Southard JH, Belzer FO. Evaluation of simplified variants of the UW solution using the isolated perfused rabbit liver. Transplant Proc 1989; 21:1294–1295.

171. Sumimoto R, Jamieson NV, Kamada N. Examination of the role of the impermeants lactobionate and raffinose in a modified UW solution. Transplantation 1990; 50:573–576.

172. Wahlberg JA, Jacobsson J, Tufveson G. Relevance of additive components of university of wisconsin cold-storage solution An experimental study in the rat. Transplantation 1989; 48:400–403.

173. Howden BO, Jablonski P, Thomas AC, Walls K, Biguzas M, Scott DF, Grossman H, Marshall VC. Liver preservation with UW solution. I. Evidence that hydroxyethyl starch is not essential. Transplantation 1990; 49:869–872.

174. Ar'Rajab A, Ahrén B, Sundberg R, Bengmark S. The function of a colloid in liver cold–storage preservation. Transplantation 1991; 52:34–38.

175. Sumimoto R, Jamieson NV, Wake K, Kamada N. 24–hour rat liver preservation using UW solution and some simplified variants. Transplantation 1989; 48:1–5.

176. Moen J, Claesson K, Pienaar H, Lindell S, Ploeg RJ, McAnulty JF, Vreugdenhil PK, Southard JH, Belzer FO. Preservation of dog liver, kidney, and pancreas using the Belzer–UW solution with a high–sodium and low–potassium content. Transplantation 1989; 47:940–945.

177. Sumimoto R, Kamada N, Jamieson NV, Fukuda Y, Dohi K. A comparison of a new solution combining histidine and lactobionate with UW solution and eurocollins for rat liver preservation. Transplantation 1991; 51:589–593.

178. Belzer FO, Hoffmann RM, Stratta RJ, D'Alessandro AM, Pirsch JD, Kalayoglu M, Sollinger HW. Combined cold storage–perfusion preservation of the kidney with a new synthetic perfusate. Transplant Proc 1989; 21:1240–1241.

179. Francavilla A, Brown TH, Fiore R, Cascardo S, Taylor P, Groth CG. Preservation of organs for transplantation: evidence of detrimental effect of rapid cooling. Eur Surg Res 1973; 5:384–388.

180. Pienaar BH, Stapleton GN, Bracher M, Lotz Z, Innes CR, Fourie J, Hickman R. Six–hour porcine liver storage without flushing or perfusion. Transplantation 1991; 52:38–43.

181. Barbier PA, Luder PJ, Wagner HE, Barbier A, Mettler D. Orthotopic liver transplantation in pigs without cold perfusion of the donor liver. Eur Surg Res 1986; 18:293–301.

182. Nedelec JF, Capron–Laudereau M, Adam R, Dimicoli J, Gugenheim J, Patry J, Pin ML, Fredj G, Bismuth H, Lhoste JM. Liver preservation: ^{31}P and ^{13}C NMR spectroscopic assessment of liver energy and metabolism after cold storage in Collins, Marshall, Ringer's lactate and modified UW solutions. Transplant Proc 1989; 21:1327–1329.

183. Kamiike W, Watanabe F, Hashimoto T, Tagawa K, Ikeda Y, Nako K, Kawashima Y. Changes in cellular levels of ATP and its catabolites in ischemic rat liver. J Biochem 1982; 91:1349–1356.

184. Sakurada M, Ohkohchi N, Kato H, Koizumi M, Fujimori K, Satomi S, Sasaki T, Taguchi Y, Mori S. Mitochondrial respiratory function, adenine nucleotides and antioxygenic enzymes in pig liver transplantation. Transplant Proc 1989; 21:1321–1322.

185. Kamiike W, Burdelski M, Steinhoff G, Ringe B, Lauchart W, Pichlmayr R. Adenine nucleotide metabolism and its relation to organ viability in human liver transplantation. Transplantation 1988; 45:138–143.

186. Palombo JD, Hirschberg Y, Pomposelli JJ, Blackburn GL, Zeisel SH, Bistrian BR. Decreased loss of liver nucleotides and energy charge during hypothermic preservation by donor pretreatment with glucose--a preliminary report. Transplant Proc 1989; 21:1299–1300.

187. D'Alessandro AM, Kalayoglu M, Sollinger HW, Hoffmann RM, Reed A, Knechtle SJ, Pirsch JD, Hafez GR, Lorentzen DF, Belzer FO. The Predictive Value of Donor Liver Biopsies on the Development of Primary Nonfunction After Orthotopic Liver Transplantation. Transplant Proc 1991; 23:1536–1537.

188. Oellerich M, Burdelski M, Ringe B, Wittekind Ch, Lamesch P, Lautz HU, Gubernatis G, Beyrau R, Pichlmayr R. Functional state of the donor liver and early outcome of transplantation. Transplant Proc 1991; 23:1575–1578.

189. Currin RT, Toole JG, Thurman RG, Lemasters JJ. Evidence that Carolina rinse solution protects sinusoidal endothelial cells against reperfusion injury after cold ischemic storage of rat liver. Transplantation 1990; 50:1076–1078.

190. Gao W, Takei Y, Marzi I, Lindert KA, Caldwell-Kenkel JC, Currin RT, Tanaka Y, Lemasters JJ, Thurman RG. Carolina rinse solution – a new strategy to increase survival time after orthotopic liver transplantation in the rat. Transplantation 1991; 52:417–424.

191. Currin RT, Thurman RG, Lemasters JJ. Carolina rinse solution protects adenosine triphosphate-depleted hepatocytes against lethal cell injury. Transplant Proc 1991; 23:645–647.

Chapter 4

EXPERIMENTAL DESIGN AND GENERAL RESULTS

4.1 INTRODUCTION

This thesis principally deals with intraoperative hemodynamic and hemostatic changes during liver transplantation. These data are reported in the following chapters. In this chapter the experimental design and the techniques used will be summarized. In addition, the characteristics of the different operative procedures and other general intraoperative measurements will be described, together with morphological changes, survival and mortality.
It is not possible to give a meaningful comparative analysis of late postoperative liver function parameters, as almost all pigs in the long-term preservation experiment died the first or second day after the transplantation. On the other hand, most of the animals from the experiments with short-term (2 hr) preservation survived the first postoperative days. The postoperative biochemical, morphological and scintigraphic results of the 2-hr preservation study are reported elsewhere.[1]

4.2 MATERIALS AND METHODS

4.2.1 Animals and perioperative management

One-hundred-and-thirty-eight female, cross bred Yorkshire cross Danish Landrace pigs were commercially obtained from one farm (HVC, Hedel, The Netherlands). The median body weight was 26 kg (interquartilate range: 22.5-28.5). Donor and recipient were matched according to a negative reaction in the mixed lymphocyte culture test.[2] Body weight of the donor and recipient were mostly similar. If there was a difference in body weight, the heavier was chosen as recipient. One and two days before surgery, the bowel was cleansed by daily, oral administration of 150 ml of lactulose. The animals were fasted for 1 day preoperatively, with free access to water.
Anesthesia was induced with an intramuscular injection of ketamine chloride (35 mg/kg) or thiopentone sodium (2 mg/kg). The animals were intubated and ventilated using a Siemens 900B Servo ventilator, and placed on the operating table in a supine position. Anesthesia was maintained with a mixture of nitrous oxide-oxygen (2:1) and enflurane. The animals were paralysed with pancuroniumbromide and analgesia was added with small doses of fentanylcitrate. End-expiratory carbon dioxide was maintained between 4-5%.

During surgery, fluid support was given with Ringer's lactate, 0.9% NaCl, and Haemaccel®. Metabolic acidosis was corrected by administration of sodium bicarbonate. Depending on the amount of blood loss, 500–1000 ml donor blood was given to the recipient. Usually, this was necessary around 30 min after graft recirculation. Dopaminehydrochloride and epinefrine were given when inotropic support was needed and lidocainehydrochloride for arrhythmias. All animals received a daily intramuscular injection of 2.5 ml of a mixture of procain–penicillin (200,000 E/ml) and dihydro–streptomycin (200 mg/ml), from one day before surgery to four days afterwards. No immunosuppressive drugs were given.

Four separate experiments, in which the duration of liver preservation was varied, were performed. First, 31 transplantations were done after 2 hr of liver preservation. Next, 16 animals received a liver graft after 72 hr of preservation and in a third and fourth experiment, 7 animals were transplanted after 48 hr and 15 after 24 hr of liver preservation. In all four experiments, the animals were randomly assigned to orthotopic liver transplantation (OLT) or reduced size heterotopic auxiliary liver transplantation (HLT). Except for the experiments with 2 hr of preservation, the groups were divided into subgroups with and without the use of Prostaglandin E_1. In Table 4.1 the numbers of animals in the various subgroups are given.

Table 4.1. Numbers of animals in the various subgroups.

	EXPERIMENTAL GROUP:								TOTAL:
	2 hr[a]		24 hr		48 hr		72 hr		
	OLT[b]	HLT	OLT	HLT	OLT	HLT	OLT	HLT	
PGE_1+[c]	0	0	4	4	1	2	1	4	16
PGE_1–	16	15	4	4	1	3	5	5	53
TOTAL	16	15	8	8	2	5	6	9	69

[a] Duration of liver preservation.
[b] OLT, orthotopic liver transplantation; HLT, heterotopic liver transplantation.
[c] PGE_1+, with the use of Prostaglandin E_1; PGE_1–, without.

4.2.2 Surgical technique

4.2.2.1 Donor operation

In the 2–hr experiments, the donors were exsanguinated in one step, and then the liver was excised and *ex vivo* perfused with 4°C Eurocollins solution.
In the 24, 48 and 72–hr experiments a different technique of harvesting was used. First, ± 500 ml of blood– to be used at the recipient operation– was retrieved from a carotid artery canula. Then, this artery was used for arterial pressure monitoring to prevent hypotension, particularly when PGE_1 was given. Next, the liver and its vascular pedicles were isolated. After exsanguinating another 500 ml of blood by cannulation of the mesenteric artery, the liver was perfused *in situ* with one liter of UW solution. This was done retrograde via the mesenteric canula, into an isolated segment of the aorta. This segment had no other outflow than the coeliac axis. Another liter of UW solution was connected to the portal vein at the same time.
During bench surgery the liver graft was placed on melting ice. A cholecystectomy was performed and in case of HLT the lateral and left medial lobes were removed, leaving about 70% of the donor liver to be transplanted. The resection–surface was sutured with a large continuous atraumatic suture. Finally, the graft was preserved by simple hypothermic (4°C) storage in ± 100 ml of retained UW solution.

4.2.2.2 Transplantation

An orthotopic or an heterotopic liver transplantation was performed, as to which the animal was assigned. The required venous and arterial catheters were introduced in the neck and after midline laparotomy, a truncal vagotomy and pyloroplasty was performed to avoid gastric ulcer development and a cystostomy–catheter was placed.
Before implantation of the graft, it was rinsed via the portal vein with two liters of cold (4°C) 0.9 % saline.

Orthotopic Liver Transplantation
With OLT, the hepatic arteries and the common bile duct were dissected but not cut until hepatectomy. The rest of the hepatogastric ligament was divided. Also, the triangular ligaments were divided and the portal and infrahepatic inferior caval vein were cleared. Immediately before recipient hepatectomy, a venovenous heparin–coated,

iliac and portal vein to the right external jugular vein. For a safe introduction of the portal limb of the venovenous shunt, a cuff was anastomosed end-to-side to the recipient portal vein. This cuff was made from a separate small segment of the donor vena cava. Next, recipient hepatectomy was performed, and the graft was placed in an orthotopic position.

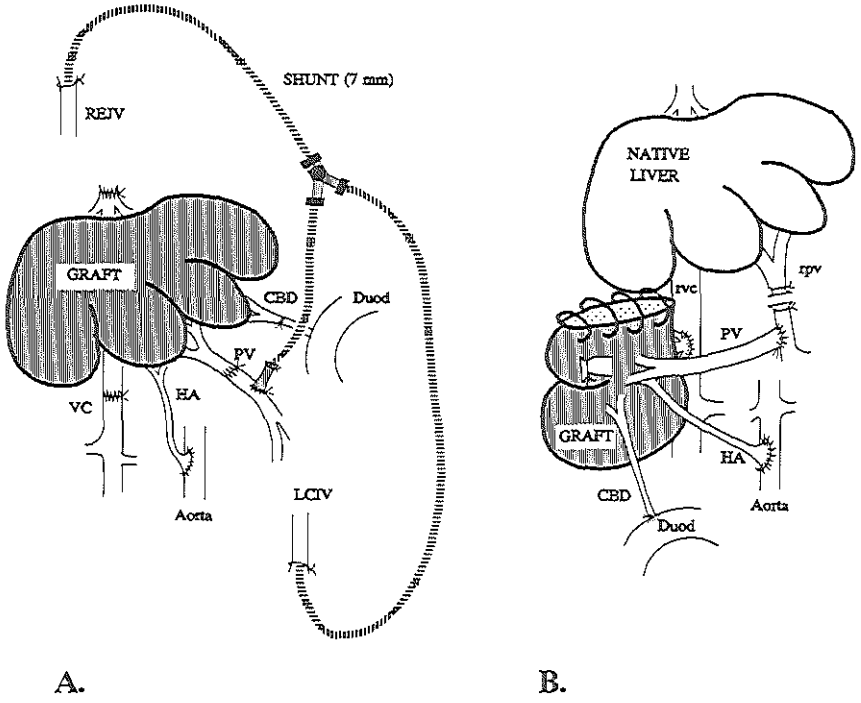


Figure 4.1. Schematic representation of the surgical techniques. A. Orthotopic liver transplantation; B. Reduced-size heterotopic auxiliary liver transplantation. (CBD) common bile duct; (Duod) duodenum; (HA) hepatic artery; (LCIV) left common iliac vein; (PV) portal vein; (REJV) right external jugular vein; (rpv) recipient portal vein; (rvc) recipient vena cava; (VC) vena cava.

The suprahepatic inferior vena cava was anastomosed but not unclamped and the portal vein anastomosis was completed. Next, the portal venous clamp was released, allowing blood to flush the graft through the infrahepatic vena cava. Only after the first 30 ml of perfusate were drawn from the infrahepatic vena cava of the graft, it was occluded with a vascular clamp. Then, the suprahepatic venous clamp was removed, allowing systemic recirculation of the graft with portal blood flow. After removal of the portal limb of the shunt, the infrahepatic vena cava was anastomosed. Then, the caval and jugular limb of the shunt were removed. The donor common hepatic artery with an aorta patch was anastomosed end–to–side to the recipient aorta, just cephalad to the superior mesenteric artery. A choledocho–choledochostomy completed the procedure (Figure 4.1A).

Heterotopic Liver Transplantation

In case of HLT, the portal and infrahepatic inferior caval vein were cleared. No venovenous bypass was used. The graft was transferred into the recipient's right subhepatic space and the suprahepatic vena cava of the graft was anastomosed to the recipient infrahepatic vena cava, proximal to the renal veins. The portal vein was anastomosed end–to–side to the host portal vein. Again, on reperfusion the first 30 ml of perfusate were drawn from the infrahepatic vena cava of the graft, before the suprahepatic venous clamp was removed. The infrahepatic part of the donor vena cava was ligated. The recipient portal vein was also ligated and divided close to the liver hilum. The common hepatic artery with an aorta patch was anastomosed end–to–side to the infrarenal aorta. The graft biliary system was connected by a choledochoduodenostomy (Figure 4.1B).

4.2.2.3 Prostaglandin

When PGE_1 was given, this was started 60 min before donor hepatectomy by an intravenous infusion rate of 100 ng/kg/min.[5] In addition, 1 mg/L PGE_1 was added to the UW solution.[6] The recipient animal was treated with a similar infusion as the donor, starting about 60 min before the recirculation of the graft. Also, 1 mg/L PGE_1 was added to the cold 0.9 % saline used to rinse the graft before implantation. PGE_1 (Prostin®) was supplied by Upjohn, The Netherlands. In three experiments, at regular intervals during cold storage, PGE_1 levels were measured in the UW solution, using specific PGE_1 antisera. This was done to verify lasting presence of PGE_1 in the preservation solution after long–term cold storage of the graft.

4.2.3 Measurements

The duration of several periods during the transplantation was recorded. The *first warm ischemic period* was defined as the time between the beginning of the exsanguination of the donor animal and the beginning of the *in situ* graft perfusion. The *cold ischemic period* was defined as the time between the end of the first warm ischemic period and the moment the graft was taken out of the refrigerator, to be transplanted. This period included the bench surgery in HLT. The *second warm ischemic period* was defined as the time between the end of the cold ischemic period and the recirculation of the graft. The *portal vein interruption time* was defined as the time between clamping of the portal vein and recirculation of the graft. In OLT, this is equal to the duration of the anhepatic phase. The *preparation time* was defined as the time needed for the introduction of the various catheters, the vagotomy and pyloroplasty and the dissection of the recipient vessels and the native liver in OLT. The preparation time ended with a short break; thereafter, the actual transplantation started. The time between the break and the closure of the abdomen was the *total transplantation time*. For OLT, this period included the time needed for the shunting procedure.

Intraoperative hemodynamic changes were monitored using an intracarotid arterial line, a jugular vein catheter, and a heparin–coated flow–directed thermodilution pulmonary artery catheter (American Edwards Laboratories, Puerto Rico, USA).

Infusions were recorded as blood–suppletion (citrated whole blood from the donor), plasma–suppletion (fresh frozen plasma and Haemaccel®) and electrolyte–suppletion (0.9% NaCl, Ringer's lactate and sodium bicarbonate). Blood loss was estimated from the amount of liquid sucked away from the surgical field and collected in Buleaux–bottles during the transplantation time.

Because certain steps in HLT and OLT take different amounts of time, the moments of measurement were synchronized about the minute of recirculation. Most measurements were performed around every important operative step (Table 4.2). In OLT, R–5 is a measurement in the anhepatic phase. In HLT, there is no anhepatic phase, but during the portal vein anastomosis there is a period of partial clamping of the portal vein. In the pig, this often implies (almost) complete clamping of this vessel. Measurements of hemoglobin, platelets, potassium (K^+), and Aspartate Aminotransferase (ASAT) were also performed at the intervals of Table 4.2.

In addition, three K^+ and ASAT samples were drawn from the rinsing solution that had passed the graft: at the beginning (R0), halfway through (after 1 liter: R1) and at the

Table 4.2. Moments of intraoperative measurements, relative to the minute of reperfusion.

R–60	Starting value: ± 1 hr before recirculation.
R–30	In HLT 5 min after the partial clamping of the subhepatic inferior. vena cava and in OLT 5 min after opening the venovenous shunt.
R–5	Anhepatic phase: ± 5 min before recirculation.
R+5	± 5 min after recirculation.
R+30	± 30 min after recirculation (during the construction of the arterial anastomosis).
R+60	± 60 after recirculation (5 min after the aorta anastomosis).
R+90	± 90 min after recirculation (closure).

end (after 2 liters: R2) of the rinsing period. On reperfusion, K^+ and ASAT samples were also taken from the first blood emerging out of the infrahepatic vena cava (FR). Finally, besides the hemodynamic assessment which will be described later, direct measurements of the venous pressure in the infrahepatic vena cava and in the portal vein were performed, before and after transplantation (except in the 2–hr experiments).

4.2.4 Morphology

Tissue sampling

Wedge tissue specimens of the liver graft were taken immediately after hepatectomy (B0), before reperfusion (B24, B48 or B72), 5 min after reperfusion (BR), and at postmortem (B†). The biopsy specimens were divided into three parts: one part was fixed in 10% buffered formalin and two parts were snap–frozen in liquid nitrogen, and stored at –70°C.

The biopsies were examined by a pathologist who had no information with respect to preservation time and use of PGE_1.

Light microscopy

Sections of the formalin–fixed, paraffin–embedded tissue were stained with hematoxylin and eosin for light microscopic examination. The condition of hepatocytes was assessed by recording loss of cohesion, cellular rounding, eosinophilic

degeneration, intracytoplasmatic vacuoles, and nuclear changes. Sinusoidal lining cells were also judged on loss of cohesion.

Electron microscopy

One of the frozen parts was prepared for electron microscopic studies by fixation of ± 1 mm^3 in glutaraldehyde.

Enzyme histochemistry

Cryostat sections (8 μm thick) were cut from the frozen tissue blocks at a cabinet temperature of −25°C. In preliminary experiments three histochemical techniques were used to judge the condition of the liver tissue. These were 5'−nucleotidase, alkaline phosphatase, and lactate dehydrogenase (LDH). 5'−Nucleotidase activity was demonstrated by the metal salt method of Wachstein and Meisel,[7] as modified by Frederiks and Marx.[8] The activity of alkaline phosphatase was demonstrated according to the method of van Noorden et al.[9] and the activity of LDH according to Frederiks et al.[10]

After interpreting the obtained results, specimens of the 24−hr preservation experiments (with and without the use of PGE_1) were analyzed for the localization and activity of alkaline phosphatase.

4.2.5 Statistics

Data were subjected to computerized statistical analysis (PATFILE statistical package). Continuous variables are given as median (mean, ± standard error of the mean) and analyzed by nonparametric tests: in case of independent samples, the Mann−Whitney test was used, in case of paired samples, the Wilcoxon rank−sum test, and in case of correlation analysis, the Spearman rank−correlation test was used. The hemodynamic, hemostatic and some of the general data were also analyzed in a multiple regression model (SPSS/PC+) using the three main variables in this study: the type of transplantation, the use of PGE_1 and the preservation time. The relative influence of these variables is represented by the T−value (ratio between B and Standard Error of B) together with the significance of T. Survival was assessed by life−table analysis as described by Kaplan and Meier[11] and survival times were compared using the log−rank test.

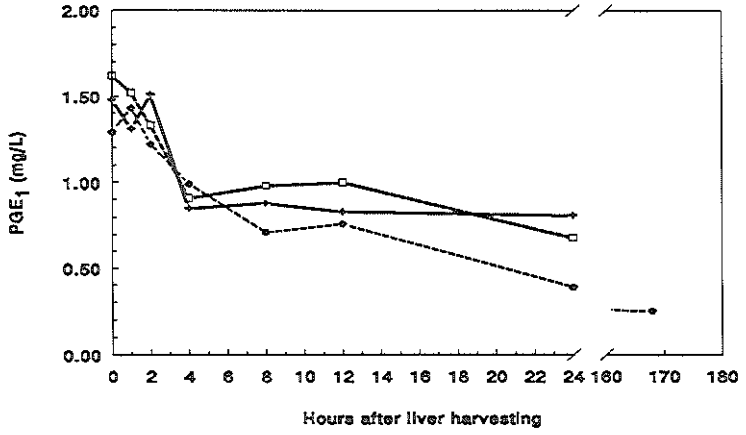


Figure 4.2. Prostaglandin E_1 (PGE_1) in UW solution during liver preservation (N=3).

4.3 RESULTS

4.3.1 General data

The PGE_1 levels in the preservation solution are depicted in Figure 4.2. A substantial amount of PGE_1 was still present after 24 hours. Even after seven days, PGE_1 could still be detected in the UW solution (only one measurement).

In Table 4.3 the durations of the various stages of the transplantation are given. The overall preparation–time was 120 (122, 2.7) min in OLT and 90 (96, 4.1) min in HLT (P<0.001). The overall total transplantation time was 173 (173, 6.4) min in OLT and 136 (136, 4.7) min in HLT (P<0.001). The overall portal vein interruption time was 53 (54, 1.8) min in OLT and 15 (16, 0.6) min in HLT (P<0.001).

The amount of fluid loss and suppletion in the different groups is given in Table 4.4. In the multiple regression analysis blood loss was significantly controlled by the preservation time (T=2.88, P<0.01) and the type of transplantation (T=–2.33, P=0.02). Thus, longer preservation times and OLT were connected with more blood loss.

Table 4.3. Median duration of the various periods of the transplantation.

DURATION OF:	EXPERIMENTAL GROUP:							
	2 hr[a]		24 hr		48 hr		72 hr	
	OLT[b]	HLT	OLT	HLT	OLT	HLT	OLT	HLT
1st warm ischemic period (min)								
–median	8	8[c]	4	4	5	5	4	6
–lowest	6	5	2	3	3	3	3	2
–highest	15	10	5	6	7	10	6	8
cold ischemic period (hr:min)								
–median	2:42	2:03[d]	25:39	24:28[d]	49:12	48:19[e]	74:02	72:28[d]
–lowest	1:52	1:15	24:46	23:42	48:28	48:10	73:24	71:18
–highest	4:20	3:12	25:53	24:59	49:55	49:55	75:08	73:19
2nd warm ischemic period (min)								
–median	52	53	58	61	57	58	57	55
–lowest	38	29	46	47	57	37	43	43
–highest	66	62	66	74	57	68	60	61
preparation (min)								
–median	120	110[d]	115	80[d]	110	75[e]	133	87[d]
–lowest	105	90	90	70	105	60	110	70
–highest	155	190	150	100	115	100	150	145
total transplantation (min)								
–median	165	128[d]	195	131[d]	170[†]	154	[‡]	148
–lowest	130	90	135	115		147		136
–highest	230	210	215	175		160		155
portal vein interruption (min)								
–median	48[f]	15[d]	65	19[d]	63	18[e]	56	15[d]
–lowest	29	11	48	12	62	10	46	11
–highest	62	18	73	27	63	22	68	19

[a] Duration of liver preservation.
[b] OLT, orthotopic liver transplantation; HLT, heterotopic liver transplantation.
[c] Significantly longer compared to 24, 48 and 72 hr ($P<0.001$); for OLT as well as HLT.
[d] Significantly different from OLT ($P<0.001$).
[e] Difference not significant because of small numbers in 48 hr group (see Table 1).
[f] Significantly shorter compared to OLT in 24, 48 and 72 hr ($P<0.001$).

[†] Only one animal survived the operation.
[‡] All animals died before closure.

Table 4.4. Median total amounts of fluid loss and suppletion during the transplantation (from induction of anesthesia to closure).

FLUID:	EXPERIMENTAL GROUP:							
	2 hr[a]		24 hr		48 hr		72 hr	
	OLT[b]	HLT	OLT	HLT	OLT[†]	HLT	OLT[‡]	HLT
Blood loss: (ml)								
–median	400	500[c]	850	425[d]	160	1125	1100	925[d]
–lowest	250	250	400	100		700	600	200
–highest	1800	1200	2200	700		1500	2000	1500
Urinary output (ml)								
–median	550	400	680	195	800	410	200	100
–lowest	170	140	300	100		215	100	25
–highest	1500	990	1400	900		1350	800	490
TOTAL OUT:	950	900	1530	620	960	1565	1300	1025
Blood suppletion (ml)								
–median	650	600	1000	600[d]	750	1100	600	1000[d]
–lowest	450	400	200	500		900	450	375
–highest	2000	1100	1200	1200		1250	1000	1500
Plasma expanders (ml)								
–median	3000	2000	2525	1900[d]	2900	2900	2900	2500[d]
–lowest	1500	1500	2000	800		2125	1900	500
–highest	5100	4000	4100	2600		3000	5000	4600
Electrolyte solutions (ml)								
–median	2675	2425	2300	2750	1500	2000	4000	3000
–lowest	1500	1500	1000	1500		1500	2000	1000
–highest	4000	5000	5850	4000		4000	5000	4000
TOTAL IN:	6325	5025	5825	5250	3750	6000	7500	6500

[a] Duration of liver preservation.
[b] OLT, orthotopic liver transplantation; HLT, heterotopic liver transplantation.
[c] The blood loss in the 2 hr experiment is lower compared to the longer preservation groups (P=0.03).
[d] Significantly different from OLT.

[†] Only one animal represented.
[‡] All animals died before closure.

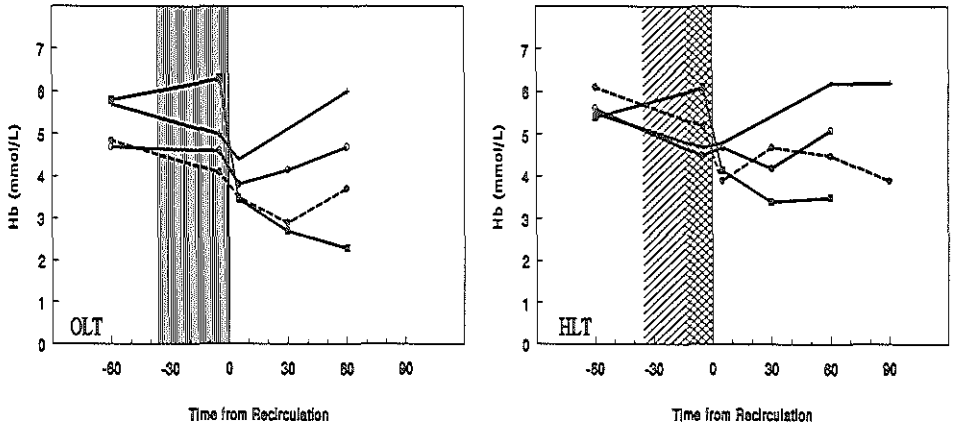


Figure 4.3. Hemoglobin (Hb; in mmol/L) during liver transplantation. Values are stated as medians. The horizontal axis gives the time in minutes from or to the moment of recirculation of the graft. Symbols: (**OLT**) *Orthotopic Liver Transplantation;* (**HLT**) *Heterotopic Liver Transplantation. Preservation time:* (+——+) *2 Hr;* (o——o) *24 Hr;* (●----●) *48 Hr (line is interrupted because this group consists of only seven animals);* (■——■) *72 Hr. Shaded areas:* (▥) *is the anhepatic period in OLT; in HLT,* (▨) *is the period in which the subhepatic inferior vena cava is partially clamped and* (▩) *in which also the portal vein is (partially) clamped.*

Diuresis was higher in OLT as measured by the multiple regression analysis (T=−3.3, P=0.001). This was still true when urinary output per hour was calculated: for all animals the median diuresis was 98 (120, 15) ml/hr in OLT and 52 (79, 11) ml/hr in HLT (P<0.01). Preservation time and PGE_1 had no effect on the urine production.

With regard to the fluid suppletion, blood suppletion was higher in OLT compared to HLT in the 24-hr experiment (P<0.001). The multiple regression analysis revealed significantly more infusion of plasma expanders in OLT (T=−3.31, P=0.001). However, when the total duration of the procedure (from induction of anesthesia to closure) was added to the multiple regression model, the latter appeared to claim most of the previous T-value (T=2.63, P=0.01). This means the difference in administration of plasma expanders between OLT and HLT, can be declared by the longer total

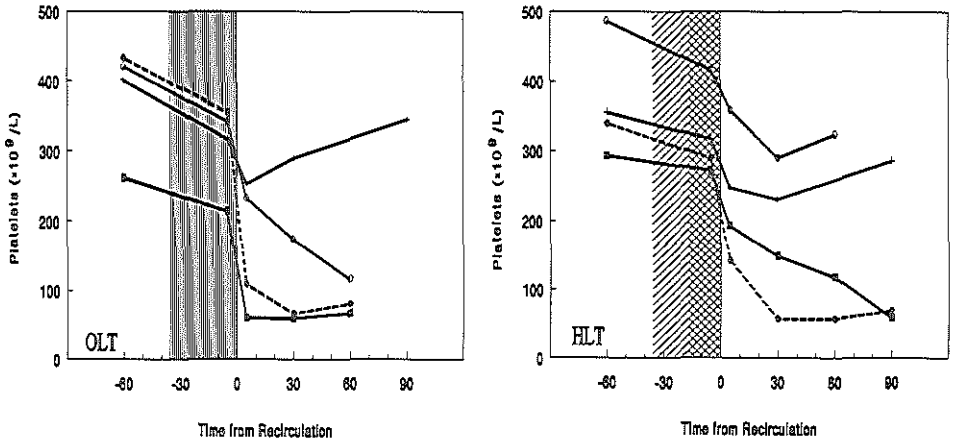


Figure 4.4. Platelets during liver transplantation. Values are stated as medians. Horizontal axis and symbols as in Figure 4.3.

duration of OLT, only. PGE_1 administration was connected with significantly more suppletion of electrolyte solutions, even when the operation time was included (T=2.22, P=0.03).

The median pressure gradient between the portal vein and inferior vena cava was higher after transplantation compared with the starting value. Before transplantation, the median portal–caval pressure gradient was 2 mm Hg (1.8, 0.2) and after 5 mm Hg (5.7, 0.7) (P<0.01). In the multiple regression analysis, no significant relation could be demonstrated between portal–caval pressure gradient and the type of transplantation, the use of PGE_1, or the duration of the preservation.

4.3.2 Hematological and biochemical data

The changes in hemoglobin and platelets are depicted in Figure 4.3 and 4.4. Reperfusion causes the hemoglobin levels and platelet counts to fall instantly to median values of around 70–80% of those before reperfusion (P<0.001). In the multiple regression analysis the decline in hemoglobin was significantly larger with longer duration of preservation (T=6.67, P<0.001), in OLT (T=–2.89, P<0.01) and when PGE_1

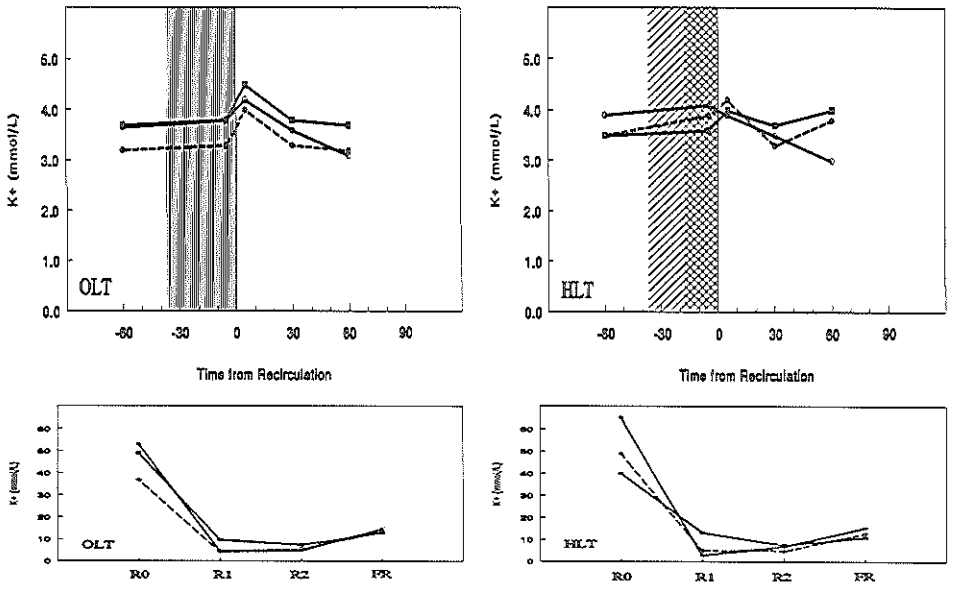


Figure 4.5. Potassium (K^+) during liver transplantation. In the upper graphs arterial blood samples are depicted. Values are stated as medians. Horizontal axis and symbols as in Figure 4.3. In the lower graphs values are given as measured in the rinsing solution after passing the graft: (R0) *at the beginning;* (R1) *halfway through; and* (R2) *at the end of the rinsing period;* (FR) *first reperfusion blood.*

was used (T=2.66, P=0.01). The decrease in platelets counts was only significantly related to the preservation time (T=4.29, P<0.001).

Five and 30 min after reperfusion, hemoglobin levels were higher in HLT (T=2.68, P<0.01 and T=1.91, P=0.07). The preservation time, however, had a far more evident effect on both hemoglobin and platelets (all T–values below –3.0 and P<0.005).

As measures of cellular damage, the changes in K^+ and ASAT levels are shown in Figure 4.5 and 4.6. In these graphs, the values in the rinsing solution washout are also given. At reperfusion both K^+ and ASAT levels quickly increased. In the multiple regression analysis, the increase of K^+ was greater in OLT (T=–2.39, P=0.02) and for both K^+ (T=2.46, P=0.02) and ASAT (T=2.78, P<0.01) the increase was significantly greater with longer preservation times. ASAT levels in the washout solution in the 72–hr experiment were higher in HLT compared to OLT in the R1, R2, and FR samples (P<0.05).

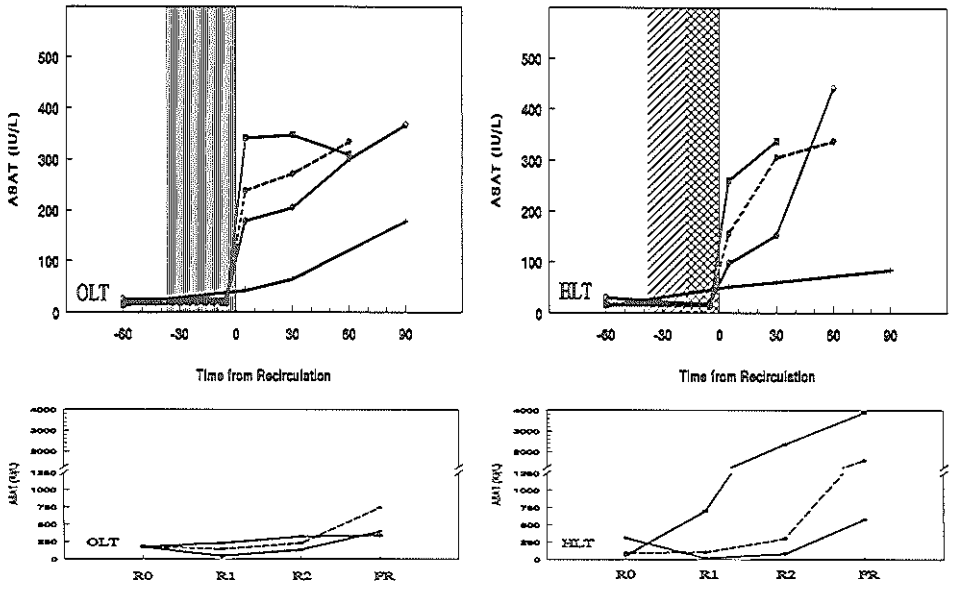


Figure 4.6. Aspartate Aminotransferase (ASAT) during liver transplantation. In the upper graphs arterial blood samples are depicted. Values are stated as medians. Horizontal axis and symbols as in Figure 4.3. In the lower graphs values are given as in Figure 4.5.

4.3.3 Morphology

Light microscopy

In Table 4.5 the characteristics of the hepatocytes and the sinusoidal lining cells are depicted.

At donor hepatectomy, vacuoles were present in some biopsies. Continuity of endothelial cell lining and normal polygonal outline of hepatocytes was well maintained (Figure 4.7A and Figure 4.8A).

Before reperfusion, after 24 hr of cold ischemia, the majority of biopsies showed normal trabecular arrangement of hepatocytes (Figure 4.7B), although some loss of cohesion of the hepatocytes was frequently recognized. At 72 hr, all but one specimen showed partial or mild loss of cohesion (Figure 4.8B). During cold ischemic storage,

Table 4.5. Light microscopic examination of the hepatocytes and sinusoidal lining cells.

			Hepatocytes								Sinusoidal lining cells		
Time:	N[a]	S.N.A[b]	Membrane Changes[c]		Eosinophilic Degeneration		Vacuoles		Nucleus dis-integration		Loss of Cohesion		Cells not Recognizable[d]
			+[e]	++	+	++	+	++	+	++	+	++	
24 hr preservation:													
B0[f]	14	2	1	0	1	0	3	1	0	0	0	0	0
B24	16		6	1	8	0	3	1	3	0	11	2	0
BR	16		9	4	13	3	6	4	9	1	10	4	1
B†	15	1	2	13	5	10	3	9	9	5	1	1	13
48 hr preservation:													
B0	7		0	0	0	0	1	0	0	0	0	0	0
B48	5	2	3	0	4	0	4	0	1	0	4	1	0
BR	7		6	1	7	0	6	1	6	0	4	3	0
B†	6	1	1	5	2	4	3	1	5	1	0	1	5
72 hr preservation:													
B0	13	2	0	0	0	0	5	0	0	0	0	0	2
B72	15		13	1	7	5	9	3	3	0	4	11	0
BR	15		7	8	9	6	10	3	10	1	3	10	2
B†	9	6	0	9	2	7	3	3	3	6	0	1	8

[a] Number of available biopsies.
[b] S.N.A.: Specimen Not Available.
[c] Membrane changes of hepatocytes: loss of cohesion and cellular rounding.
[d] Sinusoidal lining cells absent or not recognizable.
[e] Damage: +, partial or mild; ++, severe.
[f] Biopsy: B0, immediately after hepatectomy; B24/B48/B72, before reperfusion; BR, 5 min after reperfusion; B†, at post-mortem.

degenerative changes increased with time. After 24-hr storage, only a mild degree was recognized in half of the biopsies. After 72-hr preservation, most specimens showed mild or severe eosinophilic degeneration. Intracytoplasmatic vacuoles were recognized in most of the 72-hr preserved liver before reperfusion.

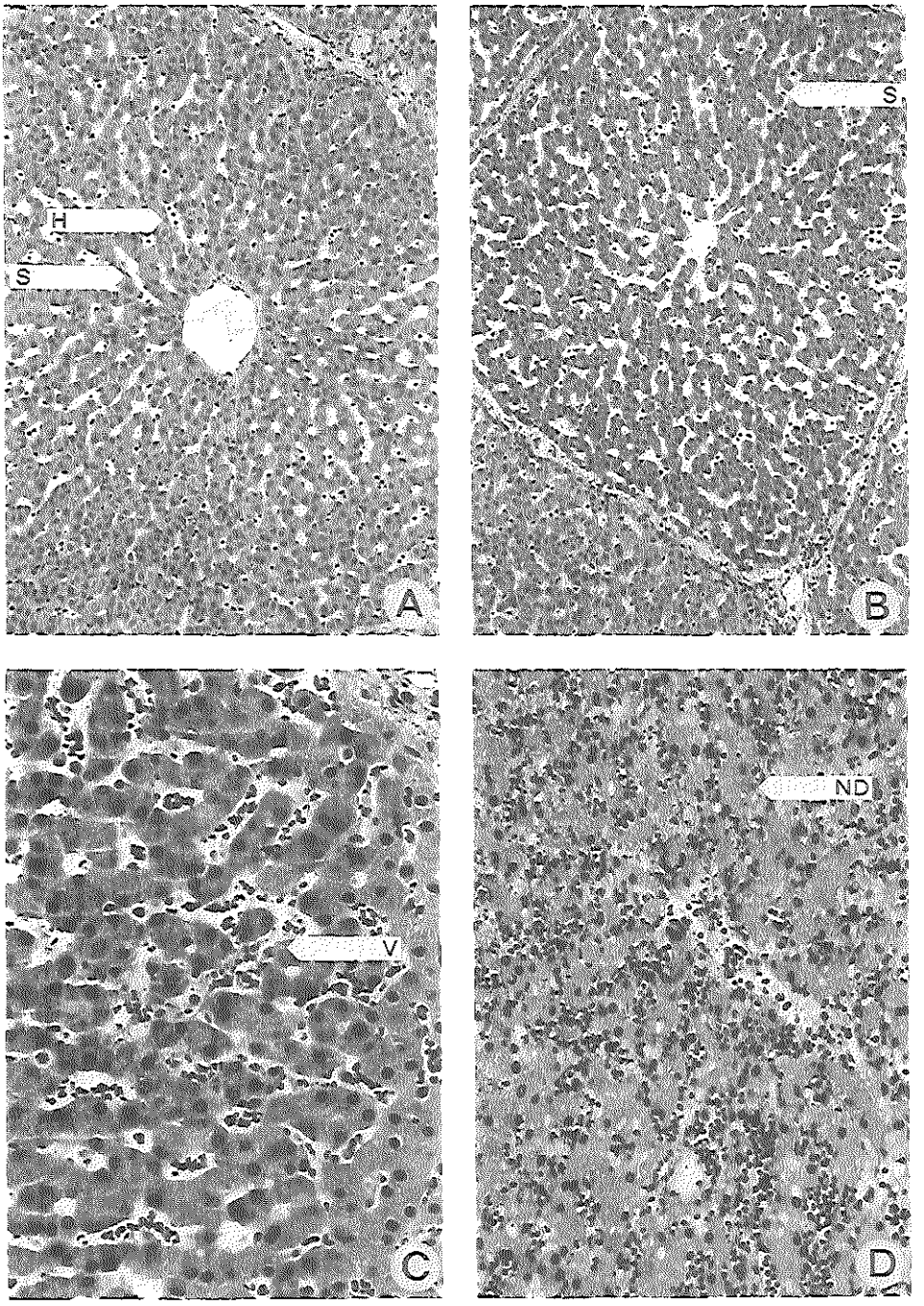
Legends to the figures on page 106 and page 107:

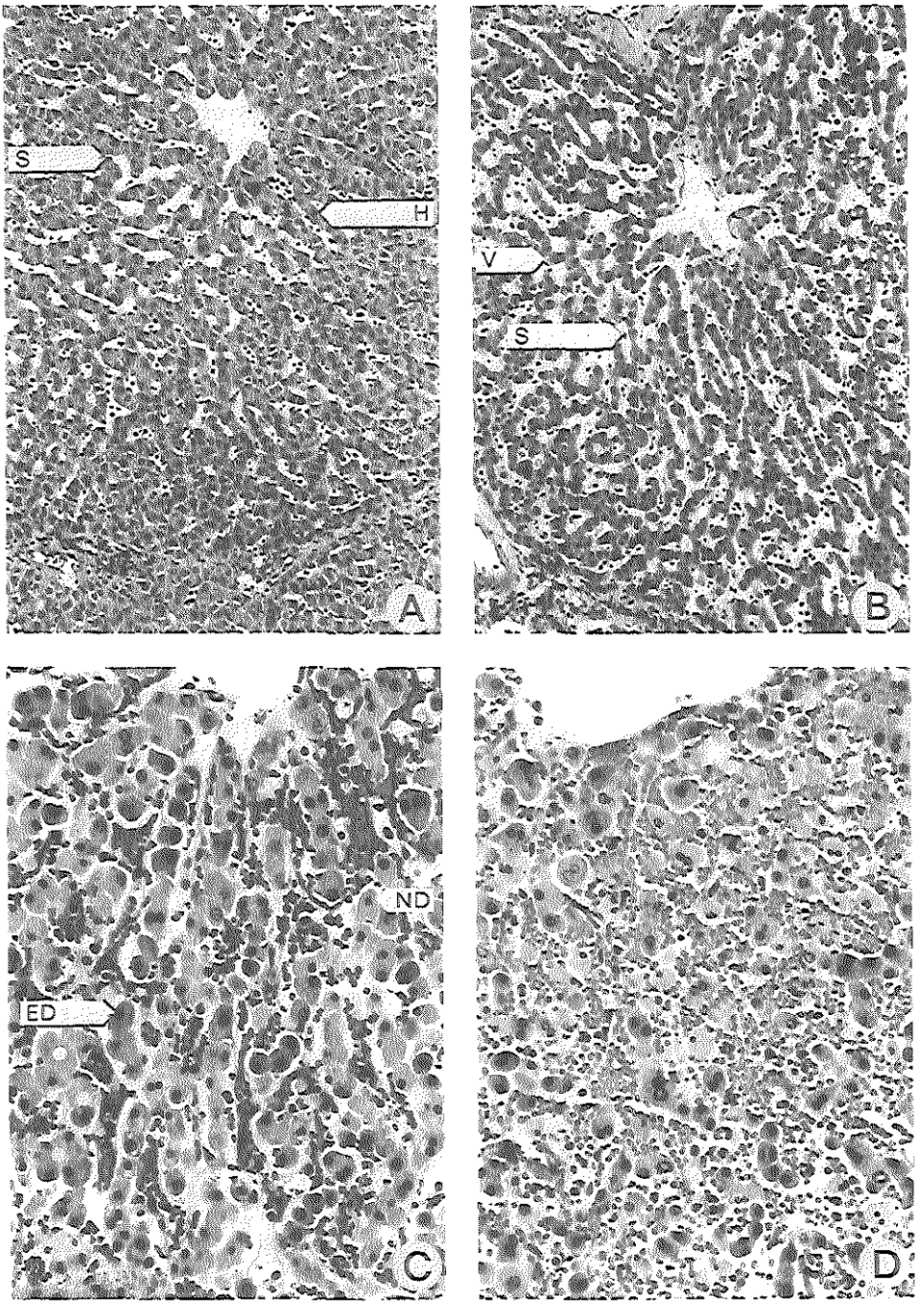
(A) Donor hepatectomy; (B) after 24-hr simple cold storage; (C) immediately after reperfusion; (D) at postmortem. (Hematoxylin-eosin stain; Magnification: 140x) Symbols at arrows: (H) Hepatocyte; (S) Sinusoidal lining cell; (ED) Eosinophylic degeneration; (V) Vacuole; (ND) Nuclear Disintergration.

Figure 4.7. (page 106) Light micrograph of a 24-hr preserved liver.

Figure 4.8. (page 107) Light micrograph of a 72-hr preserved liver.

Nuclear degenerative changes were usually not recognized during the ischemic period: most of the biopsies had intact nuclei, even after 72-hr cold ischemic storage.
Partial or mild loss of cohesion of sinusoidal lining cells was recognized in most of the 24-hr preserved grafts. After 72-hr preservation, the majority of specimens showed severe loss of cohesion.
After reperfusion, there was an immediate, further decrease of hepatocyte cohesion in the 72-hr preserved livers, and to a lesser degree in the 24-hr preserved livers (Figure 4.7C and Figure 4.8C). Eosinophilic degeneration also developed rapidly in the 24-hr preserved livers, although the extent was usually mild. As with eosinophilic degeneration, most of the vacuoles appeared at reperfusion in the 24-hr preserved livers, while already recognized in most of the 72-hr preserved liver before reperfusion. The majority of biopsies showed mild nuclear degenerative shrinkage, unrelated to the antecedent duration of cold ischemia.
In most specimens, reperfusion did not add to the extent of loss of cohesion of sinusoidal lining cells.
At postmortem, virtually all available biopsies showed severe loss of cohesion, with disintegration of the liver cell plates (Figure 4.7D and Figure 4.8D). Severe nuclear changes were frequently found. In most biopsies the sinusoidal lining cells could no longer be recognized.
PGE_1 did not correlate to any of the mentioned morphologic parameters (data not shown).


H
S
A
S
B
V
C
ND
D


S
H
A
V
S
B
ND
S
ED
C
D

Electron microscopy

Already after 24–hr cold storage, remarkable osmotic swelling of the hepatocytes and rupture of the cell membranes were found. Mitochondria showed beginning flocculent densities, as a sign of irreversible changes. Also, discontinuity of the endothelial cell lining on hepatocytes were seen. After reperfusion, flocculent densities in mitochondria increased and hepatocytes showed further cell swelling and rupture of the cell membrane. Endothelial cells were detached from the hepatocytes, and Disse's space could hardly be recognized.

Enzyme histochemistry

The preliminary experiments showed that 5'–nucleotidase activity was not localized in the plasma membrane of hepatocytes in pig liver. Activity was only found in sinusoidal endothelial cells. Therefore, the localization of this enzyme at the light microscopic level could not be used as a sensitive parameter for ischemic damage, as was proposed for the rat liver.[8] In accordance with the findings in the rat liver, alkaline phosphatase was found in the bile canalicular membranes and LDH was found in the cytoplasm of the porcine hepatocytes. Ischemia per se did not affect the localization or activity of LDH. After reperfusion, however, a decreased activity of LDH was found in certain areas.

Regarding the alkaline phosphatase activity and localization, a clear correlation was found between the condition of the liver before and after reperfusion, although in most cases a deterioration occurred as a consequence of reperfusion. Livers were qualified as good, moderate or bad according to alkaline phosphatase activity and localization. No significant effect of adding PGE_1 to the UW solution was found.

4.3.4 Mortality and survival

In Figure 4.9 the cumulative survival curves of the first 24 hr after reperfusion are given. In the multiple regression analysis, the duration of preservation was a significant determinant of the survival ($T=-3.59$, $P<0.001$). In the 24–hr experiments, survival was significantly longer after HLT compared to OLT ($P<0.05$). Survival in the extended preservation experiments was dismal: all animals died the day of the operation after an OLT or after 72–hr graft preservation. After HLT in the 24–hr preservation group,

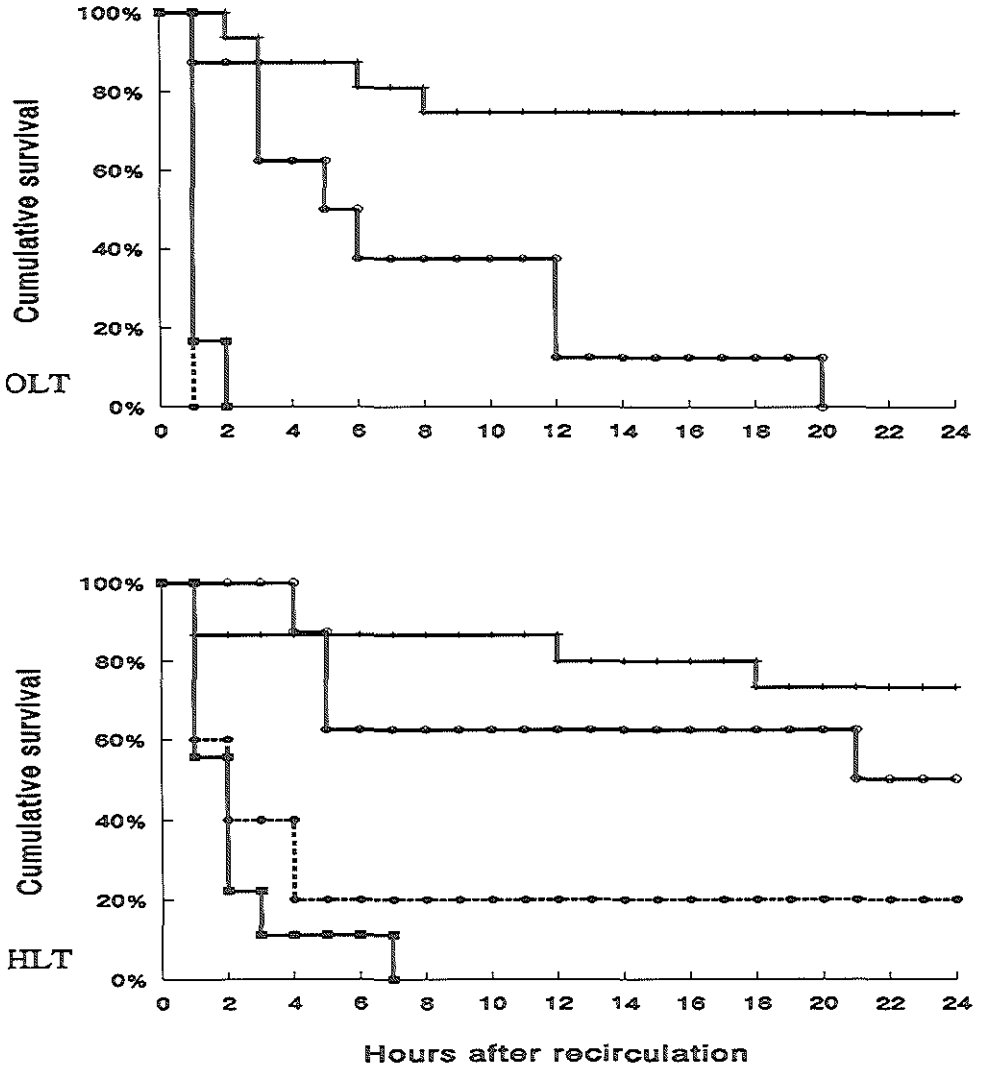


Figure 4.9. Cumulative survival in the first 24 hr after liver transplantation. The horizontal axis gives the time in hours from the moment of recirculation of the graft. Preservation time: (+——+) 2 Hr; (○——○) 24 Hr; (●----●) 48 Hr (line is interrupted because this group consists of only seven animals); (■——■) 72 Hr.

Table 4.6. Causes of death after liver transplantation.

CAUSE:	EXPERIMENTAL GROUP:								TOTAL:
	2 hr[a]		24 hr		48 hr		72 hr		
	OLT[b]	HLT	OLT	HLT	OLT	HLT	OLT	HLT	
Number:	**16**	**15**	**8**	**8**	**2**	**5**	**6**	**9**	**69**
Hemorrhage	3	1	4	2	2	5	3	6	26
Portal vein block[c]						3	1		4
Heart failure			1	1				1	3
Cholangitis[d]	1			1					2
Volvulus	3	2		1					6
Rejection[e]	2								2
Technical[f]		4						1	5
Sacrificed	3	5							8
Others[g]	2	2		1					5
Unknown	2	1	3	2					8

[a] Duration of liver preservation.
[b] OLT, orthotopic liver transplantation; HLT, heterotopic liver transplantation.
[c] Total intrahepatic thrombosis of the portal venous system.
[d] In combination with obstruction of the biliary tract.
[e] Only if rejection was thought to be the direct cause of death.
[f] Technical errors during surgery or investigations; pulmonary embolism at angiography (N=3).
[g] Pulmonary sepsis (N=3); Pneumothorax (N=1); Peritonitis (N=1).

3 animals survived more than 3 weeks. At postmortem, these grafts were necrotic and life was sustained by the native liver. Two more animals survived after HLT for two days, one in 24-hr and one in the 48-hr preservation group. In Table 4.6 the causes of mortality are categorized.

4.4 DISCUSSION

The starting values of the PGE_1 levels in the UW solution at procurement varied around 1.5 mg/L, whereas 1 mg/L was planned. We are not aware of any methodological error responsible for this deviation. If it was a problem of the PGE_1–analysis used, a correction factor of 0.7 would still calculate a concentration of about 0.5 mg/L at 24 hours. It is therefore evident that PGE_1 is not broken down as rapidly in cold UW solution, as it is in the circulation. Within the blood the half–live of most prostaglandins is less than 1 minute.[12]

In the 2–hr experiment, the donor liver was perfused *ex vivo*, as opposed to the *in situ* flushing in the other groups. This resulted in a somewhat longer first warm ischemia in the 2–hr experiments. Furthermore, by our definition, the first warm ischemic period started at the beginning of the exsanguination. However, ischemia during exsanguination is only present in severe hypotension, which never occurred in the procurement of the 24, 48 and 72–hr experiments. Therefore, with the *in situ* perfusion technique, the duration of warm ischemia was almost negligible.

Due to the longer preparation and transplantation time, the cold ischemic period was longer in OLT. In the statistical analysis, this was taken into account by using the actual duration of the cold ischemic period instead of the hours of the preservation group.

In the 2nd warm ischemic period, the liver was first rinsed with 4°C 0.9% saline. Then it was placed in the abdomen, where it was subject to surface warming during the construction of the anastomoses (about 45 min). Naturally, this caused the parenchymal temperature of the graft to rise, but this is not true *warm* ischemia.

In OLT, the anhepatic phase (portal vein interruption period) was significantly shorter in the 2–hr group compared to the other experiments. As the anhepatic period included the time needed to rinse the graft, a shorter rinsing period in 2–hr experiment compared to the other groups could be responsible for this. For instance, a higher hepatic vascular resistance – due to storage damage–[13] in the extended preservation groups could extend the duration of rinsing. On the other hand, it could also be explained by the lower viscosity of Eurocollins, used in the 2–hr experiment, making it easier to be rinsed from the graft.

Blood loss was higher in OLT compared to HLT. This is in agreement with our clinical experience[14] and it corresponds well with the concept of the native liver function protecting the recipient of an auxiliary graft from hemostatic disturbances. It may be argued that in clinical transplantation the blood loss in HLT is mainly reduced

by the limited dissection and by not removing the recipient liver instead of the protective native liver function. However, in the absence of portal hypertension in the healthy pig model we used, these clinical advantages appeared of lesser importance. Furthermore, the difference in blood loss between OLT and HLT was more prominent with increasing storage damage to the graft (Chapter 6). On the other hand, it should be stressed that the assessment of blood loss in these experiments was no more than a rough estimate.

Because none of the animals survived OLT after 72-hr graft storage, many never received the second unit of blood. This explains why it appeared that HLT required more blood transfusion in the 72-hr experiment, despite higher blood loss in OLT.

The increased portal-caval pressure gradient after transplantation might be a result of an increase in hepatic vascular resistance. Congestion of blood in the sinusoids or cellular swelling, induced by ischemic or reperfusion damage, could be responsible for this.[13]

On reperfusion, there was an abrupt fall in hemoglobin values and platelet counts, while it was not usually accompanied by large amounts of blood loss. Obviously, at that moment many red blood cells and platelets got stuck in the graft. This effect was especially noteworthy in HLT. This might be related to the resection surface in HLT created at the donor procedure after the blood was flushed from the liver.

The immediate increase of K^+ could be explained by an incomplete washout of the preservation solution, which is rich in K^+.[15] On the other hand, increased ASAT and K^+ levels might have been present in the portal blood, before entering the graft. However, these explanations are opposed by the fact that an increase in the duration of graft preservation -and thereby storage damage- enlarged the postreperfusion increase of K^+ and ASAT values. More likely, the sudden increase of both K^+ and ASAT levels is an expression of cellular damage of the graft. The higher ASAT levels in the washout solution in the 72-hr experiment in HLT compared to OLT could also be an effect of the resection in HLT.

Degenerative features of the hepatocyte membrane (cellular rounding and loss of cohesion) paralleled both prolonged ischemia and reperfusion. Using electron microscopy, signs of irreversible damage could be detected in the 24-hr preserved grafts, already. This is inconsistent with the report of Momii and Koga who observed almost no ultrastructural damage in the hepatocytes after 48-hr cold storage of the rat liver in UW solution.[16]

Cytoplasmatic degenerative changes of hepatocytes were more striking with increasing cold ischemic duration. These changes did not progress directly after reperfusion. On

the other hand, evidence of irreversible cell death (nuclear disintegration) occurred, with few exceptions, exclusively after reperfusion.

The pathogenesis of storage damage is not clearly understood. Recently, attention has been focused on nonparenchymal damage.[17–20] It is generally accepted that hepatocytes are especially susceptible to warm ischemia and that endothelial cells are particularly vulnerable when exposed to cold ischemia and reperfusion.[20,21]

Shedding of endothelial cells appeared to be present in most of the 24–hr preserved livers. This is in agreement with earlier reports,[20,21] in which the sinusoidal lining cells were shown to be more vulnerable to cold ischemia than the hepatocyte. In the present experiments, reperfusion did not worsen the histological degenerative changes. The fact that endothelial cells could not be demonstrated in virtually all postmortem specimens, suggests that damage was already irreversible, before reperfusion. On the other hand, reperfusion damage might have interfered with restoration of the normal coherence between hepatocyte and sinusoidal lining cell.

It is not clear whether endothelial cells are detached from the hepatocytes by an intrinsic disturbance, or whether degenerative changes of the hepatocytes itself are responsible.[20] Holloway et al.[22] established that detached endothelial cells are not necessarily nonviable. This suggests that the primary site of lethal cold storage injury may be localized to the attachment mechanism of sinusoidal cells to the extracellular matrix.

Enzyme histochemistry showed significant variation in reaction patterns. Generally speaking, changes present after 24–hr cold storage were not clearly different from those found after 48 and 72–hr preservation. Still, reperfusion worsened enzyme activity and localization in a few minutes, indicating that the nature of reperfusion injury is explicitly different from ischemic injury.

It is stressed, that the morphological changes found between the biopsies taken after cold ischemia and those taken immediately after reperfusion, occurred in no more than 5 or 10 min. This emphasizes the acute nature of reperfusion injury.

Mortality and survival

The poor survival in these experiments made all comparisons of postoperative tests impossible. Although initial experimental results with UW solution held out hope for preservation times well over 24 hr,[23] it has become clear that human livers enter the danger zone of storage injury once 20 hr of simple cold storage has passed,[24] or even earlier.[25,26] While successful liver preservation did reach the one–day limit in man[27–29] and also in dogs[23] and rats[30], the pig is probably more susceptible to the

consequences of storage damage. This is in agreement with others who use the porcine liver transplantation model to study long-term preservation.[31] In our experiments, the better survival after HLT compared to OLT must exclusively be attributed to the remaining function of the native liver.

4.5 CONCLUSIONS

In the healthy pig model, HLT was a shorter procedure compared to OLT, with shorter portal vein interruption. Intraoperative blood loss was lower in animals that underwent HLT and this effect was more prominent after longer periods of graft preservation. This suggests that the native liver function protects the recipient of an auxiliary graft from hemostatic disturbances. Immediately after reperfusion there was an instant fall in hemoglobin levels and platelet counts, without excessive fluid suppletion. Since this effect was more obvious with longer periods of preservation, it suggests that these cells adhere to damaged components of the graft. There was no evidence PGE_1 provided protection from storage damage.

Despite the absence of light microscopic evidence of irreversible cell death before reperfusion, irreversible changes could be detected in the 24-hr preserved grafts using electron microscopy. Still, enzyme histochemistry showed an abrupt deterioration immediately after reperfusion indicating that the nature of reperfusion injury is different from the preceding ischemic damage. The assumption endothelial cells are more vulnerable to cold ischemia and reperfusion than hepatocytes could be confirmed as notable endothelial shedding was already found after 24-hr ischemia. At reperfusion, morphological appearance instantaneously worsened to an extent correspondeding the poor graft and animal survival.

4.6 REFERENCES

1. Blankensteijn JD, Groenland THN, Baumgartner D, Vos LP, Kerkhofs LGM, Terpstra OT. Intraoperative hemodynamics in liver transplantation comparing orthotopic with heterotopic transplantation in the pig. Transplantation 1990; 49:665–668.

2. Bijnen AB, Dekkers–Bijma AM, Vriesendorp HM, Westbroek DL. Value of the mixed lymphocyte reaction in dogs as a genetic assay. Immunogenetics 1979; 8:287–297.

3. Denmark SW, Shaw BW, Griffith BP, Starzl TE. Venous–venous bypass without systemic anticoagulant in canine and human liver transplantation. Surg Forum 1983; 34:380–382.

4. Griffith BP, Shaw BW,Jr., Hardesty RL, Iwatsuki S, Bahnson HT, Starzl TE. Veno–venous bypass without systemic anticoagulant for transplantation of the human liver. Surg Gynecol Obstet 1985; 160:271–272.

5. Tobimatsu M, Konomi K, Saito S, Tsumagari T. Protective effect of prostaglandin E_1 on ischemia induced acute renal failure in dogs. Surgery 1985; 98:45–52.

6. Gaber AO, Thistlethwaite JR,Jr., Busse–Henry S, Aboushloe M, Emond JC, Rouch D, Broelsch CE. Improved results of preservation of hepatic grafts preflushed with albumin and prostaglandins. Transplant Proc 1988; 20:992–993.

7. Wachstein M, Meisel E. Histochemistry of hepatic phosphatase at a physiologic pH. Am J Clin Pathol 1957; 27:13–23.

8. Frederiks WM, Marx F. A quantitative histochemical study of 5'–nucleotidase activity in rat liver using the lead salt method and polyvinyl alcohol. Histochem J 1988; 20:207–214.

9. van Noorden CJ, Frederiks WM, Aronson DC, Marx F, Bosch K, Jonges GN, Vogels IM, James J. Changes in the acinar distribution of some enzymes involved in carbohydrate metabolism in rat liver parenchyma after experimentally induced cholestasis. Virchows Arch [B] 1987; 52:501–511.

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

11. Kaplan EL, Meier EA. Nonparametric estimation from incomplete observations. J Am Statistical Assoc 1958; 53:457–481.

12. Goldyne ME. Prostaglandins & other eicosanoids. In: Katzung BG, ed. Basic & clinical pharmacology. Los Altos:Lange medical publications, 1984:217–226.

13. Ikeda T, Yanaga K, Lebeau G, Higashi H, Kakizoe S, Starzl TE. Hemodynamic and biochemical changes during normothermic and hypothermic sanguinous perfusion of the porcine hepatic graft. Transplantation 1990; 50:564–567.

14. Terpstra OT, Schalm SW, Weimar W, Willemse PJA, Baumgartner D, Groenland THN, ten Kate FJW, Porte RJ, de Rave S, Reuvers CB, Stibbe J, Terpstra JL. Auxiliary partial liver transplantation for end-stage chronic liver disease. N Engl J Med 1988; 319:1507–1511.

15. Arias J, Aller MA, Lorente L, Rodriguez JC, Fernandez X, Brandau D, Duran H. Washout of the pig liver with Haemaccel after hypothermic preservation. Int Surg 1990; 75:78–83.

16. Momii S, Koga A. Time-related morphological changes in cold-stored rat livers. A comparison of Euro-Collins solution with UW solution. Transplantation 1990; 50:745–750.

17. Bühren V, Marzi I, Walcher F, Menger M, Hower R. Effects of Euro-Collins, University-of-Wisconsin, and Histidine-Tryphtophane-Ketoglutarate Solution on Hepatic Microcirculation Following Liver Transplantation in the Rat. Transplant Proc 1991; 23:643–644.

18. Koyamada N, Ohkohchi N, Koizumi M, Katoh H, Sakurada M, Hirano T, Orii T, Terashima T, Fujimori K, Satomi S, Oguma S, Taguchi Y, Mori S. Prevention of Ischemic Sinusoidal Lining Cell Injury During Liver Transplantation in Pigs Using an Artificial Heart and Lung. Transplant Proc 1991; 23:721–725.

19. Gao W, Takei Y, Marzi I, Lindert KA, Caldwell-Kenkel JC, Currin RT, Tanaka Y, Lemasters JJ, Thurman RG. Carolina rinse solution – a new strategy to increase survival time after orthotopic liver transplantation in the rat. Transplantation 1991; 52:417–424.

20. Caldwell-Kenkel JC, Currin RT, Tanaka Y, Thurman RG, Lemasters JJ. Reperfusion injury to endothelial cells following cold ischemic storage of rat livers. Hepatology 1989; 10:292–299.

21. Koizumi M, Ohkohchi N, Katoh H, Koyamada N, Fujimori K, Sakurada M, Andoh T, Satomi S, Sasaki T, Taguchi Y, Mori S, Kataoka S, Yamamato TY. Preservation and reflow damage in liver transplantation in the pig. Transplant Proc 1989; 21:1323–1326.

22. Holloway CM, Harvey PR, Strasberg SM. Viability of sinusoidal lining cells in cold-preserved rat liver allografts. Transplantation 1990; 49:225–229.

23. Jamieson NV, Sundberg R, Lindell S, Claesson K, Moen J, Vreugdenhil PK, Wight DGD, Southard JH, Belzer FO. Preservation of the canine liver for 24–48 hours using simple cold storage with UW solution. Transplantation 1988; 46:517–522.

24. Furukawa H, Todo S, Imventarza O, Wu YM, Scotti C, Day R, Starzl TE. Cold ischemia time vs outcome of human liver transplantation using UW–solution. Transplant Proc 1991; 23:1550–1551.

25. Sanchez–Urdazpal L, Gores G, Ward E, Maus T, Wahlstrom H, Wiesner RH, Krom RAF. Non–anastomotic biliary strictures after orthotopic liver transplantation. 5th Congres European Society for Organ Transplantation 1991; 73.(Abstract)

26. Adam R, Morino M, Diamond T, Astarcioglu I, Johann M, Azoulay D, Bao YM, Bismuth H. Influence of prolonged cold ischaemia using UW solution on graft function and outcome following liver transplantation. 5th Congres European Society for Organ Transplantation 1991; 140.(Abstract)

27. Kalayoglu M, Sollinger HW, Stratta RJ, D'Alessandro AM, Hoffmann RM, Pirsch JD, Belzer FO. Extended preservation of the liver for clinical transplantation. Lancet 1988; 1:617–619.

28. Todo S, Tzakis A, Starzl TE. Preservation of livers with UW or Eurocollins' solution. Transplantation 1988; 46:925–926.

29. Todo S, Nery JR, Yanaga K, Podesta L, Gordon RD, Starzl TE. Extended preservation of human liver grafts with UW solution. JAMA 1989; 261:711–714.

30. Sumimoto R, Jamieson NV, Wake K, Kamada N. 24–hour rat liver preservation using UW solution and some simplified variants. Transplantation 1989; 48:1–5.

31. Manner M, Shult W, Senninger N, Machens G, Otto G. Evaluation of preservation damage after porcine liver transplantation by assessment of hepatic microcirculation. Transplantation 1990; 50:940–943.

Chapter 5

HEMODYNAMIC CHANGES

An adapted version of this chapter
has been accepted for publication in
TRANSPLANTATION.

Also related to this chapter:

Blankensteijn JD, Groenland ThHN, Baumgartner D, Vos LP, Kerkhofs LGM, Terpstra OT. Intraoperative hemodynamics in liver transplantation comparing orthotopic with heterotopic liver transplantation in the pig. Transplantation 1990;49:665–668.

THE EFFECTS OF LONG-TERM GRAFT PRESERVATION AND PROSTAGLANDIN E_1 ON INTRAOPERATIVE HEMODYNAMIC CHANGES IN LIVER TRANSPLANTATION

A comparison between orthotopic and heterotopic transplantation in the pig

Jan D. Blankensteijn[1], Peter M. Schlejen[1],
Theo H.N. Groenland,[2] and Onno T. Terpstra[1]

Departments of Surgery[1] and Anesthesiology[2], University Hospital
Dijkzigt and the Laboratory for Surgical Research,
Erasmus University, Dr. Molewaterplein 40
Rotterdam, The Netherlands

5.1 INTRODUCTION

Liver transplantation compromises the intraoperative hemodynamic condition of the recipient. The simultaneous clamping of the inferior caval and portal vein leads to a major decrease in venous return, even when a venovenous bypass is used.[1] The removal of the cirrhotic liver in combination with portal hypertension and preexisting coagulation disorders may lead to significant blood loss.[2] Furthermore, during the anhepatic phase, vasoactive and myocardial depressant substances may be released from the congested mesenteric vessels.[3] These substances may also be important determinants of the hemodynamic condition after reperfusion of the graft.[4] For patients with end–stage liver disease in particular, these hemodynamic effects are a threat to a successful outcome.

Heterotopic transplantation avoids many of the above mentioned aspects. Recently, we reported results form a study on the hemodynamic changes of experimental orthotopic (OLT) and reduced size, heterotopic liver transplantation (HLT).[5] We demonstrated definite advantages of HLT over OLT. However, the grafts used in our experiment were almost free of storage damage, since they were stored for no more than 2 hr in cold Eurocollins' solution.

The high incidence of primary graft nonfunction, which is thought to be related to storage damage, indicates that ischemia/reperfusion injury remains a problem in clinical transplantation, despite improvement in preservation techniques.[6] Vasoactive substances released by a damaged graft upon reperfusion may act on the circulatory system of the recipient. In HLT, these substances may be cleared from the blood by the native *in situ* liver.

The primary aim of the present study was to evaluate the hemodynamic changes in both OLT and HLT after various periods of cold storage of the graft.

Protection of the graft by adding prostaglandin E_1 (PGE_1) to the flush solution has been demonstrated in human liver transplantation.[7] However, successful liver transplantations after long–term simple cold storage with the University of Wisconsin (UW) solution without prostaglandins have also been reported: for 24 hr and more in man[8] and for 48 hr in dogs.[9] It is not known if cytoprotective agents can give additional improvement after simple cold storage for 24 to 48 hr or more. Furthermore, the effect of prostaglandins on long–term preservation followed by HLT, has never been studied. Thus, we also assessed the effect of PGE_1, both in OLT and HLT after various periods graft preservation.

5.2 METHODS

An extensive description of the materials and methods is given in paragraph 4.2. In short: 69 female Yorkshire pigs were randomly allocated to OLT (N=32) or HLT (N=37). Thirty-one grafts were transplanted after 2 hr of cold storage, 16 after 24 hr, 7 after 48 hr and 15 after 72 hr. Sixteen transplantations in the different preservation groups were performed using PGE_1. Body weights of the donor and recipient animals were similar, about 25 Kg. Histocompatibility was matched by the mixed lymphocyte culture-test.[10]

Donor and recipient operation were performed as depicted in Figure 4.1 (Page 92). A venovenous bypass between the common iliac and portal vein to the jugular vein was used in OLT. To maintain pulmonary capillary wedge pressure during surgery, fluid support was given with Ringer's lactate, 0.9% NaCl, and Haemaccel®. Metabolic acidosis was corrected by administration of sodium bicarbonate. Depending on the amount of blood loss, 500-1000 ml donor blood was given to the recipient.

PGE_1 was given intravenously to the donor and the recipient and in the flushing and rinsing solutions as described in paragraph 4.2.2.3.

The following hemodynamic changes were monitored using an intracarotid arterial line, a jugular vein catheter, and a thermodilution pulmonary artery catheter: cardiac output (CO), central venous pressure (CVP), heart rate (HR), mean arterial pressure (MAP), mean pulmonary artery pressure (MPAP), and pulmonary capillary wedge pressure (PCWP).

Table 5.1. Intraoperative moments of hemodynamic measurements.

R-60	Starting value: ± 1 hr before recirculation.
R-30	In HLT 5 min after the partial clamping of the subhepatic inferior. vena cava and in OLT 5 min after opening the venovenous shunt.
R-5	Anhepatic phase: ± 5 min before recirculation.
R+5	± 5 min after recirculation.
R+30	± 30 min after recirculation (during the construction of the arterial anastomosis).
R+60	± 60 after recirculation (5 min after the aorta anastomosis).
R+90	± 90 min after recirculation (closure).

Cardiac output measurements were performed in triplets and the mean of the two most identical values was recorded. For graphical depiction the moments of measurement were synchronized, relative to the moment of recirculation. Cardiac output measurements were performed around every important operative step (Table 5.1). In OLT, R–5 is a measurement in the anhepatic phase. Although there is no anhepatic phase in HLT, during the portal vein anastomosis there is a period of clamping of the portal vein. In the pig this often implies (almost) complete clamping of this vessel.
The calculations of ventricular minute work and vascular resistance are given in Table 5.2.
Data were subjected to computerized statistical analysis as described in paragraph 4.2.4. Additionally, the multiple regression analysis was also performed with models including hemoglobin, potassium, and ASAT.

5.3 RESULTS

All median values are depicted in Table 5.3. Grouped as to the moment of measurement some data are elaborated on in the following paragraph. Also, meaningful changes from time to time and results of the multiple regression analysis are presented.

Table 5.2. Definition of the hemodynamic parameters.

Abbrev:	Parameter:	Units:	Calculation:
CO	Cardiac Output	L/min	
CVP	Central Venous Pressure	mm Hg	
HR	Heart Rate	Beats/min	
MAP	Mean Arterial Pressure	mm Hg	
MPAP	Mean Pulmonary Artery Pressure	mm Hg	
PCWP	Pulmonary Capillary Wedge Pressure	mm Hg	
LVMW	Left Ventricular Minute Work	Kg x meters/min	(MAP–PCWP) x 0.0136 x CO
RVMW	Right Ventricular Minute Work	Kg x meters/min	(MPAP–CVP) x 0.0136 x CO
PVR	Pulmonary Vascular Resistance	Dyne x sec/cm^5	(MPAP–PCWP) x 80/CO
SVR	Systemic Vascular Resistance	Dyne x sec/cm^5	(MAP–CVP) x 80/CO

Table 5.3. Hemodynamic parameters at the various operative moments. Values are given as median (95% confidence limits of the mean).

Parameter:	R-60	R-30	R-5	R+5	R+30	R+60	R+90
CO (L/min)							
OLT	3.1 (3.0-3.8)	2.5 (2.3-2.7)	2.6 (2.4-2.8)	2.8 (2.3-3.1)	2.8 (2.6-3.4)	2.7 (2.4-3.2)	2.7 (2.4-2.8)
HLT	3.9 (3.7-4.1)	2.8 (2.6-3.4)	2.0 (2.1-2.5)	3.3 (3.1-3.5)	2.5 (2.4-2.8)	3.1 (2.5-3.3)	2.9 (2.3-3.1)
CVP (mm Hg)							
OLT	4 (3.0-5.0)	3 (2.8-5.2)	5 (3.8-6.2)	5 (3.8-6.2)	7 (5.6-8.4)	4 (3.8-6.2)	4 (3.6-6.4)
HLT	3 (3.2-4.8)	3 (2.0-4.0)	2 (2.2-3.8)	3 (2.2-3.8)	3 (2.2-3.8)	3 (2.2-3.8)	4 (3.2-4.8)
HR (Beats/min)							
OLT	120 (116-133)	160 (153-173)	128 (122-146)	138 (130-148)	123 (111-133)	140 (124-148)	155 (138-166)
HLT	120 (117-139)	140 (139-163)	132 (131-161)	140 (136-156)	144 (141-165)	165 (153-173)	178 (160-184)
MAP (mm Hg)							
OLT	103 (98-109)	92 (84-96)	93 (88-100)	66 (64-82)	117 (107-121)	76 (68-84)	81 (72-88)
HLT	105 (100-110)	95 (87-99)	68 (64-76)	81 (71-87)	85 (76-88)	80 (69-85)	76 (66-80)
MPAP (mm Hg)							
OLT	12 (12.2-15.8)	9 (7.4-12.6)	11 (9.4-12.6)	22 (19.4-24.6)	22 (19.6-24.4)	21 (18.40-23.60)	16 (15.00-21.00)
HLT	13 (11.8-14.2)	10 (8.8-13.2)	8 7.8-10.2)	22 (18.2-23.8)	16 (14.2-19.8)	18 (13.2-30.8)	15 (14.4-19.6)
PCWP (mm Hg)							
OLT	5 (3.8-6.2)	4 (3.6-6.4)	5 (3.8-6.2)	6 (5.8-8.2)	10 (9.8-14.2)	6 (5.2-8.8)	6 (4.6-7.4)
HLT	6 (5.2-6.8)	5 (3.0-5.0)	4 (3.2-4.8)	6 (5.6-8.4)	5 (3.6-6.4)	6 (4.8-7.2)	5 (4.4-7.6)
RVMW (Kg*meters/min)							
OLT	0.50 (0.40-0.60)	0.15 (0.13-0.33)	0.20 (0.21-0.29)	0.70 (0.52-0.76)	0.55 (0.52-0.76)	0.60 (0.52-0.80)	0.40 (0.33-0.53)
HLT	0.50 (0.46-0.62)	0.29 (0.25-0.41)	0.20 (0.24-1.56)	0.90 (0.77-1.05)	0.50 (0.42-0.66)	0.70 (0.52-0.76)	0.40 (0.40-0.60)
LVMW (Kg*meters/min)							
OLT	4.3 (4.1-5.3)	3.1 (2.4-3.6)	3.0 (2.8-3.6)	2.8 (2.1-3.3)	4.4 (3.7-4.9)	2.6 (2.3-3.1)	3.0 (2.4-3.2)
HLT	5.3 (5.1-5.9)	3.7 (3.0-4.6)	1.7 (1.8-3.0)	3.0 (2.9-4.1)	2.8 (2.6-3.4)	3.3 (2.6-3.8)	2.8 (2.3-3.1)
PVR (Dyne*sec/cm^5)							
OLT	207 (186-246)	184 (160-212)	187 (183-263)	533 (405-665)	215 (222-522)	429 (384-552)	406 (325-565)
HLT	152 (143-179)	143 (159-239)	211 (175-239)	386 (315-495)	311 (304-532)	299 (286-458)	312 (274-414)
SVR (Dyne*sec/cm^5)							
OLT	2589 (2253-2657)	2864 (2630-3042)	2960 (2683-3319)	1754 (1878-2606)	2986 (2745-3893)	2143 (1921-2489)	2375 (2170-2742)
HLT	2050 (1971-2299)	2383 (2352-2744)	2467 (2349-2789)	1861 (1699-2231)	2534 (2341-2885)	1972 (1922-2214)	2046 (1971-2355)

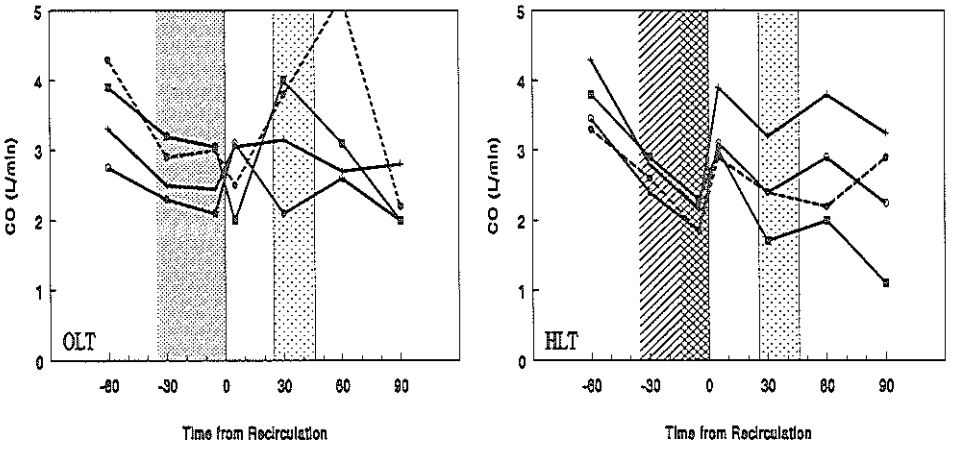


Figure 5.1. Cardiac Output (CO) during liver transplantation. Values are stated as medians. The horizontal axis gives the time in minutes from or to the moment of recirculation of the graft. Symbols: (**OLT**) *Orthotopic Liver Transplantation;* (**HLT**) *Heterotopic Liver Transplantation. Preservation time:* (+——+) *2 Hr;* (○——○) *24 Hr;* (●——●) *48 Hr (line is interrupted since this group consists of only seven animals);* (■——■) *72 Hr. Shaded areas:* (▤) *is the anhepatic period in OLT; in HLT,* (▨) *is the period in which the subhepatic inferior vena cava is partially clamped and* (▩) *in which also the portal vein is (partially) clamped;* (▣) *is the period of clamping of the aorta.*

For both HLT and OLT the CO, MAP, RVMW, and LVWM are graphically depicted in Figure 5.1 – 5.4.

Sixty minutes before recirculation, there were no differences in venous, pulmonary, and arterial pressures. However, the CO was higher in HLT compared to OLT ($P<0.01$). Simultaneously, the SVR ($P=0.01$) and PVR ($P<0.01$) were lower in HLT than in OLT.

Thirty minutes before recirculation, the MPAP, the PCWP and CO in both procedures decreased significantly ($P<0.001$).

Five minutes before recirculation, the CVP, MPAP and PCWP all showed a further decrease to around 60–70% of the initial recordings in HLT. Together with these changes, both the MAP and the CO decreased significantly (both $P<0.001$).

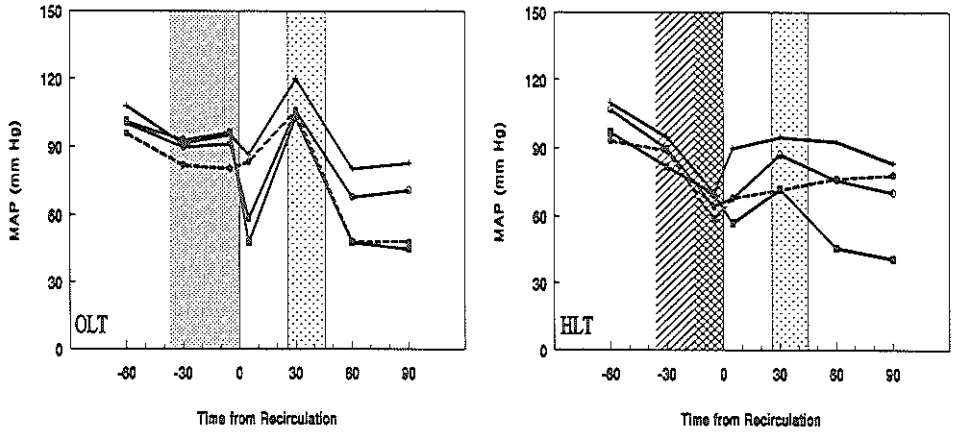


Figure 5.2. Mean arterial pressure (MAP) during liver transplantation. Values are stated as medians. Horizontal axis and symbols as in Figure 5.1.

In OLT, there was no further decrease of the CVP, MPAP, and PCWP and most values returned towards the starting values. As a consequence, all these pressure parameters as well as the CO, the SVR, and the LVMW were significantly higher in OLT than in HLT. During the anhepatic phase in OLT, the HR significantly decreased (P=0.02), but in HLT there was no significant difference (P=0.20).

Multiple regression analysis confirmed these effects of the type of transplantation on the hemodynamic parameters, five minutes before recirculation.

Five minutes after recirculation, the PVR increased significantly for both OLT and HLT (P<0.001). The proportional change for OLT was 240% (310%, 50%) and for HLT 180% (350%, 100%) (P=0.39). In contrast, the SVR decreased significantly in both OLT (P<0.001) and HLT (P=0.02).

HR did not significantly change from five minutes before to five minutes after recirculation.

In HLT, the CO showed a quick recovery from the depressed values of the previous measurement, five before recirculation. In OLT, the recovery of the CO was not so obvious. As a result, five minutes after recirculation, CO was significantly higher in HLT compared to OLT (P=0.02).

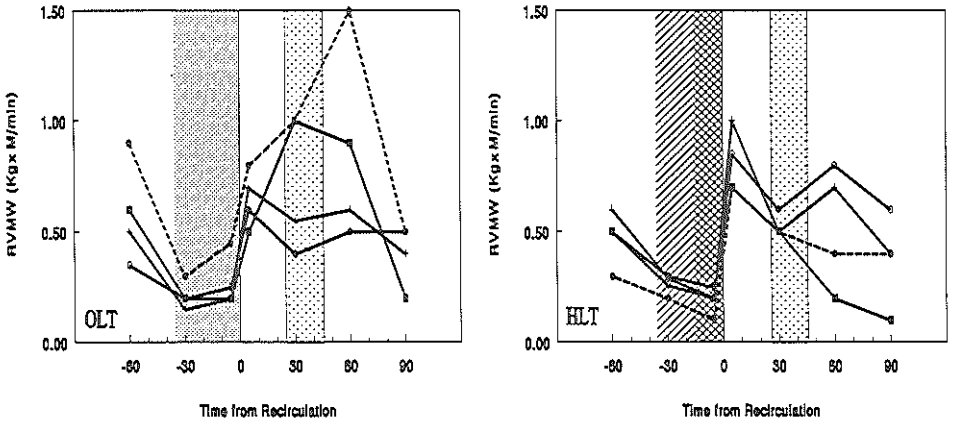


Figure 5.3. Right ventricular minute work (RVMW) during liver transplantation. Values are stated as medians. Horizontal axis and symbols as in Figure 5.1.

As a result of the above mentioned changes, both the RVMW (P=0.01) and the LVMW (P=0.03) were lower in OLT. Compared to five minutes before recirculation, the RVMW increased with a 280% (300%, 30%) in OLT and 460% (540%, 70%) in HLT (P<0.01).

In the multiple regression analysis, the type of transplantation significantly influenced CO, RVMW, and LVMW (all T-values about 3.5, P<0.001). In addition, the MAP was higher in HLT compared to OLT (T=2.19, P=0.03). When PGE_1 was used, the CO was lower, although not statistically significant (T=−1.37, P=0.18), while the MAP was significantly lower (T=−2.96, P<0.01). Regarding the preservation times, longer graft storage had a negative effect on the CO (T=−2.49, P=0.02) and on the MAP (T=−4.25, P<0.001). Both RVMW and LVMW were not only significantly controlled by the type of transplantation, but also by the duration of graft-storage. However, the T-value of the preservation time for the RVMW reached only marginal statistical significance (T=−2.07, P=0.04), while this effect on the LVWM was highly significant (T=−4.07, P<0.001).

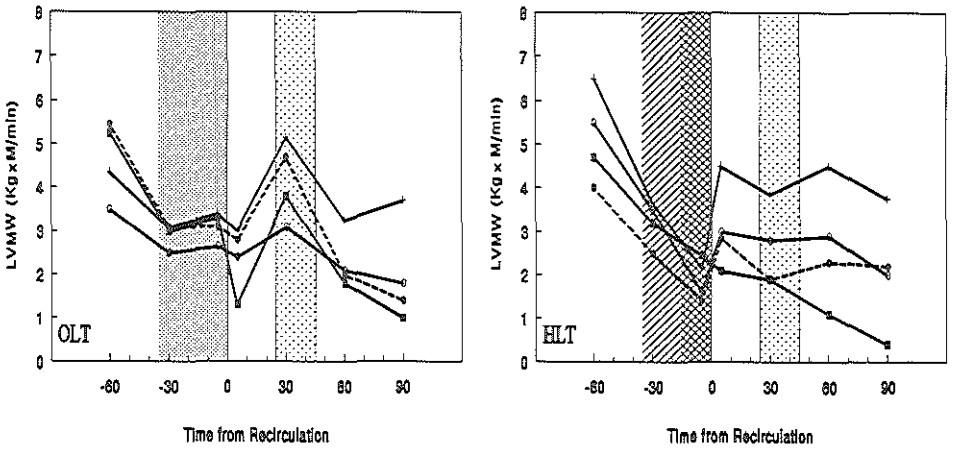


Figure 5.4. Left ventricular minute work (LVMW) during liver transplantation. Values are stated as medians. Horizontal axis and symbols as in Figure 5.1.

The multiple regression analysis with models including hemoglobin, potassium, and ASAT, did not show any statistically significant relation with the hemodynamic parameters.

Thirty minutes after recirculation, upon clamping of the aorta, CVP increased in OLT ($P<0.01$), but did not change in HLT. The PCWP also increased in OLT ($P=0.01$) and decreased in HLT ($P=0.01$). This resulted in a higher PCWP for OLT compared to HLT, thirty minutes after recirculation ($P<0.001$).

The SVR increased for both OLT ($P<0.01$) and HLT ($P=0.02$). In HLT, CO decreased ($P<0.001$), but in OLT it did not as clearly. As a result the MAP sharply increased in OLT ($P<0.001$), while in HLT only a slight change was found. Related to the changes in MAP and CO, the LVMW also markedly increased in OLT ($P<0.01$) and decreased in HLT ($P=0.03$).

In the multiple regression analysis, all the above mentioned parameters were indeed significantly influenced by the type of transplantation as already pointed out by the non-parametric analysis, except for the CO ($T=-0.83$, $P=0.41$).

Furthermore, the multiple regression analysis of the hemodynamic effects of PGE_1 at thirty minutes after recirculation revealed CO to be lower when PGE_1 was used

(T=–3.07, P<0.01). Despite a positive effect of PGE_1 on the SVR (T=2.00, P<0.01) the MAP was lower with PGE_1 (T=–2.53, P=0.01). Also, the PVR was higher when PGE_1 was used (T=2.68, P<0.01). In accordance with the lower CO and MAP, the use of PGE_1 was also connected with a lower LVWM (T=–3.34, P<0.01).
Furthermore, thirty minutes after recirculation, the MAP (T=–5.30, P< 0.001) and the LVMW (T=–3.03, P<0.01) were significantly lower with longer preservation times.
Sixty and ninety minutes after recirculation, in OLT, CVP (P<0.01) and PCWP (P<0.001) showed a significant decrease and restored to levels equivalent to those in HLT. Adequate preload was maintained in both HLT and OLT (Table 5.3). SVR dropped in both OLT (P<0.01) and HLT (P=0.01), while in OLT it concurred with a significant decrease in MAP (P<0.001).
The multiple regression analysis did not reveal any significant effects related to PGE_1, at this interval. A longer preservation time resulted in a lower CO (T=–2.71, P<0.01), MAP (T=–6.60, P<0.001), and LVMW (T=–5.47, P<0.001).

5.4 DISCUSSION

Sixty minutes before the recirculation, there was a difference in CO and SVR between OLT and HLT. This first measurement was most frequently taken at the moment of the dissection of the hepatogastric ligament in OLT. This dissection was done with the liver retracted (and compressed) in the right subdiaphragmatic space. In addition, transection of the autonomic nerves and spasm of the hepatic artery could also contribute to the elevated SVR and the related decreased CO in OLT.
After the introduction of the iliacoportajugular bypass in OLT or the partial clamping of the subhepatic inferior vena cava in HLT, the preload decreased. Especially in HLT, this was important enough to lower the CO, the RVMW and the LVMW.
In HLT, five minutes before recirculation, with the portal vein clamped, there was a further reduction in venous return and as a result also in MAP, CO, and ventricular minute work. In clinical HLT for liver cirrhosis, these changes are negligible, since portacaval collaterals can shunt the mesenteric blood flow. Moreover, in man the portal vein can be partially clamped, which is rarely the case in the piglet. Indeed, our clinical experience with HLT is that CO hardly responds to partial clamping of the portal vein.[11] The changes found in our pig model are to be anticipated in patients without portal hypertension– e.g., in children with a hepatic inborn error of metabolism.

In OLT, in the anhepatic phase, the SVR increased and the CO decreased, due to the removal of the (arterial bed of the) liver. The PCWP restored towards pre-bypass levels and the CVP even exceeded the starting value. An increase in CVP at the end of the anhepatic phase was also found by other authors.[4,12,13] Kalpokas et al. [4] assumed that the elevated CVP was due to their enthusiastic blood volume replacement, before recirculation. However, the replacement of the portal hepatic vascular resistance by a shunt with a lower resistance -in itself- increases the CVP. In addition, on removal of the recipient liver, its blood content is squeezed out before the suprahepatic vena cava is clamped. This leads to a sudden autotransfusion which can amount to 10% of the circulating volume.[14] These elements, together with the downregulation of the CO by the increased SVR, could be responsible for the elevated CVP at the end of the hepatic phase. Despite this improved preload, only the MPAP increased, and not the CO and MAP. Other reports,[12,13] studying the hemodynamic effects of venovenous bypass in man, also showed a 15% decrease in MAP and an increase in MPAP (15%) and CVP (27%).

The SVR in HLT also increased, although the native liver was not removed. This increase, however, is probably a compensatory effect of the decreased venous return and the resulting decrease in CO.

Hemodynamic changes were most apparent after recirculation of the graft. There was a marked increase in PVR in both HLT and OLT, but a decrease in SVR. Paulsen et al.[12] postulated that the decrease in SVR can also be explained by the opening of a parallel branch of the circulation, at recirculation of the graft. However, first the graft is reperfused by portal blood only and the portal hepatic vascular bed is a serial resistance in the mesenteric circulation. This increases the total SVR compared to the bypass-period. A better explanation for the combination of changes is the release of vasoactive substances from the graft, causing a decrease in SVR.[15] However, when vasodilators are involved, also a decrease in PVR should be expected. Therefore other factors are speculated to be responsible for an increase in PVR. These include (micro-) air-emboli and cellular debris (exploded blebs[16,17]) from marginally preserved grafts. These factors will be trapped in the pulmonary vascular bed and so they will have no effect on the systemic circulation.

Besides vasoactive substances, many other factors are considered to be responsible for this so called postreperfusion syndrome.[18,19] Among these are the release of acid metabolites,[15] prostanoids,[20] anaphylatoxins (C5a),[21] potassium,[22] cold perfusate from the donor liver,[22] a decrease in ionized calcium[23] and acidosis.[4] As long as the exact

nature of these substances is unidentified, they may be called myocardial depressant factors.

Some authors found bradycardia associated with the postreperfusion syndrome.[12,13] In agreement with other investigators[4,24] we found the heart rate to be relatively constant after recirculation.

Furthermore, at five minutes after recirculation, an increase in PCWP, MPAP and CO was measured. These findings agree with other reports.[4,12,13,24] With regard to the type of transplantation, the CO was higher in HLT compared to OLT. This may be due to the implantation of an auxiliary organ with metabolic demands. On the other hand this difference can be explained by the presence of an intact donor liver in HLT after recirculation. This organ is probably able to clear many of the above mentioned toxic and vasoactive substances from the circulation. In case of OLT, the graft may not be able to give the same clearance of these myocardial depressant factors, immediately after recirculation. This effect is expected to be more distinct when storage damage increases. This theory is supported by the observation from the multiple regression analysis that increased preservation time was related to a decreased CO, in OLT in particular.

In the multiple regression analysis, the LVMW was predominantly influenced by factors related to the duration of graft preservation– and, to a lesser degree to the type of transplantation. The opposite was true for the RVMW, which also supports the theory that the pulmonary vascular bed is the primary target of factors related to the reperfusion itself and not particularly related to the extent of storage damage– i.e., (micro–) air–emboli or temperature.

In the multiple regression analysis, PGE_1 had a negative effect on the MAP, after recirculation. Although not individually statistically significant, the combination of the negative effects on CO and SVR could be responsible for this phenomenon. Also, it could be speculated that PGE_1 promotes the release of myocardial depressant factors. Although PGE_1 infusion was stopped at the moment of recirculation and the plasma half–life of PGE_1 is very short, the multiple regression analysis showed a significant positive effect on the SVR and a negative effect on CO at thirty minutes after recirculation. It may be hypothesized that the flow–distribution was changed by the earlier administration of PGE_1 to such an extent that clamping of the aorta was tolerated worse.

Although other parameters than MAP and CO were influenced by the duration of graft storage, these did not reach significance because in the 72–hr preservation group at thirty minutes after recirculation only 3 animals receiving an OLT were still alive.

After unclamping of the aorta, parameters leveled for HLT and OLT. Significant effects of PGE_1 could no longer be detected. Sixty and ninety minutes after recirculation, the ischemia-related effects on MAP, CO, and LVWM were highly significant. Again, the presence of an adequate preload shows these ischemia-related effects to be interpreted as myocardial depressant effects.

5.5 CONCLUSION

In HLT, during the portal vein interruption, there was an important reduction in venous return and as a result also in MAP, CO, and ventricular minute work. In OLT, in the anhepatic phase, the SVR increased and the CO decreased due to the removal of the arterial bed of the liver. After recirculation of the graft, there was a marked increase in PVR in both HLT and OLT, but a decrease in SVR. We hypothesized the pulmonary vascular bed to be the primary target of factors related to the reperfusion itself and not particularly related to the extent of storage damage, and the systemic vascular bed to be primarily compromised by myocardial depressant factors.
Furthermore, we showed a longer preservation time to be related with a decreased CO in the post-reperfusion period. This effect was more prominent in OLT. We hypothesized that myocardial depressant factors are responsible for this phenomenon and that in HLT the native *in situ* liver may be capable of clearing these substances from the blood.
With respect to the use of PGE_1, we did not find any significant beneficial effects on the hemodynamic intraoperative condition, and the expected vasodilatory effects of PGE_1 were not found.

5.6 REFERENCES

1. Paulsen AW, Whitten CW, Ramsay MA, Klintmalm GB. Considerations for anesthetic management during veno-venous bypass in adult hepatic transplantation. Anesth Analg 1989; 68:489-496.

2. Lewis JH, Bontempo FA, Cornell FW, Kiss JE, Larson P, Ragni MV, Rice EO, Spero JA, Starzl TE. Blood use in liver transplantation. Transfusion 1987; 27:222-225.

3. Falcini F, Martini E, Marsili M, Benassai C, Fabbri LP, Tanini R, Linden M, Simoncini R, Filipponi F, Cataliotti L. Veno-venous bypass in experimental liver transplantation: portal-jugular versus caval-portal-jugular. G Chir 1990; 11:206-210.

4. Kalpokas M, Bookallil M, Sheil AG, Rickard KA. Physiological changes during liver transplantation. Anaesth Intensive Care 1989; 17:24-30.

5. Blankensteijn JD, Groenland THN, Baumgartner D, Vos LP, Kerkhofs LGM, Terpstra OT. Intraoperative hemodynamics in liver transplantation comparing orthotopic with heterotopic transplantation in the pig. Transplantation 1990; 49:665-668.

6. Bismuth H, Castaing D, Ericzon BG, Otte JB, Rolles K, Ringe B, Slooff M. Hepatic transplantation in Europe. First report of the European liver transplant registry. Lancet 1987; 2:674-676.

7. Gaber AO, Thistlethwaite JR,Jr., Busse-Henry S, Aboushloe M, Emond JC, Rouch D, Broelsch CE. Improved results of preservation of hepatic grafts preflushed with albumin and prostaglandins. Transplant Proc 1988; 20:992-993.

8. Todo S, Tzakis A, Starzl TE. Preservation of livers with UW or Eurocollins' solution. Transplantation 1988; 46:925-926.

9. Jamieson NV, Sundberg R, Lindell S, Claesson K, Moen J, Vreugdenhil PK, Wight DGD, Southard JH, Belzer FO. Preservation of the canine liver for 24-48 hours using simple cold storage with UW solution. Transplantation 1988; 46:517-522.

10. Bijnen AB, Dekkers-Bijma AM, Vriesendorp HM, Westbroek DL. Value of the mixed lymphocyte reaction in dogs as a genetic assay. Immunogenetics 1979; 8:287-297.

11. Groenland THN, Visser L, Terpstra OT, Terpstra JL, Reuvers CB, Baumgartner D, Schalm SW. Stable hemodynamics during heterotopic auxiliary partial liver transplantation for end-stage liver cirrhosis. Transplant Proc 1988; 20:538-540.

12. Paulsen AW, Valek TR, Blessing WS, Johnson DD, Parks RI, Pyron JT, Ramsay MA, Simpson BR, Swygert T, Walling P, Klintmalm GB. Hemodynamics during liver transplantation with veno-venous bypass. Transplant Proc 1987; 19:2417-2419.

13. Ringe B, Bornscheuer A, Blumhardt G, Bechstein WO, Wonigeit K, Pichlmayr R. Experience with veno-venous bypass in human liver transplantation. Transplant Proc 1987; 19:2416.

14. Guyton AC. Muscle blood flow during exercise; cerebral, splanchnic, and skin blood flows. In: Guyton AC, ed. Textbook of medical physiology. Philadelphia, London, Toronto:W.B. Saunders Company, 1976:375-381.

15. Carmichael FJ, Lindop MJ, Farman JV. Anaesthesia for hepatic transplantation: cardiovascular and metabolic alterations and their management. Anesth Analg 1985; 64:108-116.

16. Lemasters JJ, Stemkowski CJ, Ji S, Thurman RG. Cell surface changes and enzyme release during hypoxia and reoxygenation in the isolated, perfused rat liver. J Cell Biol 1983; 97:778-786.

17. Momii S, Koga A. Time-related morphological changes in cold-stored rat livers. A comparison of Euro-Collins solution with UW solution. Transplantation 1990; 50:745-750.

18. Aggarwal S, Kang YG, Freeman JA, Fortunato FL, Pinsky MR. Postreperfusion syndrome: cardiovascular collapse following hepatic reperfusion during liver transplantation. Transplant Proc 1987; 19(Suppl 3):54-55.

19. Aggarwal S, Kang YG, Freeman JA, DeWolf AM, Begliomini B. Is there a post-reperfusion syndrome. Transplant Proc 1989; 21:3497-3499.

20. Post S, Goerig M, Otto G, Manner M, Senninger N, Kommerell B, Herfarth C. Prostanoid release in experimental liver transplantation. Transplantation 1990; 49:490-494.

21. Henriksson B-Å, Stenqvist O, Bengtsson J-P, Bengtsson A. Release of anaphylatoxins during orthotopic liver transplantation. Transplant Proc 1991; 23:1949-1950.

22. Marino IR, De Luca G. Orthotopic liver transplantation in pigs. An evaluation of different methods of avoiding the revascularization syndrome. Transplantation 1985; 40:494-498.

23. Kost GJ, Jammal MA, Ward RE, Safwat AM. Monitoring of ionized calcium during human hepatic transplantation. Critical values and their relevance to cardiac and hemodynamic management. Am J Clin Pathol 1986; 86:61-70.

24. Rettke SR, Janossy TA, Chantigian RC, Burritt MF, Van Dyke RA, Harper JV, Ilstrup DM, Taswell HF, Wiesner RH, Krom RAF. Hemodynamic and metabolic changes in hepatic transplantation. Mayo Clin Proc 1989; 64:232–240.

Chapter 6

HEMOSTATIC CHANGES

An adapted version of this chapter has been submitted for publication.

Also related to this chapter:

Porte RJ, Blankensteijn JD, Knot EAR, de Maat MPM, Groenland THN, Terpstra OT. A comparative study on changes in hemostasis in orthotopic and auxiliary transplantation in pigs. Transplant Int 1991; 4:12–17.

THE EFFECTS OF LONG-TERM GRAFT PRESERVATION AND PROSTAGLANDIN E_1 ON INTRAOPERATIVE HEMOSTATIC CHANGES IN LIVER TRANSPLANTATION

A comparison between orthotopic and heterotopic transplantation in the pig

C. Minke Bakker,[1] Jan D. Blankensteijn,[2] Peter M. Schlejen,[2] Robert J. Porte,[1] Maria J. Gomes,[1] Harald I.H. Lampe,[2] Jeanne Stibbe,[3] and Onno T. Terpstra[2]

Departments of Internal Medicine II,[1] Surgery,[2] and Hematology,[3]
University Hospital Dijkzigt, Erasmus University,
Rotterdam, The Netherlands

6.1 INTRODUCTION

Orthotopic liver transplantation (OLT) has become an accepted method to treat patients with end–stage chronic liver disease. However, the procedure is frequently complicated by severe changes in hemostasis, as reflected by a decrease in platelets, fibrinogen and clotting factors as well as a shortening of the euglobulin clot lysis time. Both increased fibrinolysis[1,2] and disseminated intravascular coagulation[3–5] have been implicated in playing a role. The most striking abnormalities in hemostasis occur late in the anhepatic period and become more marked after reperfusion of the graft. This suggests, besides lack of hepatic clearance of activated hemostasis and fibrinolytic factors during the anhepatic period, revascularization of the ischemically damaged graft is also involved.[6,7] Previous studies have already demonstrated the relation between the quality of the donor liver and the severity and duration of hemostasis abnormalities.[8,9]
Auxiliary, heterotopic liver transplantation (HLT) has been proposed as an alternative to hepatic replacement. In HLT, the host liver is left *in situ* and the graft is transplanted in a heterotopic position. The anhepatic period is avoided and the function of the host liver retained. Hence, substances released from the ischemically damaged allograft at reperfusion might be cleared and the recipient is not from the outset dependent on the function of the graft. In spite of these theoretical advantages, the clinical results of HLT were initially discouraging. After experimental studies using a graft reduced in size by partial hepatectomy, provided with both arterial and portal blood inflow, an HLT model was developed in our institution.[10,11] This has been applied successfully in clinical practice.[12]
Recently, we reported results from a comparative study on the hemostatic changes of experimental OLT and HLT in pigs, after 2 hours of simple cold storage of the graft.[13] Fibrinolytic activity after reperfusion was more severe and sustained in OLT than in HLT. The native liver in HLT was suggested to protect the recipient from changes induced by storage damage. As we were also interested in the effects on hemostasis of longer cold storage times, we studied the hemostatic changes in both porcine OLT and HLT after various periods of graft preservation.
Graft protection by addition of prostaglandin E_1 (PGE_1) to the flush solution has been demonstrated in human liver transplantation.[14] Successful liver transplantations have also been reported after long–term simple cold storage with University of Wisconsin (UW) solution without prostaglandins: for 24 hr and more in man[15] and for 48 hr in dogs.[16] It is not known if cytoprotective agents give additional protection after simple cold storage for 24 to 48 hr or more. In addition, the effect of prostaglandins on

long-term preservation followed by HLT have not been investigated before. So, we also studied the effects of adding PGE_1 to the preservation solution.

6.2 METHODS

An extensive description of the materials and methods is given in paragraph 4.2. In short: 69 female Yorkshire pigs were randomly allocated to OLT (N=32) or HLT (N=37). Thirty-one grafts were transplanted after 2 hr of cold storage, 16 after 24 hr, 7 after 48 hr and 15 after 72 hr. Sixteen transplantations in the different preservation groups were performed using PGE_1. Body weights of the donor and recipient animals were similar, about 25 Kg. Histocompatibility was matched by the mixed lymphocyte culture-test.[17]

Donor and recipient operation were performed as depicted in Figure 4.1 (Page 92). In OLT, a venovenous heparin-coated iliacoportajugular bypass was introduced.[18] Depending on the amount of blood loss, 500-1000 ml donor blood was given to the acceptor.

PGE_1 was given intravenously to the donor and the recipient and in the flushing and rinsing solutions as described in paragraph 4.2.2.3.

Data were subjected to computerized statistical analysis as described in paragraph 4.2.4.

Table 6.1. Intraoperative moments of hemostatic measurements.

R-60	Starting value: ± 1 hr before recirculation.
FR	First hepatic outflow after reperfusion.
R-5	Anhepatic phase: ± 5 min before recirculation.
R+5	± 5 min after recirculation.
R+30	± 30 min after recirculation (during the construction of the arterial anastomosis).
R+60	± 60 after recirculation (5 min after the aorta anastomosis).
R+90	± 90 min after recirculation (closure).

6.2.1 Blood samples

For graphical depiction, the moments of measurement were synchronized, relative to the moment of recirculation. Blood samples were drawn around every important operative step (Table 6.1) to assess hemoglobin levels, platelet counts, and various hemostasis parameters. Additionally, on reperfusion, the first 30 ml of perfusate were drawn from the infrahepatic vena cava of the graft. These first hepatic outflow samples (FR) after reperfusion were also subjected to hemostasis studies.
In OLT, R–5 is a measurement in the anhepatic phase. Although there is no real anhepatic phase in HLT, during the portal vein anastomosis there is a period of partial clamping of the portal vein. In the pig, this often implies (almost) complete clamping. Blood samples (20 ml) were taken from an arterial line. Eighteen ml of blood was divided equally into two polystyrene test tubes, containing 1 ml ice–cold trisodium citrate (0.11 mol/l) and immediately placed on melting ice. Plasma was collected after centrifugation (2800 g, 4°C, 20 min), snap–frozen and stored in small aliquots at –70°C until used. Two ml blood was also collected into 0.045 ml 15% sol 6.75 mg EDTA.
A normal plasma pool was prepared from equal volume samples obtained from 10 animals immediately after the induction of anaesthesia. This pool was defined as having 100% of normal coagulation and fibrinolysis proteins.

6.2.2 Hemostasis studies

Fibrinolysis: Tissue–type plasminogen activator (t–PA) was assayed according to Verheijen et al.[19] using plasminogen, t–PA stimulator and S–2251 (Kabi Diagnostica, Woerden, The Netherlands) and anti (porcine–heart) t–PA immunoglobulin (from Biopool AB, Umea, Sweden).
To determine the euglobulin clot lysis time (ECLT), standard euglobulin fractions of plasma were prepared at pH 5.9 with a plasma dilution of 1:10.[20] Precipitates were redissolved in Tris/Tween buffer (0.1 M Tris/Hcl, containing 0.1% Tween 80 [v/v] pH 7.5) and to 0.2 ml aliquots of the dissolved euglobulin fractions 0.1 ml portions of calcium–thrombin solution ($CaCl_2$ 25 mmol/l and thrombin 10 NIH U/l) were added to induce clot formation. The lysis time of the clot was recorded. The disappearance of air bubbles was regarded as the endpoint of lysis.
α_2–Antiplasmin (α_2–AP) activity was measured according to Friberger et al.[21] using Coatest® antiplasmin (Kabi Diagnostica, Woerden, The Netherlands).

Coagulation: The activated partial thromboplastin time (aPTT) and prothrombin time (PT) were performed using routine procedures. Reagents used were Actin® (Baxter Dade AG, Düdingen, Switzerland) for the aPTT and Thromborel-S® (Behring Diagnostica, Amsterdam, The Netherlands) for the PT.
The Normotest® (NT) (Nyegaard Diagnostica, Pharmachemie, Haarlem, The Netherlands) was determined according to the manufacturer's instruction. The NT measures coagulation factors VII, X as well as II.
Fibrinogen was measured according to Clauss[22] using thrombin from DiaLab, Leusden, The Netherlands.

6.3 RESULTS

All median values are depicted in Table 6.2. Changes from time to time, grouped according to the hemostasis parameters, are elaborated on in the following paragraph. Data are presented separately for each of the three main variables in this study: the type of transplantation, the effect of graft preservation time, and the effect of PGE_1.

6.3.1 Type of transplantation

Fibrinolysis parameters: The changes in t-PA activity (Figure 6.1) were reciprocal to those in ECLT. In OLT, t-PA activity levels rose significantly during the anhepatic period ($P<0.02$), and increased sharply after reperfusion ($P<0.05$) till the end of the operation. In HLT, t-PA activity levels remained low before reperfusion, increased temporarily after reperfusion ($P<0.01$) and normalized again thereafter. The proportional increase in t-PA activity was similar in HLT to that in OLT immediately after reperfusion. At all sampling points, except R-60 and R+5, the t-PA activity in OLT was significantly higher than in HLT (at R-5: $T=-2.20$, $P=0.03$ and at R+30 through R+90: T between -2.79 and -3.12, $P<0.01$).
t-PA activity levels were higher in the first venous outflow of the graft (FR) in HLT compared to OLT ($T=2.39$, $P=0.02$), while this difference was not yet present in the portal vein blood entering the graft ($T=1.06$, $P=0.32$).

Table 6.2. Hemostatic parameters at the various operative moments. Values are given as median (95% confidence limits of the mean).

Parameter:	R–60	R–5	FR	R+5	R+30	R+60	R+90
t–PA (IU/ml)							
OLT	68 (50–133)	330 (351–1014)	358 (339–1352)	613 (465–1892)	1083 (1053–4259)	1355 (0–9591)	1864 (250–8219)
HLT	48 (39–128)	124 (88–237)	1219 (1231–4356)	563 (527–1203)	220 (336–1240)	498 (295–1091)	255 (94–502)
ECLT (min)							
OLT	>180 >180	>180 (127–168)	121 (100–144)	126 (97–147)	105 (79–132)	134 (65–168)	115 (67–158)
HLT	>180 >180	>180 (179–181[a])	119 (79–137)	170 (119–161)	>180 (138–173)	>180 (139–181[a])	>180 (159–181[a])
α_2–AP (U/ml)							
OLT	99 (84–102)	64 (53–67)	52 (40–53)	43 (41–53)	49 (38–54)	55 (35–67)	57 (49–65)
HLT	99 (90–103)	73 (67–82)	66 (53–71)	52 (49–63)	60 (52–66)	62 (55–69)	69 (62–76)
aPTT (sec)							
OLT	16 (15–17)	18 (18–20)	33 (28–50)	25 (27–61)	31 (33–73)	35 (31–97)	32 (7–145)
HLT	16 (15–16)	16 (11–35)	34.4 (37–78)	25 (23–59)	24 (21–39)	25 (25–40)	21 (17–33)
PT (sec)							
OLT	13 (13–14)	14 (14–16)	20 (19–33)	18 (17–21)	21 (19–28)	25 (21–29)	23 (20–27)
HLT	14 (13–15)	15 (12–24)	23 (24–38)	17 (12–37)	17 (17–19)	19 (17–20)	19 (18–21)
NT (%)							
OLT	50 (45–59)	35 (31–39)	17 (16–22)	21 (20–26)	24 (19–26)	20 (16–27)	24 (19–26)
HLT	52 (45–56)	36 (31–40)	17 (17–22)	27 (23–31)	27 (25–29)	26 (22–29)	29 (24–34)
Fibrinogen (G/L)							
OLT	1.55 (1.36–1.92)	0.80 (0.75–0.99)	0.45 (0.34–0.58)	0.60 (0.45–0.72)	0.64 (0.48–0.75)	0.65 (0.41–0.78)	0.70 (0.58–0.86)
HLT	1.40 (1.34–1.62)	0.85 (0.71–1.02)	0.59 (0.45–0.74)	0.72 (0.59–0.88)	0.70 (0.64–0.94)	0.72 (0.63–0.91)	0.81 (0.67–1.15)
Hemoglobin (mmol/L)							
OLT	5.5 (5.1–5.7)	4.9 (4.5–5.9)	5.0 (4.0–5.3)	4.0 (3.8–4.3)	3.7 (3.1–4.1)	5.3 (4.5–5.6)	[b]
HLT	5.5 (5.4–6.1)	5.1 (4.7–5.5)	5.6 (3.9–5.9)	4.6 (4.2–4.8)	4.1 (3.6–4.5)	5.2 (4.6–5.4)	
Platelets (*10^9/ml)							
OLT	392 (338–432)	315 (264–346)	228 (199–294)	220 (176–282)	171 (153–245)	99 (66–230)	[b]
HLT	370 (327–469)	291 (275–430)	191 (158–296)	223 (199–308)	170 (169–253)	150 (125–267)	

[a] Upper 95%–confidence limit exceeds 180 min.
[b] No samples available.

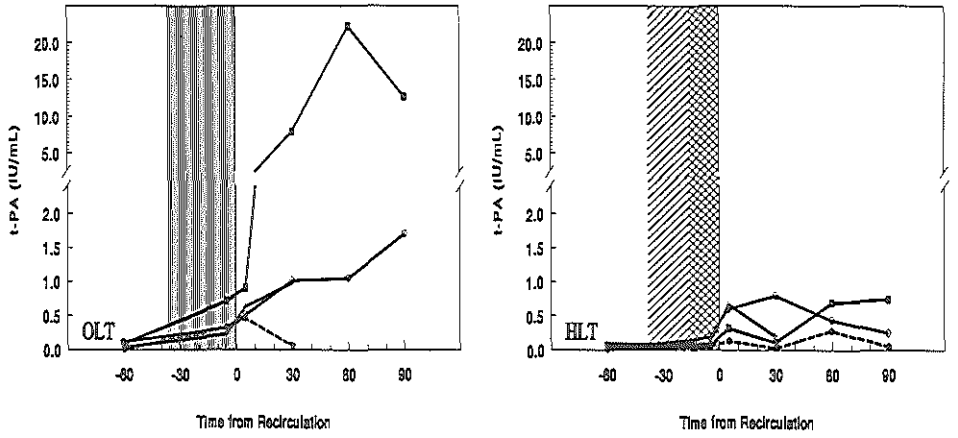


Figure 6.1. Tissue–type Plasminogen Activator (t–PA) during liver transplantation. Values are stated as medians. The horizontal axis gives the time in minutes from or to the moment of recirculation of the graft. Symbols: (OLT) *Orthotopic Liver Transplantation;* (HLT) *Heterotopic Liver Transplantation. Preservation time:* (+——+) *2 Hr;* (o——o) *24 Hr;* (●---●) *48 Hr (line is interrupted since this group consists of only seven animals);* (▪——▪) *72 Hr. Shaded areas:* (▨) *is the anhepatic period in OLT; in HLT,* (▨) *is the period in which the subhepatic inferior vena cava is partially clamped and* (▨) *in which also the portal vein is (partially) clamped.*

α_2–AP levels (Figure 6.2) declined significantly from R–60 through R+5 (P<0.001), and remained almost unchanged thereafter. α_2–AP levels were lower in OLT than in HLT in the postreperfusion period from R+30 through R+90 (T between 2.08 and 3.37, P<0.05).

Coagulation parameters: The PT (Figure 6.3) and aPTT (Figure 6.4) increased slightly in the period before reperfusion and increased significantly thereafter (P<0.001). Before reperfusion and immediately thereafter, there was no significant difference between OLT and HLT for these tests, but in the postreperfusion period, at R+30 and R+60, the PT (T=–3.48 and T=–3.55, P=0.001) and aPTT (T=–3.83 and T=–4.11, P<0.001) were prolonged in OLT compared to HLT.

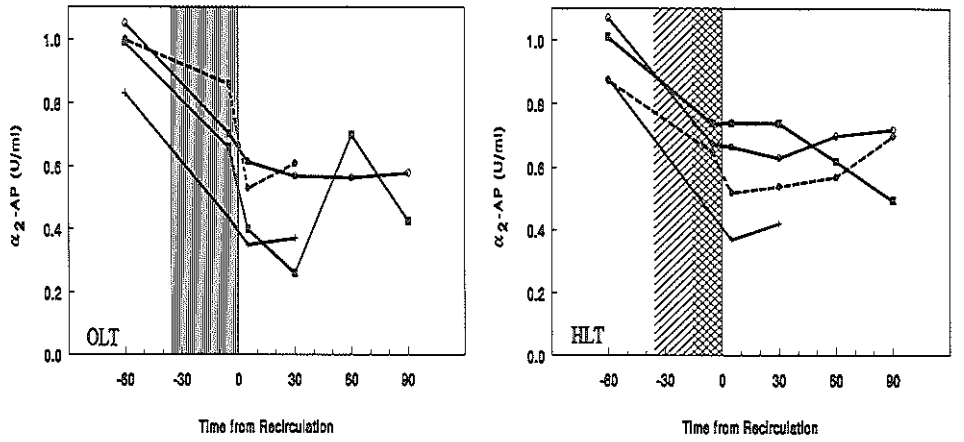


Figure 6.2. α_2–Antiplasmin (α_2–AP) during liver transplantation. Horizontal axis and symbols as in Figure 6.1.

The Normotest (Figure 6.5) declined significantly from the start of the operation till R+5 (P<0.001), but although levels were lower in OLT than in HLT, the difference between OLT and HLT attained no statistical difference (T=0.61, P=0.55). After reperfusion, the Normotest remained stable and after R+5 the difference between OLT and HLT was significant (T between 2.38 and 2.91, P<0.05), to the advantage of HLT. Fibrinogen levels (Figure 6.6) practically halved before reperfusion (P<0.001) without reaching a significant difference between both types of transplantation (P=0.98). After reperfusion, fibrinogen levels remained stable and at R+5 and R+30 the fibrinogen level in OLT was significantly lower compared to HLT (T=2.05 and T=2.09, P<0.05).

6.3.2 Graft storage time

Fibrinolysis parameters: The preservation time had an evident effect on the fibrinolytic parameters, although not yet apparent at R+5 (for t–PA: T=0.45, P=0.66). Especially at R+60 and R+90, the ECLT, the t–PA activity, and α_2–AP deteriorated with increasing preservation times (Figures 6.1 and 6.2).

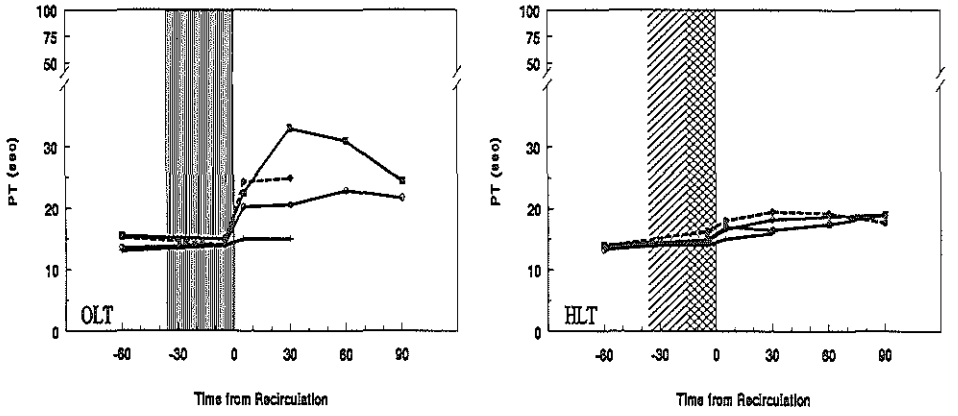


Figure 6.3. Prothrombin Time (PT) in during liver transplantation. Horizontal axis and symbols as in Figure 6.1.

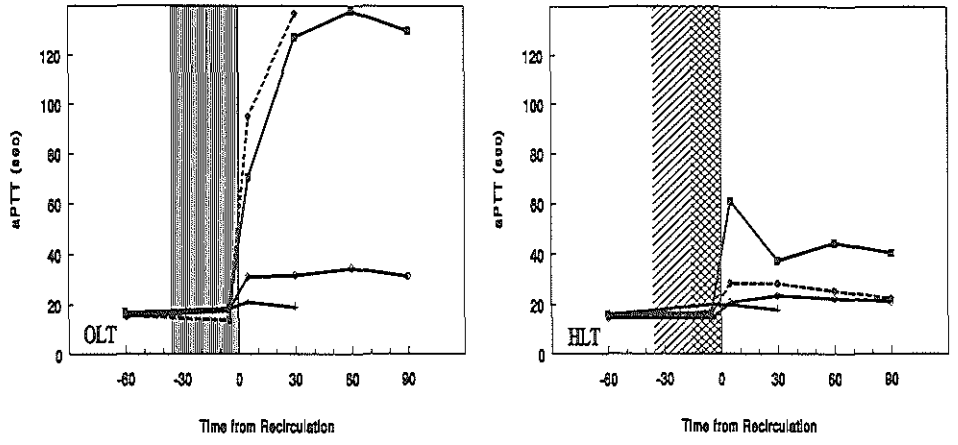


Figure 6.4. Activated Partial Thromboplastin Time (aPTT) during liver transplantation. Horizontal axis and symbols as in Figure 6.1.

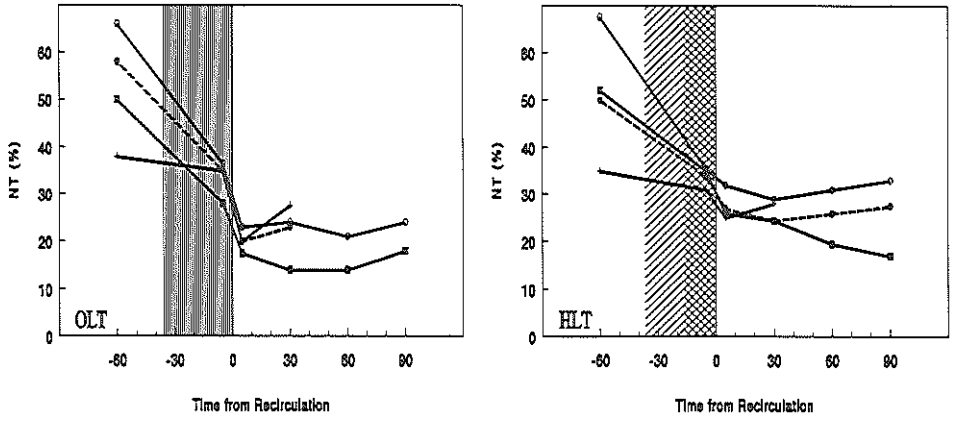


Figure 6.5. Normotest (NT) during liver transplantation. Horizontal axis and symbols as in Figure 6.1.

Coagulation parameters: The multiple regression analysis for the effect of the graft storage time revealed a clear influence on the PT and aPTT levels (Figures 6.3 and 6.4). This was evident in the period after reperfusion, starting at R+5 (T=2.38, P=0.02 and T=3.88, P<0.001) and continuing till R+30 for PT (T=3.17, P<0.01) and R+60 for aPTT (T=3.93, P<0.001).

Also, the Normotest (Figure 6.5) was significantly influenced by the preservation time, although this effect was not present immediately after reperfusion (R+5: T=−0.20, P=0.85), but only from R+30 till R+90 (T around −3.3, P<0.01).

In contrast, fibrinogen levels (Figure 6.6) were not significantly influenced by graft storage time. At R+5 and R+30 a longer ischemic period correlated with a decreased fibrinogen level, but the difference did not reach significance.

The preservation time had an evident effect on the platelet count (all T−values below −3.0 and P<0.005).The first venous outflow (FR) from the 2−hr and 24−hr preserved grafts contained high levels of t−PA activity. No increased t−PA levels could be demonstrated in the first venous outflow from the 48−hr and 72−hr preserved grafts.

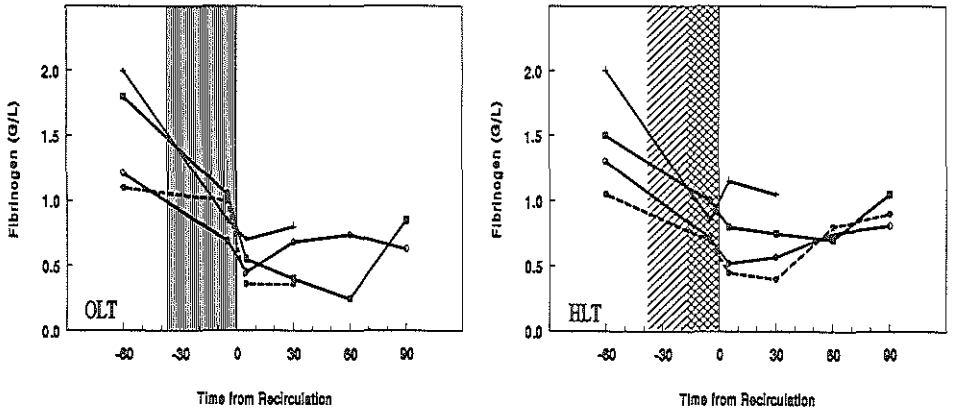


Figure 6.6. Fibrinogen during liver transplantation. Horizontal axis and symbols as in Figure 6.1.

6.3.3 Use of PGE_1

At none of the measured points, PGE_1 had any effect on the coagulation tests or on the fibrinolysis tests (results not shown).

6.4 Discussion

In agreement with previous studies, we found an increase in fibrinolytic activity in the anhepatic period in OLT and not in the corresponding period of HLT.[13,23] Immediately after reperfusion, there was an increase in t–PA activity both in OLT and HLT, but only in OLT the increase in fibrinolytic activity was sustained. These observations suggest that the healthy host liver in HLT prevents the development of hyperfibrinolysis. However, even in HLT, an initial increase in t–PA activity after reperfusion was found, probably because t–PA release after reperfusion is too abundant to be instantaneously cleared by the host liver.

There are several explanations for the increase of t–PA after reperfusion. Endothelial cells, an important source of t–PA, are more susceptible to cold hypoxia and

reperfusion than parenchymal cells.[24,25] The damaged endothelium may release t–PA directly into the circulation. Free oxygen radicals have been implicated in this mechanism of graft injury.[26,27] Also, tissue damage may lead to the attraction and activation of inflammatory cells, including macrophages.[28,29] When activated, these cells release a wide variety of toxic substances, including tumor necrosis factor, interleukin–1, interleukin–6, leukotrienes, platelet activation factor, prostanoids and proteases. These agents may actively interfere with coagulation and fibrinolysis.[30,31] Recently, pretreatment with cyclosporine, a powerful inhibitor of the function of macrophages, has been shown to ameliorate the hemostatic changes after reperfusion in pigs, as measured by prothrombin times, platelet counts, and fibrinogen levels.[32] Riess et al.[5] demonstrated increased levels of lysosomal proteinases (from Kupffer cells and leukocytes) paralleled by the plasma levels of thrombin–antithrombin III complexes as well as by decreasing activities of antithrombin III and C_1–inhibitor after reperfusion of the human liver graft. This suggested intravascular thrombin generation and a dissiminated intravascular coagulation–like constellation in the early postreperfusion period.

The duration of the graft storage and the related damage had an effect on t–PA activity levels after reperfusion. The effects during HLT were much less dramatic than during OLT, which demonstrates that the protective effect of the host liver on the development of hyperfibrinolysis in HLT also holds true when grafts with long cold storage times are used.

Surprisingly, the effect of the duration of the cold storage on the t–PA activity levels was not only found after but also before reperfusion of the graft, when the graft had not yet been connected to the circulation. A possible explanation is provided by the ischemically damaged graft which is placed in the abdominal cavity during the vascular anastomoses. Substances may leak into the abdominal cavity, either t–PA itself or cytokines, which subsequently evoke t–PA release.

High levels of t–PA were found in the first venous outflow from the graft upon reperfusion (FR). Comparison of t–PA activity levels in the portal vein inflow and the venous outflow revealed a gradient across the graft, so t–PA is released by the graft. It is difficult to explain the higher t–PA levels in the first hepatic outflow of the graft in HLT compared to OLT. It might be explained by the partial resection of the liver graft in HLT. This extra handling could make endothelial cells more liable to release t–PA.[33] Furthermore, the resection was performed *ex vivo*, after the graft was flushed with preservation solution. Therefore, clotting at the resection surface might be postponed till the moment of reperfusion.

Notable are the high t–PA activity levels in the 2–hr and 24–hr cold storage grafts. This was not observed in the 48–hr and 72–hr grafts. Damage provoked by a prolonged preservation period might have depleted t–PA from the endothelial cells. Their contents, including t–PA, may have leaked to either the preservation fluid, which is subsequently washed out during flushing before reperfusion or to the abdominal cavity (see above). Despite this t–PA depletion, subsequent contact activation of the blood with the injured graft endothelium may evoke systemic t–PA release as suggested above. Probably, the same process occurred in HLT, but now either t–PA or the cytokines may be cleared by the host liver.

The prereperfusion period in both OLT and HLT was characterized by a decline in the measured coagulation parameters. Fibrinogen declined most serious, with no difference between OLT and HLT. The changes in coagulation parameters are therefore related to the surgical procedure in general, and not specifically to the anhepatic period.

Previous investigators suggested that fibrino*geno*lysis or the use of an external bypass might also contribute to the decrease in fibrinogen levels.[18,34] These factors are unlikely to be of major influence, as in HLT neither signs of fibrinogenolysis were present nor a shunt was used. Although part of the decline can be ascribed to hemodilution, there has to be another explanation since the hematocrit, a measure of hemodilution, did not equally decrease. Most likely a surgically induced activation of the coagulation system and extravasation of hemostasis proteins to the extravascular space are involved.[33]

Reperfusion induced a more serious deterioration in coagulation factors in OLT than in HLT and there was an apparent influence of the graft storage time on coagulation tests. It was observed that the aPTT increased dramatically after reperfusion, while the PT and the Normotest did only mildly. This may be the result of the release of heparin (–like substances) from the graft, as has been suggested earlier.[8,35]

Physiological effects of PGE_1 include vasodilatation, inhibition of platelet–aggregation and stabilization of lysosomal membranes. Since remarkable effects of PGE_1 were found in preventing warm ischemia/reperfusion injury[36] in both experimental[37] and clinical[14] studies, we implemented a trial of PGE_1 in our experiment with long–term preservation. As determined by fibrinolytic and coagulation tests, our study provides no evidence of a protection against long–term cold ischemia/reperfusion damage or the resulting hemostatic disturbances.

As opposed to the multitude of positive studies on PGE_1, we found no protective effect at all. Besides having included not enough animals to detect small differences, there is probably only a narrow margin of therapeutic efficacy, if it exists, between trivial

and irreversible damage. Apparently, the upper limit is surpassed with 24 hr preservation of the porcine liver. In addition, prostaglandins may counteract a physiologic protection mechanism which secludes marginally functioning areas in the graft. This may well prove useful in retrieving reversibly injured parenchyma,[38] but in contrast, it could promote the entrance into the bloodstream of deleterious substances originating from injured cells. Furthermore, PGE_1 was demonstrated to accelerate thrombolysis by t-PA.[39] Obviously, this might have detrimental effects in case of substantial ischemia/reperfusion injury, as shown above.

6.5 CONCLUSION

It was demonstrated that changes in hemostasis in the pig are less pronounced in HLT compared to OLT, in the presence of a healthy host liver (although it became deprived of portal blood after the transplantation). Fibrinolytic activity, especially, was increased in the anhepatic period and postreperfusion period of OLT compared to HLT.

The duration of the graft storage time was related to the severity of the hemostatic changes. This was more apparent in OLT than in HLT, so the host liver in HLT also protects the recipient from the effects of longer preserved grafts. Notably, the cold storage time already exerted an effect on fibrinolytic activity before reperfusion. This is probably due to leakage of humoral factors from the ischemically damaged graft, during the vascular anastomoses.

In the first venous hepatic outflow of the graft, t-PA activity was higher in the HLT than in the OLT group. Next, t-PA activity could only be detected in venous hepatic outflow of the 2-hr and 24-hr preserved grafts and not of the 48-hr and 72-hr preserved grafts. This suggests that after 24-hr cold storage endothelial cells are destroyed and no longer capable of releasing t-PA. However, subsequent contact activation of the blood with the injured endothelium may lead to the production of cytokines, evoking t-PA release by the intact systemic endothelium.

Addition of PGE_1 to the preservation fluid had no effect on the hemostatic changes in both OLT and HLT.

6.6 REFERENCES

1. Kang YG, Martin DJ, Marquez J, Lewis JH, Bontempo FA, Shaw BW,Jr., Starzl TE, Winter PM. Intraoperative changes in blood coagulation and thrombelastographic monitoring in liver transplantation. Anesth Analg 1985; 64:888–896.

2. Von Kaulla KN, Kayne H, von Kaulla E, Marchioro TL, Starzl TE. Changes in blood coagulation before and after hepatectomy or transplantation in dogs and man. Arch Surg 1966; 92:71–79.

3. Blecher TE, Terblanche J, Peacock JH. Orthotopic liver homotransplantation. Arch Surg 1968; 96:331–339.

4. Böhmig HJ. The coagulation disorder of orthotopic hepatic transplantation. Semin Thromb Hemostas 1977; 4:57–82.

5. Riess H, Jochum M, Machleidt W, Himmelreich G, Blumhardt G, Roissaint R, Neuhaus P. Possible role of extracellularly released phagocyte proteinases in coagulation disorder during liver transplantation. Transplantation 1991; 52:482–490.

6. Porte RJ, Bontempo FA, Knot EAR, Lewis JH, Kang YG, Starzl TE. Systemic effects of tissue plasminogen activator–associated fibrinolysis and its relation to thrombin generation in orthotopic liver transplantation. Transplantation 1989; 47:978–984.

7. Dzik WH, Arkin CF, Jenkins RL, Stump DC. Fibrinolysis during liver transplantation in humans: role of tissue–type plasminogen activator. Blood 1988; 71:1090–1095.

8. Homatas J, Wasantapruek S, von Kaulla KN, Eiseman B. Clotting abnormalities following orthotopic and heterotopic transplantation of marginally preserved pig livers. Acta Hepato–splenol 1969; 2:14–27.

9. Mieny CJ, Homatas J, Moore AR, Eiseman B. Limiting functions of a preserved liver homograft. Gastroenterology 1968; 55:179–182.

10. Reuvers CB, Terpstra OT, Boks AL, De Groot GH, Jeekel J, ten Kate FJW, Kooy PPM, Schalm SW. Auxiliary transplantation of part of the liver improves survival and provides metabolic support in pigs with acute liver failure. Surgery 1985; 98:914–921.

11. Reuvers CB, Terpstra OT, ten Kate FJW, Kooy PPM, Molenaar JC, Jeekel J. Long–term survival of auxiliary partial liver grafts in DLA–identical littermate beagles. Transplantation 1985; 39:113–118.

12. Terpstra OT, Schalm SW, Weimar W, Willemse PJA, Baumgartner D, Groenland THN, ten Kate FJW, Porte RJ, de Rave S, Reuvers CB, Stibbe J, Terpstra JL. Auxiliary partial liver transplantation for end-stage chronic liver disease. N Engl J Med 1988; 319:1507-1511.

13. Porte RJ, Blankensteijn JD, Knot EAR, de Maat MPM, Groenland THN, Terpstra OT. A comparative study on changes in hemostasis in orthotopic and auxiliary liver transplantation in pigs. Transplant Int 1991; 4:12-17.

14. Gaber AO, Thistlethwaite JR,Jr., Busse-Henry S, Aboushloe M, Emond JC, Rouch D, Broelsch CE. Improved results of preservation of hepatic grafts preflushed with albumin and prostaglandins. Transplant Proc 1988; 20:992-993.

15. Todo S, Tzakis A, Starzl TE. Preservation of livers with UW or Eurocollins' solution. Transplantation 1988; 46:925-926.

16. Jamieson NV, Sundberg R, Lindell S, Claesson K, Moen J, Vreugdenhil PK, Wight DGD, Southard JH, Belzer FO. Preservation of the canine liver for 24-48 hours using simple cold storage with UW solution. Transplantation 1988; 46:517-522.

17. Bijnen AB, Dekkers-Bijma AM, Vriesendorp HM, Westbroek DL. Value of the mixed lymphocyte reaction in dogs as a genetic assay. Immunogenetics 1979; 8:287-297.

18. Denmark SW, Shaw BW, Griffith BP, Starzl TE. Venous-venous bypass without systemic anticoagulant in canine and human liver transplantation. Surg Forum 1983; 34:380-382.

19. Verheyen JH, Mullaart E, Chang GTG, Kluft C, Wijngaards G. A simple, spectrophotometric assay for the extrinsic (tissue-type) plasminogen activator applicable to measurement in plasma. Thrombos Haemost 1982; 48:266-269.

20. Kluft C, Brakman P, Veldhuijzen-Stolk EC. Screening of fibrinolytic activity in plasma euglobulin fractions on the fibrin plate. In: Davidson JF, Samama MM, Desnoyers PC, eds. Progress in chemical fibrinolysis and thrombolysis. New York:Raven Press, 1976:57-65.

21. Friberger P, Knos M, Gustavsson S, Aurell L, Claeson G. Methods for the determination of plasmin, antiplasmin and plasminogen by means of substrate S-2251. Haemostasis 1978; 7:138-145.

22. Clauss A. Gerinnungsphysiologische Schnell Methode zur Bestimmung des Fibrinogens. Acta Haematol (Basel) 1957; 17:237-246.

23. Bakker CM, Porte RJ, Knot EAR, de Maat MPM, Stibbe J, Terpstra OT. Fibrinolysis in auxiliary partial liver transplantation. Transplant Proc 1990; 22:2305.

24. Marzi I, Zhong Z, Lemasters JJ, Thurman RG. Evidence that graft survival is not related to parenchymal cell viability in rat liver transplantation. The importance of nonparenchymal cells. Transplantation 1989; 48:463–468.

25. Caldwell–Kenkel JC, Currin RT, Tanaka Y, Thurman RG, Lemasters JJ. Reperfusion injury to endothelial cells following cold ischemic storage of rat livers. Hepatology 1989; 10:292–299.

26. De Groot H, Brecht M. Reoxygenation injury in rat hepatocytes – mediation by O_2^- /H_2O_2 liberated by sources other than xanthine oxidase. Biol Chem Hoppeseyler 1991; 372:35–41.

27. Southard JH, Marsh DC, McAnulty JF, Belzer FO. The importance of O_2–derived free radical injury to organ preservation and transplantation. Transplant Proc 1987; 19:1380–1381.

28. Takei Y, Marzi I, Kauffman FC, Currin RT, Lemasters JJ, Thurman RG. Increase in survival time of liver transplants by protease inhibitors and a calcium channel blocker, nisoldipine. Transplantation 1990; 50:14–20.

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

30. Nawroth PP, Handley D, Stern DM. The multiple levels of endothelial cell – coagulation factor interactions. In: Chesterman CN, ed. Clinics in Haematology. 1986:293–321.

31. Nawroth PP, Stern DM. Interaction of vitamin–K–dependent coagulation factors and the endothelium. Cardiovascular Drugs and Therapy 1989; 3:117–119.

32. Kim YI, Kawano K, Nakashima K, Goto S, Kobayshi M. Alleviation of 3.5–hour warm ischemic injury of the liver in pigs by cyclosporine pretherapy. Transplantation 1991; 51:731–733.

33. Hickman R, Bracher M, Pienaar BH, Terblanche J. Heparin as the cause of coagulopathy which may complicate grafting of the liver. Surg Gynecol Obstet 1991; 172:197–206.

34. Rettke SR, Janossy TA, Chantigian RC, Burritt MF, Van Dyke RA, Harper JV, Ilstrup DM, Taswell HF, Wiesner RH, Krom RAF. Hemodynamic and metabolic changes in hepatic transplantation. Mayo Clin Proc 1989; 64:232–240.

35. Stremple JF, Hussey CV, Ellison EH. Study of clotting factors in liver transplantation. Am J Surg 1966; 111:862–869.

36. Ueda Y, Matsuo K, Kamei T, Kayashima K, Konomi K. Protective effect of prostaglandin E_1 (PGE_1) on energy metabolism and reticuloendothelial function in the ischemically damaged canine liver. Liver 1989; 9:6–13.

37. Ueda Y, Matsuo K, Kamei T, Ono H, Kayashima K, Tobimatsu M, Konomi K. Prostaglandin E_1 but not E_2 is cytoprotective of energy metabolism and reticuloendothelial function in the ischemic canine liver. Transplant Proc 1987; 19:1329–1330.

38. Greig PD, Woolf GM, Sinclair SB, Abecassis M, Strasberg SM, Taylor BR, Blendis LM, Superina RA, Glynn MFX, Langer B, Levy GA. Treatment of primary liver graft nonfunction with prostaglandin E_1. Transplantation 1989; 48:447–453.

39. Vaughan DE, Plavin SR, Schafer AI, Loscalzo J. PGE_1 accelerates thrombolysis by tissue plasminogen activator. Blood 1989; 73:1213–1217.

Chapter 7

GENERAL DISCUSSION AND CONCLUSIONS

7.1 INTRODUCTION

Not so very long ago patients with only three types of liver disease were seen by hepatologists: acute hepatitis, chronic hepatitis and cirrhosis. Hepatic cancer was mostly left to the oncologist. Accordingly, attention was only focussed at the complications of end-stage liver disease like ascites, encephalopathy, and variceal bleeding and occasionally at fulminant hepatic failure. The provision of a new liver with or without removal of the affected native organ was considered the ultimate therapeutic step.[1]
Nowadays, liver transplantation is universally accepted, not only for the classic types of liver disease but also for many metabolic hepatic disturbances, as well as for some patients with primary malignancies of the liver. Two major prototypes of liver transplantation are employed. The first one is orthotopic liver transplantation (OLT), in which the diseased host liver is removed and the liver graft is implanted in its place. The other consists of the insertion of an extra liver at a heterotopic –i.e., a non-anatomical- position (auxiliary, heterotopic liver transplantation, HLT). This approach leaves the recipient's liver intact. Auxiliary liver segments may also be transplanted in an orthotopic position after resecting a portion of the recipient liver. Although this method was occasionally performed in man,[2,3] it is still in an early experimental phase.[4]
For a long time, the theoretical advantages of leaving the native liver *in situ* could not be translated into satisfactory clinical results. As good results of HLT have been reported,[5] this period may come to an end. Now it should be established which type of transplantation is preferable when both OLT and HLT are optional.
A clear indication for OLT is hepatic malignancy. HLT may be considered for high-risk, chronic patients, for some metabolic diseases, and for acute hepatic failure with a potential for regeneration. However, clinical and experimental comparative studies are needed to support the decision between OLT and HLT.
Intraoperative hemodynamic and hemostatic changes, the tolerance of preservation injury, immediate and long-term graft function of synthesis, clearance, and excretion, the effects on portal hypertension and variceal bleeding, the regeneration and atrophy of the graft and the diseased native liver, the risk of carcinoma in the host liver, and long-term graft and patient survival are only few of the multitude of aspects of liver transplantation which should be dealt with.

This thesis reports on the intraoperative hemodynamic and hemostatic changes in the pig during OLT and HLT, on the effects of long-term graft preservation and on the value of prostaglandins in protecting the graft from storage damage. Our experimental model resembles liver transplantation in patients without portal hypertension- e.g., in children with a hepatic inborn error of metabolism.
In this chapter, the main issues and possible clinical consequences will be reviewed per intraoperative stage: the preparation period, the period of portal flow interruption, the early postreperfusion period, and the late postreperfusion period.

7.2 PREPARATION PERIOD

The removal of the host liver is potentially dangerous for a patient with end-stage liver disease as severe hemostatic disturbances and portal hypertension often exist. In our animal model healthy pigs were transplanted and therefore the theoretical advantages of omitting the dissection of the native liver in clinical OLT could not be assessed. Still, we reported an elevated systemic vascular resistance (SVR) and decreased cardiac output (CO) in OLT during dissection of the host liver. This could be related to the retracted and compressed position of the liver in that period, although intersection of autonomic, vasoregulatory nerves might also be involved.[6]
Preoperatively, in patients with cirrhosis, commonly a hyperdynamic circulation state exists, with increased CO and decreased SVR.[7-9] This could be a result of a vasodilator substance, either released by or not metabolized by the diseased liver, or a physiologically necessary vasoconstrictor which is inappropriately metabolized by the diseased liver.[10] Other substances, including prostaglandins, false neurotransmitters, and vasoactive intestinal polypeptide are mentioned in the pathogenesis of the hyperdynamic state.[11,12] Besides vasoactive substances the presence of abundant portacaval collateral circulation or the inability of the cirrhotic liver to respond to vasoregulatory nerve stimuli could also be responsible. Irrespective the cause of the hyperdynamic state, an elevation of the SVR as a result of dissection of a cirrhotic liver will hardly be of clinical importance. On the contrary, when liver transplantation is performed for metabolic deficiencies -and therefore with a normal host liver- the SVR might respond the same way as in our experiment. Also, in fulminant hepatic failure with intracranial hypertension, a fall in cardiac output during the preparation period may rapidly lead to cerebral edema and death.
Besides hemodynamic differences between OLT and HLT in this stage, another clear advantage of HLT is the shorter duration of the preparation period. While in clinical

OLT, recipient hepatectomy can be very time–consuming, preparations for HLT are not very different from a portacaval anastomosis. Time–saving is also important in transplanting patients with fulminant hepatic failure.
Prostaglandin E_1 (PGE_1) administered during the preparation period will cause splanchnic vasodilatation and decreased SVR.[13] Particularly in patients with portal hypertension, this could be a drawback regarding blood loss and hemodynamic condition in this phase. These effects could not be demonstrated in our experiments.

7.3 PORTAL FLOW INTERRUPTION PERIOD

In OLT, in the anhepatic phase, hemodynamic changes are dictated by the introduction of the venovenous bypass and the removal of the liver. The removal of the arterial bed will decrease the venous return from the liver and this will almost instantaneously decrease CO since the heart maintains permissive pumping capacity. Furthermore, with removal of the liver, vasoactive substances[14] and gut–derived bacterial endotoxin,[15,16] that would otherwise be removed from the blood by the liver, might have significant effects on the hemodynamic condition. In addition, in OLT the portal vascular resistance is substituted by the resistance of the portavenous bypass. Together with the effect of local mesenteric regulatory mechanisms, the combination of these factors makes it difficult to predict or explain the hemodynamic changes in the anhepatic phase, the more so in case of portal hypertension. In our animal model in OLT, SVR increased and CO dropped. This is in agreement with others in human[17] and animal transplantation,[18,19] although bypass techniques were different.
A venovenous bypass can provide enhanced cardiovascular stability compared to procedures without bypass,[20] although adequate decompression and maintenance of circulating blood volume are not assured. Typically, even with the use of a pump–assisted bypass, the CO and shunt flow continuously decrease as a result of fluid extravasation from the vascular space to the interstitium.[17] Nevertheless, most centers nowadays routinely make use of a venovenous bypass.
From our HLT experiments, it became clear that even partial clamping of the portal vein in combination with (partial) clamping of the caval vein in the healthy pig often leads to significant hemodynamic impediment. In OLT, we therefore used an iliacoportajugular bypass. As stable hemodynamics were achieved during passive bypass, a pumping device was expendable.
In HLT, the portal vein interruption lasted only 15 min while the anhepatic phase in OLT continued for almost an hour. Still, the hemodynamic condition in HLT was

worse compared to OLT, in which the bypass provided sufficient venous return. With liver cirrhosis, venous return is maintained by portacaval collaterals shunting the mesenteric blood flow. Moreover, in man the portal vein can be partially clamped, which is rarely the case in the piglet. Indeed, our clinical experience with HLT is that CO hardly responds to partial clamping of the portal vein.[21]

These experimental results are of clinical importance when liver transplantation is performed in patients without portacaval collaterals –e.g., in acute liver failure or in children with an inborn error of hepatic metabolism.

Concerning changes in the hemostatic mechanism, HLT takes definite advantage of the remaining synthetic and clearing function of the host liver. Additionally, a decrease in the fibrinogen level has been attributed to the use of a venovenous bypass previously.[9,22]

The earliest reports on liver transplantation already described increased fibrinolytic activity during manipulation of the liver and after its removal.[23] Since t–PA–assays are available, the relation between hemostatic disturbances, high t–PA levels and increased fibrinolysis can be studied.[24] Normally, t–PA is removed from the circulation by the liver. In OLT, t–PA can accumulate in the anhepatic phase, while additional release is also likely.

However, we found a similar decline of fibrinogen levels in both OLT and HLT in the portal vein interruption period. Moreover, Hickman et al.[25] demonstrated no significant changes in the hemostasis profile from laparotomy, hypothermia, or the insertion of a portasystemic bypass. The decline in coagulation factors may be a simple reflection of the general decrease in plasma protein during hepatic transplantation.[26]

The clinical implications of our hemostatic data especially pertain to patients with extensive parenchymal liver damage (chronic active hepatitis), who have low concentrations of most plasmatic coagulation and fibrinolysis factors. When these patients are exposed to additional hemodynamic and hemostatic derangements during the anhepatic stage, they are compromised already as they embark upon the critical phase of reperfusion.

7.4 Early postreperfusion period

This is the most crucial period in liver transplantation. In HLT, but also in OLT with venovenous bypass, congestion of the obstructed portal and systemic venous beds exists. Stagnant blood from the (partially) obstructed venous beds may be rich in –amongst others– acid substances and potassium but also endotoxin[16]. At reperfusion,

it passes a more or less ischemically damaged graft and probably takes with it remnants of the cold preservation solution, air–emboli, cellular debris, activated cytokines and pro– and anticoagulants. The newly perfused graft is probably not immediately capable of clearing the portal blood from endotoxin, released by intestinal bacterial flora.[27]

Furthermore, depending on the amount of preservation damage, neutrophils but also platelets can adhere to the sinusoidal lining cells as will be discussed below. With severe preservation damage or subsequent reperfusion injury, this may cause obstruction of hepatic blood flow. In our experiments, this corresponded with the abrupt fall in hemoglobin levels and platelet counts at reperfusion and with the increased portal vein pressure after transplantation.

Although in first instance restoration of the normal venous return from the portal vein re–establishes mean arterial pressure (MAP) and cardiac output (CO), other hemodynamic effects also occur at recirculation. In earlier reports, vasoactive substances were mainly found to have vasodilatory effects on both pulmonary and systemic vascular beds.[9,18,19] Although we confirmed the release of vasodilatory substances at reperfusion by recording a decrease in SVR, we demonstrated an increase in pulmonary vascular resistance (PVR). Assuming the vasoactive substances are released by the graft, this seems inconsistent.

We demonstrated that the effect on the SVR intensified with increasing preservation damage, while the effect on the PVR did not. We therefore hypothesized that factors related to liver reperfusion itself that cannot pass the pulmonary vascular bed (like cellular debris and air–emboli, and possibly temperature), surpass the vasodilatory effects on the PVR of other, also systemically effective, substances.

As early as 5 min after recirculation, depression of myocardial function was found in OLT. In HLT, this effect could not be demonstrated and it was postulated that the responsible substances –which we called myocardial depressant factors (MDF)– are effectively cleared from the circulation by the native liver.

A similar theory is conceivable about activated clotting and fibrinolytic factors, although most hemostatic disturbances did not emerge as early as the hemodynamic alterations.

The endothelial cells play an important role in the regulation of the coagulation and fibrinolysis systems. First, they cover the subendothelial tissue which is highly thrombogenic. Second, they are able to inhibit platelet aggregation by the production of prostaglandin I_2. Third, in the endothelial membrane, thrombin–binding receptors are present (thrombomodulin). The thrombomodulin–thrombin–complex both inhibits

further thrombin formation and enhances fibrinolysis. Finally, t–PA and its inhibitor PAI are present in the endothelial cells and release of these substances can be initiated by a variety of mediators.

Earlier, t–PA levels have been demonstrated to increase rapidly after reperfusion,[28,29] while other investigators believed that t–PA–release from the graft was not a major determinant of hemostatic disorders in liver transplantation. [30,31]

In the present study, t–PA measurements showed significant changes during transplantation. Particularly after reperfusion, systemic t–PA activity levels increased, but there were no immediate differences between OLT and HLT. We demonstrated that this early raise in t–PA levels was most likely caused by its release from the endothelium of the graft and that this could be seen as a manifestation of preservation or reperfusion injury.

However, with extreme preservation times, t–PA–release was not so high as endothelial cells were no longer viable to produce t–PA. This correlated well with the morphological findings of endothelial lining cells showing severe loss of cohesion after 72 hr of preservation.

t–PA is quickly removed from the circulation by the liver. Therefore, t–PA levels did not escalate in HLT, while they did in OLT. On the other hand, when measured in the first reperfusion blood, t–PA levels were higher in HLT compared to OLT. This appeared to be caused by an intrinsic t–PA–release in the graft in HLT, since t–PA levels in OLT and HLT were similar in the flushing solution and in the portal blood immediately before entering the graft. One theory is that this phenomenon is related to the resection in HLT, which is performed during bench surgery in the absence of normal clotting blood. The raw endothelial surface could release large quantities of t–PA at the moment that platelets and leukocytes arrive. This theory is supported by the fact that the decline of hemoglobin and platelet counts at reperfusion was more obvious in HLT. The accumulation of platelets in the newly grafted liver was demonstrated earlier.[32]

The differences in t–PA levels are of importance in human liver transplantation. Although t–PA can be inactivated by PAI, the clinical effect may extend over a longer period, as t–PA may become incorporated in hemostatic clots, resulting in early lysis and insufficient hemostatic function.

We demonstrated that after long–term preservation, endothelial cells loose their contact with the underlying hepatocytes. This exposure of the subendothelial space can be expected to activate coagulation. Also monocytes and macrophages and possibly Kupffer cells could be stimulated to release a variety of mediators, including tumor

necrosis factor (TNF), interleukin-1 (IL-1), interleukin-6, leukotrienes, platelet activation factor, prostanoids and proteases. Some of these agents (e.g. TNF) may induce expression of thromboplastin at the endothelial surface, which in its turn can activate the plasma coagulation factor VII. Furthermore, coagulation may be enhanced and fibrinolysis impaired by TNF and IL-1 by down-regulation of thrombomodulin. These reactions are supposedly not limited to the endothelial cells of the graft and could therefore be responsible for the development of disseminated intravascular coagulation (DIC).[33,34]

7.5 LATE POSTREPERFUSION PERIOD

Despite adequate preload, MAP and CO continued to decline 30 - 90 min after recirculation. This effect was related to the preservation time and more prominent in OLT. Again, HLT was considered to protect the circulation from MDF.

In OLT, we also found continuously increasing t-PA levels in the postreperfusion period. This effect was particularly evident after 72-hr preservation. Since we postulated that the graft-endothelium is no longer capable of producing large quantities of t-PA after 72-hr preservation, t-PA is most likely produced by the recipient endothelium. This endothelium -in its turn- should have been stimulated to do so. Therefore, we hypothesize that late escalation of t-PA in OLT is caused by cytokines that are produced in the damaged graft and subsequently activate the intact recipient systemic endothelium to release (probably amongst others) t-PA. As this is a relatively slow effect it can be assumed to be related to reperfusion injury rather than ischemic damage. Probably, the same process occurred in HLT, but this effect was masked as t-PA or the activating cytokines were cleared from the blood by the native liver.

Early experience with UW solution suggested that preservation times of 24 hr or more were attainable.[35] However, evidence accumulates that -even in UW solution- preservation injury arises much earlier.[36-38] In our animal model, increased portal vein pressure after transplantation, as a result of ischemic or reperfusion damage, corresponded well with the morphological findings, indicating that the extent of preservation injury already passed the point of no return. In man,[39-41] but also in dogs[35] and rats[42], successful liver transplantation was accomplished after more than 24-hr preservation. It may be postulated that the pig is more susceptible to the consequences of preservation damage. This is in agreement with others who use the porcine liver transplantation model to study long-term preservation.[43]

In our experiments, the better survival after HLT compared to OLT must exclusively be attributed to the remaining function of the native liver.

7.6 PROSTAGLANDINS

In the late seventies, the term cytoprotection was introduced to describe the protective effect of prostaglandins on the gut mucosa.[44] Although the exact mechanism was not known, it soon became clear that this effect also pertained to the liver and kidney.[45] Various prostaglandins (I_2,[46], E_2[47], and E_1[48]) were tested for their protective property. Preservation and reperfusion damage have been attributed to destruction of microvascular beds, microsludging, vascular spasm,[49] and TNF–release by Kupffer cells.[50,51] Therefore, the effects of prostaglandins –vasodilatation, inhibition of platelet–aggregation, stabilization of lysosomal membranes, and inhibition of TNF–production[52]– are attractive properties in preventing liver preservation damage.
It is debatable at which moment prostaglandins should be given, for how long and in what dose. Improved results were described by donor pre–treatment[49] and addition to the preservation[53] and flushing solutions.[54] Since remarkable effects of PGE_1 and not of PGE_2 were found in both experimental[48] and clinical[53] studies, we implemented a trial of PGE_1 in our experiment with long–term preservation. To achieve maximum effect we included prostaglandins in both preservation and flushing solutions and pre–treated the donor and acceptor animal.
We showed that the use of PGE_1 was related with increased blood loss and amounts of infusion fluids and with lower MAP after reperfusion in the absence of a significant effect on the SVR. The increased blood loss corresponded with the accelerating effect of PGE_1 on thrombolysis by t–PA.[55] In addition, we were not able to demonstrate significant morphological signs of cytoprotection.
Considering the multitude of proposed beneficial effects of prostaglandins in organ preservation, it is difficult to explain why we found no protective effect at all. Besides having included not enough animals to detect small differences, there is probably only a narrow margin of therapeutic efficacy, if it does exist. Below a certain degree of preservation injury, changes are too small to be detected, let alone be prevented, while above a certain extent of storage time, irreversible injury causes inevitable failure of the graft, with or without prostaglandins. This upper limit is apparently surpassed with 24–hr preservation of the porcine liver.
In addition, prostaglandins can be assumed to counteract a physiological protective mechanism that secludes marginally functioning areas in the graft. This may well prove

useful in retrieving reversibly injured parenchyma, but in contrast it could promote the entrance in the bloodstream of deleterious substances that originated from injured cells. In this respect, it is noticeable that endogenous prostanoids were also shown to play a role in liver transplantation.[56]
Although prostaglandins may be valuable in preventing warm ischemic and reperfusion injury,[49] and although reversal of primary graft nonfunction by continuous post-operative treatment with PGE_1 was achieved,[57,58] our study provides no evidence of a remarkable protection in long-term cold ischemic and reperfusion damage.

7.7 CONCLUSIONS

7.7.1 Are hemodynamic conditions during HLT better than during OLT?

In OLT, the hemodynamic condition in the anhepatic phase was better compared to the corresponding portal vein interruption period in HLT. In the absence of adequate portacaval bypass, clamping of the portal vein decreases venous return considerably. These changes in HLT should be anticipated in liver transplantation in patients without portal hypertension, although in man total clamping of the portal vein can usually be avoided due to the larger diameter of the vessel.
After reperfusion myocardial depression was demonstrated in OLT. Substances produced or inadequately cleared by a faulty preserved graft may be responsible. In HLT, the hemodynamic condition after reperfusion was better compared to OLT, especially when preservation damage increased. The remaining (clearing) function of the native liver may play an important role.
The exact nature of the myocardial depressant factors should be the subject of further study. The dissimilar reactions of the pulmonary and systemic vascular beds on graft recirculation indicates that a multifactorial process exists.

7.7.2 Are hemostatic changes in HLT less pronounced than in OLT?

During the anhepatic phase in OLT, hyperfibrinolysis occurs as a result of reduced hepatic clearance of t-PA. In patients with pre-existent severe clotting disturbances, additional hemostatic derangements during the anhepatic stage may further deteriorate their tolerance to the forthcoming reperfusion phase. In HLT, these problems do not occur, as the anhepatic stage is absent.

Early postreperfusion hyperfibrinolysis was caused by t–PA release from the reperfused graft, to the same extent in OLT and HLT. In OLT a late escalation of t–PA was demonstrated that could not be found in HLT. This delayed increase in t–PA levels in OLT might be caused by cytokines that are produced in the damaged graft and subsequently activate the intact recipient systemic endothelium to produce t–PA.

7.7.3 Are the hemodynamic and hemostatic effects of long–term preservation better tolerated by the recipient of an auxiliary graft?

The remaining clearing function of the native liver in HLT prevents the development of hyperfibrinolysis, even with longer preservation times. Release of t–PA from the graft is an early phenomenon on reperfusion. However, in case of severe graft damage, a massive release of cytokines and expression of thromboplastin could induce DIC. This may be associated with a delayed increase of fibrinolytic activity by systemic t–PA release from activated endothelial cells, particularly in the absence of normal hepatic clearance.
Although a diseased liver may not be able to clear t–PA, thromboplastin, cytokines, as effectively as the healthy liver, some remaining liver function is always present. Thus, our results indicate that the recipient liver can provide significant support during HLT, especially when preservation damage causes a release of cytokines.

7.7.4 Are prostaglandins effective in preventing liver injury after long–term preservation with UW solution?

Administration of PGE_1 to the donor and the recipient together with PGE_1 in the preservation and flush solutions did not yield significant beneficial effects on the intraoperative hemodynamic and hemostatic condition, nor on morphological appearance. There is probably only a narrow margin of therapeutic efficacy of prostaglandins in preventing preservation damage. Apart from any presumed value of prostaglandins, the possibility of detrimental effects, particularly after long–term preservation, should also be considered.

In conclusion, we demonstrated that the amount of preservation damage to the graft is an important determinant of the hemodynamic and hemostatic disturbances in liver transplantation. In HLT, the presence of some remaining liver function during the implantation and after recirculation of the liver graft, could give vital protection from these disturbances. This will have particular merits in high-risk patients or in case a certain degree of preservation damage is established or suspected in a liver graft.
Clinical comparative studies are necessary to support the decision between OLT and HLT when both procedures are optional. The answers provided by the experiments described in this thesis may serve as a basis for further clinical research on the potential benefits of the recipient liver.

7.8 REFERENCES

1. Van Thiel DH, Dindzans V, Schade RR, Gavaler JS, Tarter RE. Liver transplantation: the promise of the past and the future. Transplant Proc 1988; 20:490–493.

2. Bismuth H, Houssin D. Partial resection of liver grafts for orthotopic or heterotopic liver transplantation. Transplant Proc 1985; 17:279–283.

3. Gubernatis G, Pichlmayr R, Kemnitz J, Gratz K. Auxiliary partial orthotopic liver transplantation (APOLT) for fulminant hepatic failure: first successful case report. World J Surg 1991; 15:660–666.

4. Then PK, Feldman L, Broelsch CE. Flow and vascular resistance measurements in auxiliary liver segments transplanted in orthotopic position. Transplant Proc 1989; 21:2378–2380.

5. Metselaar HJ, Hesselink EJ, de Rave S, Groenland THN, Bakker CM, Weimar W, Schalm SW, Terpstra OT. A comparison between heterotopic and orthotopic liver transplantation in patients with end-stage chronic liver disease. Transplant Proc 1991; 23:1531–1532.

6. Eiseman B, Knipe P, Koh Y, Normell L, Spencer FC. Factors affecting hepatic vascular resistance in the perfused liver. Ann Surg 1963; 157:532–547.

7. Johnson G,Jr., Dart CH,Jr., Peters RM, Macfie JA. Hemodynamic changes with cirrhosis of the liver. Ann Surg 1966; 163:692–703.

8. Glauser FL. Systemic hemodynamic and cardiac function changes in patients undergoing orthotopic liver transplantation. Chest 1990; 98:1210–1215.

9. Rettke SR, Janossy TA, Chantigian RC, Burritt MF, Van Dyke RA, Harper JV, Ilstrup DM, Taswell HF, Wiesner RH, Krom RAF. Hemodynamic and metabolic changes in hepatic transplantation. Mayo Clin Proc 1989; 64:232–240.

10. Hendriksen H, Ring-Larsen H, Christensen NJ. Circulating noradrenaline and central haemodynamics in patients with cirrhosis. Scand J Gastroenterol 1985; 20:1185–1190.

11. Bruix J, Bosch J, Kravetz D, Mastai R, Rodes J. Effects of prostaglandin inhibition on systemic and hepatic hemodynamics in patients with cirrhosis of the liver. Gastroenterology 1985; 88:430–435.

12. Hendriksen H, Staun-Olsen P, Fahrenkrug P, Ring-Larsen H. Vasoactive intestinal polypeptide (VIP) in cirrhosis: arteriovenous extraction in different vascular beds. Scand J Gastroenterol 1980; 15:787-792.

13. Szczeklik A, Gryglewski RJ. Clinical pharmacology of Prostacyclin. New York: Raven Press, 1981:159-167.

14. Falcini F, Martini E, Marsili M, Benassai C, Fabbri LP, Tanini R, Linden M, Simoncini R, Filipponi F, Cataliotti L. Veno-venous bypass in experimental liver transplantation: portal-jugular versus caval-portal-jugular. G Chir 1990; 11:206-210.

15. Miyata T, Todo S, Selby R, Yokoyama I, Tzakis A, Starzl TE. Endotoxaemia,pulmonary complications, and thrombocytopenia in liver transplantation. Lancet 1989; 2:189-191.

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

17. Paulsen AW, Whitten CW, Ramsay MA, Klintmalm GB. Considerations for anesthetic management during veno-venous bypass in adult hepatic transplantation. Anesth Analg 1989; 68:489-496.

18. Ringe B, Bornscheuer A, Blumhardt G, Bechstein WO, Wonigeit K, Pichlmayr R. Experience with veno-venous bypass in human liver transplantation. Transplant Proc 1987; 19:2416.

19. Paulsen AW, Valek TR, Blessing WS, Johnson DD, Parks RI, Pyron JT, Ramsay MA, Simpson BR, Swygert T, Walling P, Klintmalm GB. Hemodynamics during liver transplantation with veno-venous bypass. Transplant Proc 1987; 19:2417-2419.

20. Shaw BW,Jr., Martin DJ, Marquez JM, Kang YG, Bugbee AC,Jr., Iwatsuki S, Griffith BP, Hardesty RL, Bahnson HT, Starzl TE. Venous bypass in clinical liver transplantation. Ann Surg 1984; 200:524-534.

21. Groenland THN, Visser L, Terpstra OT, Terpstra JL, Reuvers CB, Baumgartner D, Schalm SW. Stable hemodynamics during heterotopic auxiliary partial liver transplantation for end-stage liver cirrhosis. Transplant Proc 1988; 20:538-540.

22. Denmark SW, Shaw BW, Griffith BP, Starzl TE. Venous-venous bypass without systemic anticoagulant in canine and human liver transplantation. Surg Forum 1983; 34:380-382.

23. Starzl TE, Marchioro TL, von Kaulla KN, Herman G. Homotransplantation of the liver in humans. Surg Gynecol Obstet 1963; 117:659-676.

24. Verheyen JH, Mullaart E, Chang GTG, Kluft C, Wijngaards G. A simple, spectrophotometric assay for the extrinsic (tissue-type) plasminogen activator applicable to measurement in plasma. Thrombos Haemost 1982; 48:266–269.

25. Hickman R, Bracher M, Pienaar BH, Terblanche J. Heparin as the cause of coagulopathy which may complicate grafting of the liver. Surg Gynecol Obstet 1991; 172:197–206.

26. Böhmig HJ. The coagulation disorder of orthotopic hepatic transplantation. Semin Thromb Hemostas 1977; 4:57–82.

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

28. Porte RJ, Knot EAR, de Maat MPM, Willemse PJA, Schalm SW, Stibbe J, Groenland THN, Terpstra OT. Fibrinolysis detected by thrombelastography in heterotopic, auxiliary liver transplantation: effect of tissue-type plasminogen activator. Fibrinolysis 1988; 2 (Suppl 3):67–73.

29. Dzik WH, Arkin CF, Jenkins RL, Stump DC. Fibrinolysis during liver transplantation in humans: role of tissue-type plasminogen activator. Blood 1988; 71:1090–1095.

30. Arnoux D, Boutiere B, Houvenaeghel M, Rousset-Rouviere A, Le Treut P, Sampol J. Intraoperative evolution of coagulation parameters and t-PA/PAI balance in orthotopic liver transplantation. Thromb Res 1989; 55:319–328.

31. Suzumura N. Coagulation disorders during orthotopic liver transplantation. Nippon Geka Gakkai Zasshi 1989; 90:847–854.

32. Hutchison DE, Genton E, Porter KA, Daloze PM, Huguet C, Brettschneider L, Groth CG, Starzl TE. Platelet changes following clinical and experimental hepatic homotransplantation. Arch Surg 1968; 97:27–33.

33. Kim YI, Kawano K, Nakashima K, Goto S, Kobayshi M. Alleviation of 3.5-hour warm ischemic injury of the liver in pigs by cyclosporine pretherapy. Transplantation 1991; 51:731–733.

34. Riess H, Jochum M, Machleidt W, Himmelreich G, Blumhardt G, Roissaint R, Neuhaus P. Possible role of extracellularly released phagocyte proteinases in coagulation disorder during liver transplantation. Transplantation 1991; 52:482–490.

35. Jamieson NV, Sundberg R, Lindell S, Claesson K, Moen J, Vreugdenhil PK, Wight DGD, Southard JH, Belzer FO. Preservation of the canine liver for 24–48 hours using simple cold storage with UW solution. Transplantation 1988; 46:517–522.

36. Furukawa H, Todo S, Imventarza O, Wu YM, Scotti C, Day R, Starzl TE. Cold ischemia time vs outcome of human liver transplantation using UW-solution. Transplant Proc 1991; 23:1550-1551.

37. Sanchez-Urdazpal L, Gores G, Ward E, Maus T, Wahlstrom H, Wiesner RH, Krom RAF. Non-anastomotic biliary strictures after orthotopic liver transplantation. 5th Congres European Society for Organ Transplantation 1991; 73 (Abstract).

38. Adam R, Morino M, Diamond T, Astarcioglu I, Johann M, Azoulay D, Bao YM, Bismuth H. Influence of prolonged cold ischaemia using UW solution on graft function and outcome following liver transplantation. 5th Congres European Society for Organ Transplantation 1991; 140 (Abstract).

39. Kalayoglu M, Sollinger HW, Stratta RJ, D'Alessandro AM, Hoffmann RM, Pirsch JD, Belzer FO. Extended preservation of the liver for clinical transplantation. Lancet 1988; 1:617-619.

40. Todo S, Tzakis A, Starzl TE. Preservation of livers with UW or Eurocollins' solution. Transplantation 1988; 46:925-926.

41. Todo S, Nery JR, Yanaga K, Podesta L, Gordon RD, Starzl TE. Extended preservation of human liver grafts with UW solution. JAMA 1989; 261:711-714.

42. Sumimoto R, Jamieson NV, Wake K, Kamada N. 24-hour rat liver preservation using UW solution and some simplified variants. Transplantation 1989; 48:1-5.

43. Manner M, Shult W, Senninger N, Machens G, Otto G. Evaluation of preservation damage after porcine liver transplantation by assessment of hepatic microcirculation. Transplantation 1990; 50:940-943.

44. Robert A, Nezamis JE, Lancaster C, Hanchar J. Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury. Gastroenterology 1979; 77:433-443.

45. Araki H, Lefer AM. Cytoprotective actions of prostacyclin during hypoxia in the isolated perfused cat liver. Am J Physiol 1980; 238:H176-H181.

46. Monden M, Fortner JG. Twenty-four- and 48-hour canine liver preservation by simple hypothermia with prostacyclin. Ann Surg 1982; 196:38-42.

47. Sikujara O, Monden M, Toyoshima K, Okamura J, Kosaki G. Cytoprotective effect of prostaglandin I_2 on ischemia-induced hepatic cell injury. Transplantation 1983; 36:238-243.

48. Ueda Y, Matsuo K, Kamei T, Ono H, Kayashima K, Tobimatsu M, Konomi K. Prostaglandin E_1 but not E_2 is cytoprotective of energy metabolism and reticuloendothelial function in the ischemic canine liver. Transplant Proc 1987; 19:1329–1330.

49. Ueda Y, Matsuo K, Kamei T, Kayashima K, Konomi K. Protective effect of prostaglandin E_1 (PGE_1) on energy metabolism and reticuloendothelial function in the ischemically damaged canine liver. Liver 1989; 9:6–13.

50. Nagakawa J, Hishinuma I, Hirota K, Miyamoto K, Yamanaka T, Tsukidate K, Katayama K, Yamatsu I. Involvement of tumor necrosis factor–α in the pathogenesis of activated macrophage–mediated hepatitis in mice. Gastroenterology 1990; 99:758–765.

51. Takei Y, Marzi I, Kauffman FC, Currin RT, Lemasters JJ, Thurman RG. Increase in survival time of liver transplants by protease inhibitors and a calcium channel blocker, nisoldipine. Transplantation 1990; 50:14–20.

52. Moran M, Mozes MF, Maddux MS, Veremis S, Bartkus C, Ketel B, Pollak R, Wallemark C, Jonasson O. Prevention of acute graft rejection by the prostaglandin E_1 analogue misoprostol in renal–transplant recipients treated with cyclosporine and prednisone. N Engl J Med 1990; 322:1183–1188.

53. Gaber AO, Thistlethwaite JR,Jr., Busse–Henry S, Aboushloe M, Emond JC, Rouch D, Broelsch CE. Improved results of preservation of hepatic grafts preflushed with albumin and prostaglandins. Transplant Proc 1988; 20:992–993.

54. Ontell SJ, Makowka L, Mazzaferro V, Trager J, Ove P, Starzl TE. The protective effect of SRI 63–441 on ischemic liver injury using the isolated perfused rat liver:combined protocol with superoxide dismutase. Transplant Proc 1988; 20:972–973.

55. Vaughan DE, Plavin SR, Schafer AI, Loscalzo J. PGE_1 accelerates thrombolysis by tissue plasminogen activator. Blood 1989; 73:1213–1217.

56. Post S, Goerig M, Otto G, Manner M, Senninger N, Kommerell B, Herfarth C. Prostanoid release in experimental liver transplantation. Transplantation 1990; 49:490–494.

57. Greig PD, Woolf GM, Sinclair SB, Abecassis M, Strasberg SM, Taylor BR, Blendis LM, Superina RA, Glynn MFX, Langer B, Levy GA. Treatment of primary liver graft nonfunction with prostaglandin E_1. Transplantation 1989; 48:447–453.

58. Grazi GL, Mazziotti A, Sama C, Stefanini GF, Gozzetti G. Reversal of primary liver graft non–function using prostaglandins. Hepato–Gastroenterol 1991; 38:254–256.

SUMMARY

Chapter 1

Since the first successful liver transplantation in man, progress in various aspects of the procedure has rendered orthotopic liver transplantation (OLT) a well established therapy for end–stage liver disease. Two elements continue to inspire researchers in the field of liver transplantation: the potential advantages of heterotopic liver transplantation (HLT) and the prospects of long–term graft preservation. In the introduction to this thesis, these two issues are presented as the principal topics of the study. Problems related to the role of HLT and to storage damage are discussed and focussed into four basic questions. With these questions as guidelines the objectives of the study are defined.

The theoretical advantages of HLT are mainly associated with leaving the recipient liver *in situ*, while it is removed in OLT. HLT not only reduces the extent of the surgical procedure, but also allows the recipient to benefit from the remaining function of the native liver during the operation. Comparative studies are necessary to define the determinants in the decision between OLT and HLT.

A milestone in the history of liver transplantation is the development at the University of Wisconsin of a solution for liver preservation that prolonged the safe storage times of donor organs considerably. This UW solution eased the emergency aspects of liver transplantation and may turn it into a semielective procedure, similar to kidney transplantation. Still, storage damage is regarded as an important determinant of failure of liver transplantation and improvement of preservation methods is the objective of numerous studies.

In case of important storage damage, the remaining host liver function in HLT could theoretically provide some support in the period of establishment of graft function. Therefore, the tolerance of long–term preservation should be studied separately for HLT and OLT.

Another strategy in preventing storage injury is the use of cytoprotective drugs. In particular, prostaglandins have been described as valuable additives to the preservation solutions.

This thesis describes the studies on the hemodynamic and hemostatic changes in both OLT and HLT in the pig, after various periods of graft preservation, with and without the use of prostaglandin E_1.

Chapter 2

In this chapter, the history and clinical results of HLT are reviewed and some special aspects of current research on HLT are highlighted.

The first laboratory experiments on liver transplantation were performed with auxiliary heterotopic grafts. The initial clinical results of HLT, however, were disappointing and OLT evolved to be the procedure of choice.

Of all patients receiving a heterotopic graft before 1980 only two survived. In the next decade a new concept of auxiliary partial liver transplantation was developed in the Laboratory for Experimental Surgery in Rotterdam and subsequent clinical results were encouraging.

After 1980, 50 HLTs are known to be performed in 48 patients. In Chapter 2 the indications and results of these HLTs are reviewed. Results of HLTs after 1986 are distinctively better compared to the previous results and survival rates come within the range of those reported of OLT.

Various new aspects of HLT are described. Intraoperative fibrinolysis is found in the anhepatic phase of OLT, which is absent in HLT. Tissue-type plasminogen activator (t-PA) is held responsible for this phenomenon as for the postreperfusion hyperfibrinolysis. Parallel to the hemostatic changes, the intraoperative *hemodynamic* stability may be impaired by deleterious substances arising during liver transplantation. The capacity of the native liver in HLT to remove t-PA and the so-called myocardial depressant factors from the blood is an issue of this thesis. Furthermore, the interaction between two livers, the effect of HLT on the portal pressure and hypersplenism, and the possible role of HLT in inborn errors of hepatic metabolism are described.

An intriguing problem is the treatment of acute hepatic failure. OLT in an early phase of the disease negates the possibility of spontaneous recovery, while delay of the decision to transplant may lead to further deterioration of the patient's clinical condition. As the procedure of HLT is reversible the decision to transplant can be made quicker. The clinical experience with HLT for acute liver failure is reported in detail.

Chapter 3

The introduction of the UW solution produced a major impulse to the research on liver preservation. To uncover the key to the success of the UW solution, the history of organ preservation is outlined in Chapter 3. Potential mechanisms of storage damage are described and integrated into a generally applicable hypothesis on liver storage

injury. The rationale of the basic components of the UW solution and its current and possible future additives are discussed using this hypothesis.

In the early days of liver transplantation the most intriguing problem of preventing harvest injury was challenged by two different strategies: continuous perfusion and hypothermic storage. Continuous perfusion techniques have the disadvantages of being expensive, technically complex, and not easily portable. Therefore, methods of continuous perfusion found little favor in clinical practice.

Literature provides a continuous abundance of reports scrutinizing small parts of the multifactorial pathogenesis of storage injury. Anoxic damage by ischemia and reperfusion damage following recirculation of the graft should be distinguished. Furthermore, these components of storage damage have a variable effect on different cells (parenchymal and nonparenchymal) and in dissimilar circumstances (various periods of cold and warm ischemia).

In this chapter, for both anoxic and reperfusion damage, the differences between parenchymal and nonparenchymal damage and between warm and cold ischemia are discussed separately.

A hypothesis concerning the mechanism of harvest injury in which oxygen derived free radicals and calcium play pivotal roles in the process of cell death is presented.

Warm ischemia (and cold ischemia at a lower rate) causes depletion of ATP. This induces damage to the energy–requiring cytoskelet of the hepatocytes and the nonparenchymal cells. ATP is broken down systematically leading to an accumulation of hypoxanthine. With longer lasting cold ischemia, Ca^{2+} accumulates. Directly, and by the activation of various phospholipases and proteases this also leads to cytoskelet damage of the hepatocytes and the nonparenchymal cells. Simultaneously, this activation of proteases induces a rapid conversion of xanthine dehydrogenase to xanthine oxidase. Consequently, both activated enzyme (xanthine oxidase) and its substrate (hypoxanthine) are abundant. At reperfusion, oxygen is supplied in excess, leading to a burst of intracellular oxygen–derived free radicals, which also adds to the parenchymal damage. At reperfusion, polymorphonuclear leukocytes and Kupffer cells are activated by tissue antigens of endothelial cells (which have come to expression by the preceding anoxic injury) to produce extracellular oxygen–derived free radicals and other toxic mediators. This causes an escalation of nonparenchymal damage.

This hypothesis not only clarifies many seemingly contradictory results of recent experimental preservation studies, but also gives a logical explanation for the mechanism of action of many cytoprotective drugs used in the last decades. In addition

it generates speculation about prostaglandins being potentially beneficial in the protection of the liver from harvest injury.

Chapter 4

In Chapter 4, the experimental design and the techniques used are summarized. In addition, the characteristics of the different operative procedures and other general intraoperative measurements are described, together with the microscopic structural changes, survival and mortality.

Sixty–nine pigs were randomly allocated to OLT (N=32) or HLT (N=37). Transplants were performed after 2 hr, 24 hr, 48 hr, and 72 hr of simple cold storage. Sixteen transplantations in the different preservation groups were performed using prostaglandin E_1 (PGE_1).

In the pig model, HLT was a shorter procedure compared to OLT, with shorter portal vein interruption. Intraoperative blood loss was lower in animals that underwent HLT and this effect was more prominent after longer periods of graft preservation. This suggests that the native liver function protects the recipient of an auxiliary graft from hemostatic disturbances. There was no evidence that PGE_1 provided protection from storage damage.

Despite the absence of light microscopic evidence of irreversible cell death before reperfusion, irreversible changes could be detected in the 24–hr preserved grafts using electron microscopy. The assumption that endothelial cells are more vulnerable to cold ischemia and reperfusion than hepatocytes could be confirmed as notable endothelial shedding was already present after 24–hr ischemia. At reperfusion, morphological appearance instantaneously worsened to an extent corresponding with the poor graft and animal survival.

Chapter 5

In this chapter, the *hemodynamic* changes in both OLT and HLT after different periods of cold storage of the graft, with or without the use of prostaglandin E_1, are described. At various intervals during the operative procedure, several hemodynamic parameters are assessed: cardiac output (CO), mean arterial pressure (MAP), left and right ventricular minute work (LVMW, RVMW), pulmonary capillary wedge pressure (PCWP), and systemic and pulmonary vascular resistance (SVR, PVR). For the three main variables– i.e., the type of transplantation, the use of PGE_1, and the preservation time, multiple regression analysis is performed.

During HLT, portal vein clamping lowered MAP and CO, while during the anhepatic phase in OLT, SVR increased and CO dropped. After reperfusion of the graft, an increase in PVR and a decrease in SVR was found in both OLT and HLT. At different stages of the surgical procedure, longer graft storage time diminished CO and MAP, especially in OLT.
The observed differences in intraoperative hemodynamics between OLT and HLT can partly be attributed to differences in operative techniques. Extension of the graft preservation period resulted in poor cardiac performance, more so in OLT than HLT. The native liver in HLT might be able to metabolize the presumed myocardial depressant factors, released by the graft upon reperfusion. Prostaglandin E_1 did not protect against this reperfusion syndrome.

Chapter 6
In this chapter, the *hemostatic* changes in both OLT and HLT after different periods of cold storage of the graft, with or without the use of prostaglandin E_1, are described. At various intervals during the operative procedure, several coagulation and fibrinolysis parameters are assessed. For the three main variables– i.e., the type of transplantation, the use of PGE_1, and the preservation time, multiple regression analysis is performed. During the anhepatic period, fibrinolytic activity was increased in OLT, but there was no significant effect on the coagulation system. On the other hand, graft reperfusion induced a severe deterioration of the coagulation system in OLT compared to HLT. In both HLT and OLT, there was an increase in fibrinolytic activity immediately after reperfusion. In HLT, t–PA activity levels quickly returned to normal, while a continuous increase was found after OLT.
These observations suggest that the remaining (clearance) function of the host liver prevents the development of hyperfibrinolysis in HLT, even with longer preservation times. Probably, early hyperfibrinolysis was caused by t–PA release from the reperfused graft, in OLT and HLT to the same extent. The late escalation of t–PA activity levels demonstrated in OLT, might be caused by cytokines that are produced in the damaged graft and subsequently activate the intact recipient systemic endothelium.
No positive or negative effect of PGE_1 on coagulation or fibrinolytic parameters was noticed.

Chapter 7

In this chapter, the main issues and possible clinical consequences are reviewed for the preparation period, the period of portal flow interruption, and the early and late postreperfusion period. The prostaglandins are discussed and answers to the questions stated in the introduction of this thesis are given.

In the preparation period, minor hemodynamic differences between OLT and HLT were measured. However, the most important difference was the shorter duration of this period in HLT. Especially in patients with fulminant hepatic failure, time–saving is important.

The differences between OLT and HLT in the portal vein interruption period were determined by the absence of an anhepatic stage in HLT and the use of a venovenous bypass in OLT. Consequently, the hemodynamic condition in this period was more stable in OLT. In HLT, a larger decrease in venous return resulted from the fact that in this model healthy animals were used. In clinical transplantation, partial clamping of the portal vein is better tolerated by the presence of portacaval collateral circulation and because of the larger diameter of the human portal vein compared to that of the piglet. With regard to changes in the hemostatic mechanism in the portal vein interruption period, HLT takes definite advantage of the remaining synthetic and clearing function of the host liver.

In both the early and late postreperfusion period, depression of myocardial function was found in OLT. In HLT, this effect could not be demonstrated and it was postulated that the responsible substances (myocardial depressant factors) are effectively cleared from the circulation by the native liver.

Apparently, after long–term preservation, endothelial cells loose their contact with the underlying hepatocytes. This exposure of the subendothelial space can be expected to activate coagulation. Monocytes and macrophages and possibly Kupffer cells could be stimulated to release a variety of mediators that can interfere with the hemostatic system, such as interleukin–1 and tumor necrosis factor. These reactions are supposedly not limited to the endothelial cells of the graft and could be responsible for a systemic derangement of hemostasis. Again, the remaining function of the native liver in HLT protected the recipient from these hemostatic changes.

Considering the multitude of proposed beneficial effects of prostaglandins in organ preservation, it is difficult to explain no protective effect was found at all. Besides having included not enough animals to detect small differences, there is probably only a narrow margin of therapeutic efficacy, if it does exist. Below a certain degree of storage injury, changes are too small to be detected, let alone be prevented, while

above a certain extent of storage time, irreversible injury causes inevitable failure of the graft, with or without prostaglandins. This upper limit is apparently surpassed with 24–hr preservation of the porcine liver.

In OLT, the hemodynamic condition in the anhepatic phase was better compared to the corresponding portal vein interruption period in HLT. After reperfusion, the hemodynamic condition was better in HLT, especially when storage damage increased.

Fibrinolytic activity was increased in the anhepatic stage in OLT. More profound hemostatic disturbances were found after reperfusion. In HLT, t–PA activity levels quickly returned to normal, while a continuous increase was found after OLT.

Long–term graft preservation is an important determinant of the hemodynamic and hemostatic disturbances in liver transplantation. In HLT, the presence of some remaining liver function during the implantation and after recirculation of the liver graft, provided protection from these disturbances.

Administration of PGE_1 to the donor and the recipient together with PGE_1 in the preservation and flush solutions did not yield significant beneficial effects on the intraoperative hemodynamic and hemostatic condition, nor on morphological appearance.

SAMENVATTING

Hoofdstuk 1

Sinds de eerste succesvolle levertransplantatie bij de mens heeft vooruitgang in vele aspecten van orthotope lever transplantatie (OLT) deze procedure tot een gevestigde behandeling van terminale leverinsufficiëntie gemaakt. Twee elementen die tot de verbeelding van onderzoekers op het gebied van levertransplantatie blijven spreken, zijn de potentiële voordelen van heterotope lever transplantatie (HLT) en de mogelijkheid van lange-termijn leverpreservatie. In de inleiding van dit proefschrift worden deze twee onderwerpen gepresenteerd als de hoofdthema's van de studie. Problemen welke gerelateerd zijn aan de rol van HLT en aan schade veroorzaakt door het bewaren van de lever (bewaarschade), worden besproken en in vier basale vragen vertaald. Met deze vragen als leidraad worden de doelstellingen van het proefschrift gedefinieerd.

De theoretische voordelen van HLT berusten in hoofdzaak op het *in situ* blijven van de lever van de ontvanger. Bij OLT wordt de ontvangerlever verwijderd. HLT is niet alleen een minder ingrijpende chirurgische procedure, maar geeft de ontvanger tevens de kans om tijdens de transplantatie te profiteren van de resterende functie van de eigen lever. Vergelijkende studies zijn noodzakelijk om de beslissing tussen OLT en HLT te onderbouwen.

Een mijlpaal in de geschiedenis van levertransplantatie is de ontwikkeling op de Universiteit van Wisconsin van een vloeistof voor leverpreservatie welke de veilige preservatietijd van donor-organen aanzienlijk verlengde. Het spoedkarakter van de procedure werd door deze UW vloeistof verminderd en evenals bij niertransplantatie wordt levertransplantatie hierdoor mogelijk een semi-electieve operatie. Niettemin wordt bewaarschade gezien als een belangrijke oorzaak van het falen van een levertransplantatie en vele studies hebben het doel de preservatiemethoden te verbeteren.

In geval van belangrijke bewaarschade kan de resterende functie van de lever van de ontvanger bij HLT theoretisch enige reserve geven zolang het transplantaat nog onvoldoende functioneert. Daarom dient de tolerantie van lange-termijn preservatie voor HLT en OLT apart te worden bestudeerd.

Een andere strategie ter voorkóming van bewaarschade is het gebruik van cytoprotectieve middelen. Prostaglandinen in het bijzonder zijn beschreven als waardevolle toevoegingen aan de preservatievloeistoffen.

Dit proefschrift beschrijft studies van haemodynamische en haemostatische veranderingen tijdens HLT en OLT in het varken, na verschillende preservatieduren, met en zonder het gebruik van prostaglandine E_1.

Hoofdstuk 2

In dit hoofdstuk wordt een overzicht van de geschiedenis en de klinische resultaten van HLT gegeven en worden enkele speciale aspecten van lopend onderzoek naar HLT beschreven.

De eerste experimentele levertransplantaties werden uitgevoerd met auxiliaire heterotope transplantaten. De eerste klinische resultaten waren echter teleurstellend en OLT ontwikkelde zich tot de procedure van voorkeur.

Van alle patiënten die een heterotoop transplantaat ontvingen vóór 1980, overleefden er slechts twee. In het daaropvolgende decennium werd een nieuw concept van auxiliaire partiële levertransplantatie ontwikkeld in het Laboratorium voor Experimentele Chirurgie in Rotterdam. De daaruit voortvloeiende klinische resultaten waren bemoedigend.

Na 1980 werden, voor zover bekend, 50 HLTs verricht bij 48 patiënten. De indicaties en de resultaten van deze HLTs worden beschreven. Resultaten van HLTs na 1986 zijn beduidend beter dan die vóór dat jaar en de overlevingscijfers benaderen die van de OLT.

Voorts worden enkele nieuwe aspecten van HLT beschreven. Intraoperatieve fibrinolyse wordt gevonden in de anhepatische fase van OLT, een fase die ontbreekt bij HLT. Het *tissue-type plasminogen activator* (t-PA) wordt zowel verantwoordelijk geacht voor dit fenomeen als voor de postreperfusie hyperfibrinolyse. Evenals bij de haemostatische veranderingen wordt de haemodynamische stabiliteit mogelijk ook benadeeld door stoffen welke voorkomen bij levertransplantatie. De capaciteit van de eigen lever om t-PA en de zogeheten *myocardial depressant factors* tijdens HLT te verwijderen is een onderwerp van studie in dit proefschrift. Verder worden de interactie tussen twee levers, het effect van HLT op de portale bloeddruk en hypersplenisme en de mogelijke rol van HLT bij aangeboren leverstofwisselingsziekten toegelicht.

Een intrigerend probleem is de behandeling van acute leverinsufficiëntie. OLT in een vroege fase van de ziekte negeert de mogelijkheid van spontaan herstel van de eigen lever, terwijl uitstel van de beslissing te transplanteren mogelijk leidt tot verdere aftakeling van de klinische conditie van de patiënt. Aangezien de procedure van HLT omkeerbaar is, kan de beslissing tot transplanteren hierbij sneller genomen worden. De klinische ervaringen met HLT voor acute leverinsufficiëntie worden in detail beschreven.

Hoofdstuk 3

Het introduceren van de UW vloeistof gaf een sterke impuls aan het onderzoek op het gebied van leverpreservatie. In Hoofdstuk 3 wordt een overzicht gegeven van de geschiedenis van de orgaanpreservatie, om zo de sleutel van het succes van de UW vloeistof te achterhalen. Daarna worden potentiële oorzaken van bewaarschade beschreven en tot een algemeen bruikbare hypothese van bewaarschade van de lever geïntegreerd. De achtergronden van de basiscomponenten van de UW vloeistof en de tegenwoordige en in de toekomst mogelijke toevoegingen worden belicht aan de hand van deze hypothese.

Het voorkómen van bewaarschade was van meet af aan het meest boeiende probleem van levertransplantatie. In hoofdlijnen werden twee methoden gevolgd: continue perfusie en hypothermisch bewaren (koelmethode). De continue perfusietechnieken hebben als nadeel dat ze kostbaar zijn, technisch complex en moeilijk vervoerbaar. Om die redenen werden deze technieken in de praktijk weinig toegepast en vond de koelmethode de meeste navolging.

Er is een overvloed aan literatuur beschikbaar waarin kleine facetten van de multifactoriële pathogenese van bewaarschade worden bestudeerd. Onderscheid moet worden gemaakt tussen anoxische schade en reperfusieschade; het eerste is het gevolg van ischaemie en het tweede ontstaat direct na de recirculatie van het transplantaat. Deze componenten van bewaarschade hebben bovendien een wisselend effect op verschillende cellen (parenchymale en nonparenchymale cellen) en in verschillende omstandigheden (warme en koude ischaemieduur).

In dit hoofdstuk worden de verschillen tussen schade aan parenchymale en nonparenchymale cellen en tussen warme en koude ischaemie voor zowel anoxische als voor reperfusieschade separaat besproken.

Een hypothese, waarin de mechanismen van bewaarschade worden beschreven, wordt gepresenteerd. Zuurstofradicalen en calcium (Ca^{2+}) spelen een belangrijke rol bij het optreden van celdood.

Tijdens warme ischaemie (en tijdens koude ischaemie in een lager tempo) wordt ATP snel opgebruikt. Hierdoor ontstaat schade aan het energie–afhankelijke celskelet van de hepatocyt en de nonparenchymale cel. ATP wordt stapsgewijs afgebroken en daarbij stapelt zich hypoxanthine. Met voortduren van koude ischaemie stapelt ook het Ca^{2+} zich. Direct en via de activatie van verschillende phospholipasen en proteasen ontstaat hierdoor ook schade aan het genoemde celskelet. Gelijktijdig induceert de activatie van proteasen de snelle omzetting van xanthine dehydrogenase (XD) in xanthine oxygenase

(XO). Aldus zijn zowel het geactiveerde enzym (XO) en het substraat (hypoxanthine) overvloedig aanwezig.
Bij reperfusie is er een groot aanbod van zuurstof, met wederom schade aan het celskelet als gevolg. Bij reperfusie worden segmentkernige leucocyten en Kupffer cellen geactiveerd door weefselantigeen van endotheelcellen, welke mogelijk tot expressie zijn gekomen door de eerder ontstane anoxische schade. Ook hierbij worden zuurstofradicalen en andere toxische mediatoren geproduceerd. Dit veroorzaakt een escalatie van de nonparenchymale schade.
Deze hypothese geeft niet alleen inzicht in het waarom van vele ogenschijnlijk tegenstrijdige resultaten van experimentele preservatiestudies, maar geeft ook logische verklaringen voor het werkingsmechanisme van cytoprotectieve stoffen welke in de laatste decennia werden gebruikt. Bovendien geeft de hypothese aanwijzingen voor een mogelijk gunstig effect van prostaglandinen in de bescherming van de lever voor bewaarschade.

Hoofdstuk 4
In dit hoofdstuk worden de experimentele opzet en de gebruikte methoden weergegeven. Daarnaast worden de karakteristieken van de verschillende operatieve procedures en andere algemene intraoperatieve meetresultaten beschreven, samen met de microscopische veranderingen, de overleving en de mortaliteit.
Negenenzestig varkens werden gerandomiseerd tussen OLT (N=32) en HLT (N=37). De transplantaties werden verricht na 2 uur, 24 uur, 48 uur en 72 uur hypothermisch bewaren van de donorlever. Bij 16 transplantaties in de verschillende groepen werd PGE_1 gebruikt.
In het varkensmodel was HLT een kortere procedure vergeleken met OLT, met een kortere duur van vena portae afklemming. Intraoperatief bloedverlies was lager bij varkens die een HLT ondergingen en dit effect was meer uitgesproken naarmate de preservatieduur langer was. Dit suggereert dat de leverfunctie van de ontvanger bescherming biedt tegen de gevolgen van bewaarschade.
Ondanks de afwezigheid van lichtmicroscopisch bewijs van irreversibel celversterf voorafgaand aan reperfusie, werden met de elektronenmicroscoop wèl irreversibele veranderingen gevonden in de 24 uur gepreserveerde donorlevers. De veronderstelling kon worden bevestigd dat endotheelcellen gevoeliger zijn voor koude ischaemie en reperfusie dan hepatocyten, aangezien het loslaten van de endotheelcellen van de onderlaag reeds na 24 uur ischaemie aantoonbaar was. Na reperfusie verslechterde de

microscopische structuur direct tot een ernstige vorm welke correspondeerde met de daaropvolgende transplantaat– en proefdieroverleving.

Hoofdstuk 5

In dit hoofdstuk worden de haemodynamische veranderingen tijdens OLT en HLT na verschillende perioden van hypothermische preservatie van het transplantaat en mèt en zonder het gebruik van PGE_1 beschreven. Op verschillende momenten tijdens de operatie werden enkele haemodynamische parameters onderzocht, waaronder het hartminuutvolume (CO), de gemiddelde arteriële druk (MAP) en de pulmonale en systemische vaatweerstand (PVR, SVR). Voor de drie belangrijkste variabelen, het type van transplantatie, het gebruik van PGE_1 en de preservatieduur werd een multiple regressie analyse uitgevoerd.

Tijdens HLT verlaagde de MAP en de CO door afklemming van de vena portae, terwijl tijdens de anhepatische fase bij OLT de SVR steeg en de CO daalde. Na reperfusie werd een stijging van de PVR en een daling van de SVR gevonden bij zowel OLT als HLT. Op verschillende momenten van de operatie was een langere preservatieduur in de multiple regressie analyse gerelateerd aan een daling van de CO en de MAP, vooral bij OLT.

De gevonden verschillen in intraoperatieve haemodynamische gegevens tussen OLT en HLT kunnen gedeeltelijk worden toegeschreven aan de verschillen in operatieve techniek. Verlengen van de preservatieduur resulteerde in een slechtere cardiale functie, vooral bij OLT. De veronderstelde *myocardial depressant factors* welke bij reperfusie uit het transplantaat komen worden bij HLT mogelijk door de gezonde eigen lever weggevangen. PGE_1 beschermde niet tegen dit zogeheten reperfusie–syndroom.

Hoofdstuk 6

In dit hoofdstuk worden de haemostatische veranderingen tijdens OLT en HLT na verschillende perioden van hypothermische preservatie van het transplantaat en mèt en zonder het gebruik van PGE_1 beschreven. Op verschillende momenten tijdens de operatie werden enkele coagulatie en fibrinolyse parameters onderzocht. Voor de drie belangrijkste variabelen, het type van transplantatie, het gebruik van PGE_1 en de preservatieduur werd een multiple regressie analyse uitgevoerd.

Tijdens de anhepatische periode was de fibrinolytische activiteit verhoogd in OLT, maar er was geen significant effect op het stollingssyteem. Reperfusie veroorzaakte wel een ernstige verslechtering van de coagulatieparameters bij OLT in vergelijking met HLT. Zowel bij HLT als bij OLT was er, onmiddellijk na reperfusie, een toegenomen

fibrinolytische activiteit. Bij HLT daalde de t-PA activiteit snel naar normale waarden, terwijl er een voortdurende stijging werd gevonden bij OLT.
Deze observaties suggereren dat de resterende (klarende) functie van de ontvangerlever de ontwikkeling van hyperfibrinolyse bij HLT voorkomt, zelfs bij langere preservatieduur.
Mogelijk werd de vroeg optredende hyperfibrinolyse veroorzaakt door t-PA uitscheiding uit het gereperfundeerde transplantaat, bij OLT en HLT in gelijke mate. De laat optredende escalatie van t-PA die gevonden werd bij OLT werd mogelijk veroorzaakt door cytokinen. In deze theorie worden de cytokinen geproduceerd in het beschadigde transplantaat waarna zij het intacte systemische endotheel van de ontvanger activeren.
Er werden geen positieve of negatieve effecten van PGE_1 op de coagulatie of op de fibrinolyse parameters gevonden.

Hoofdstuk 7
In Hoofdstuk 7 worden de belangrijkste punten en mogelijke klinische consequenties samengevat per operatieve periode: de voorbereidingsperiode, de periode van de onderbreking van de portale bloedstroom en de vroege en late postreperfusie periode. De prostaglandinen worden besproken en antwoorden op de vragen gesteld in de introductie worden gegeven.
In de voorbereidingsperiode werden geringe haemodynamische verschillen tussen OLT en HLT gemeten. Echter het meest belangrijke verschil was de kortere duur van deze periode tijdens HLT. Vooral bij patiënten met fulminante leverinsufficiëntie is tijdsbesparing een belangrijke factor.
De verschillen tussen OLT en HLT in de periode van de onderbreking van de portale bloedstroom werden vooral bepaald door het afwezig zijn van de anhepatische fase bij HLT en door het gebruik van een venoveneuze bypass bij OLT. Als gevolg hiervan was de haemodynamische conditie tijdens OLT in deze periode meer stabiel. Bij HLT trad een sterkere vermindering van de veneuze terugvloed op, aangezien in het model gezonde dieren als ontvanger werden gebruikt. Bij deze gezonde varkens is er geen belangrijke portacavale collateraal circulatie. Bij klinische transplantatie wordt partiële afklemming van de vena portae beter getolereerd door het bestaan van portacavale collateraal circulatie en door het grotere kaliber van de menselijke vena portae in vergelijking met die van de big. Wat betreft veranderingen in het haemostatische systeem tijdens afklemming van de vena portae, biedt HLT belangrijke voordelen door de resterende synthetische en klarende functie van de ontvanger lever.

In zowel de vroege als de late postreperfusie periode werd bij OLT depressie van de cardiale functie gevonden. Bij HLT kon dit effect niet duidelijk worden aangetoond en mogelijk worden de verantwoordelijke stoffen (*myocardial depressant factors*) uit de bloedsomloop verwijderd door de ontvangerlever.
Voorts bleek dat endotheelcellen na lange preservatieduur het contact met de onderliggende hepatocyten verliezen. Het hiermee blootgelegde subendotheliale gebied activeert de stolling. Monocyten en macrofagen en waarschijnlijk ook Kupffer cellen worden daarbij geactiveerd tot het vrijmaken van een verscheidenheid aan ontstekings-mediatoren die inwerken op het haemostatische systeem, zoals interleukine-1 en *tumor necrosis factor*. Deze reacties zijn waarschijnlijk niet beperkt tot het endotheel van het transplantaat en zouden verantwoordelijk kunnen zijn voor een systemische verstoring van de hemostase. Wederom blijkt de resterende functie van de ontvangerlever bij HLT de ontvanger voor deze haemostatische veranderingen te beschermen.
De grote hoeveelheid aan voorgestelde gunstige effecten van prostaglandinen bij orgaanpreservatie in ogenschouw nemend, is het moeilijk uit te leggen waarom in het geheel geen beschermend effect werd gevonden. Afgezien van het feit dat mogelijk een te klein aantal experimenten werd verricht om kleine verschillen te kunnen aantonen, is er waarschijnlijk een zeer nauwe therapeutische effectiviteit, als deze al bestaat. Onder een bepaald niveau van bewaarschade in het transplantaat zijn de veranderingen te gering om te detecteren, laat staan om te voorkómen, terwijl boven een bepaalde preservatieduur de schade irreversibel is geworden, mèt of zonder prostaglandinen. Deze bovengrens is schijnbaar reeds overschreden bij 24 uur preservatie van de lever van het varken.
Bij OLT was de haemodynamische conditie in de anhepatische fase beter in vergelijking met de corresponderende periode van onderbreking van de portale bloedstroom bij HLT. Na reperfusie was de haemodynamische conditie beter bij HLT dan bij OLT, vooral wanneer de bewaarschade toenam.
De fibrinolytische activiteit was toegenomen in de anhepatische fase bij OLT. Meer ernstige haemostatische veranderingen werden gezien na reperfusie. Bij HLT daalde de t-PA spiegels snel naar normaal, terwijl een voortdurende toeneming bij OLT werd gevonden.
Lange-termijn preservatie is een belangrijke determinant van de haemodynamische en haemostatische verstoringen bij levertransplantatie. Bij HLT kan de aanwezigheid van enige resterende functie van de eigen lever tijdens implantatie en na reperfusie van het transplantaat bescherming bieden tegen deze verstoringen.

Toediening van PGE_1 aan de donor en de ontvanger samen met PGE_1 in de preservatie en spoelvloeistoffen gaf geen significante positieve effecten op de intraoperatieve haemodynamische en haemostatische conditie, noch op het microscopisch uiterlijk van het transplantaat.

ABBREVIATIONS

α_2–AP	α_2–Antiplasmin
aPTT	activated partial thromboplastin time
ASAT	aspartate aminotransferase
ATP	adenosine triphosphate
Ca^{2+}	calcium
cAMP	cyclic adenosine monophosphate
Cl^-	chloride
CO	cardiac output
CVP	central venous pressure
DIC	disseminated intravascular coagulation
HLT	heterotopic liver transplantation
HR	heart rate
IL	interleukin
K^+	potassium
LVMW	left ventricular minute work
MAP	mean arterial pressure
MDF	myocardial depressant factors
MPAP	mean pulmonary artery pressure
Na^+	sodium
NT	normotest
$O_2^{-\cdot}$	oxygen–derived free radicals
OLT	orthotopic liver transplantation
PAI	plasminogen activator inhibitor
PCWP	pulmonary capillary wedge pressure
PG	prostaglandin
PNF	primary graft nonfunction
PT	prothrombin time
PVR	pulmonary vascular resistance
RVMW	right ventricular minute work
SOD	superoxide dismutase
SVR	systemic vascular resistance
TNF	tumor necrosis factor (α)
t–PA	tissue–type plasminogen activator
UW	university of Wisconsin
XD	xanthine dehydrogenase
XO	xanthine oxydase

DANKWOORD

De experimenten, waaruit dit proefschrift is voortgekomen, werden verricht op het Laboratorium voor Experimentele Chirurgie van de Erasmus Universiteit te Rotterdam. Dit gebeurde tijdens mijn opleiding tot algemeen chirurg in het Academisch Ziekenhuis Dijkzigt en later in het Zuiderziekenhuis te Rotterdam. Het combineren van experimenteel onderzoek met een opleiding was alleen mogelijk doordat ik kon rekenen op de hulp van velen.

Mijn ouders, Pa en Ma, zonder jullie was ik nergens. Bedankt voor het vertrouwen en de steun waarop ik altijd kon rekenen.
Prof. dr O.T. Terpstra, mijn promotor, wist mij enkele maanden na aanvang van mijn opleiding al te interesseren voor experimentele chirurgie. Hij heeft in alle opzichten garant gestaan voor de voortgang van de experimenten. Onno, bedankt voor de energieke wijze waarop je de operaties hebt begeleid en voor je nooit aflatende enthousiasme voor het wetenschappelijk werk dat hiermee gepaard ging.
Mijn co-promotor, **dr J. Stibbe**, heeft zich zonder te bedenken gestort op de moeilijke taak om een chirurgisch assistent haemotologische principes bij te brengen. Jeanne, bedankt voor je zeer gedegen correcties van het manuscript en voor de grote hoeveelheid tijd die je vrijmaakte voor de "stollingsvergaderingen".
Prof. dr J. Jeekel en **Prof. dr H.A. Bruining** als hoofd en opleider van de afdeling Heelkunde van het Academisch Ziekenhuis Dijkzigt, hebben mij onvoorwaardelijk in de gelegenheid gesteld om steeds weer naar het chirurgisch laboratorium te gaan. Kieje, de dingen die je me op de Intensive Care hebt geleerd zijn enorm van pas gekomen bij de interpretatie van mijn experimentele gegevens; ik ben daarom ook blij dat je in de promotie-commissie zitting hebt willen nemen.
Dr G.A.A. Olthuis en later **dr M.K.M. Salu** hebben zich, als hoofd en opleider van de afdeling Heelkunde van het Zuiderziekenhuis, van meet af aan ingezet voor de voltooiing van dit proefschrift. Dr Olthuis, Mark, ik heb veel respect voor de belangeloze wijze waarop jullie voor mijn promotie-onderzoek, tot het einde toe, ruimte wisten te creëren in de kliniek.
Prof. dr S.W. Schalm en **Prof. dr Th.J.M.V. van Vroonhoven** dank ik voor hun bereidheid het manuscript te beoordelen. Professor van Vroonhoven, U heeft aan de wieg van mijn chirurgische en wetenschappelijke loopbaan gestaan en ik ben U de meeste dank verschuldigd voor de positie die ik nu heb. Zonder U was dit proefschrift er niet geweest.
Prof. dr M.N. van der Heyde en **Prof. dr J.L. Terpstra**, ik ben zeer vereerd dat U als pioniers in levertransplantatie, zitting hebt willen nemen in mijn promotie-commissie.
Janny de Kam was als zeer bekwaam instrumenterende mijn steun en toeverlaat tijdens alle operaties. Janny, je bent de enige operatie-assistent met wie ik kan opereren als met een gezamenlijk cerebellum. **Enno Colly**, **Erik Ridderhof**, en **Rob Meijer** hebben niet alleen gezorgd voor een goede anaesthesie, maar ook voor een efficiënte meting en registratie van

de enorme hoeveelheid gegevens die tijdens de experimenten moesten worden verzameld. Ik waardeer het zeer dat jullie steeds weer met goede moed aan een operatie begonnen, waarbij het vrijwel zeker was dat het proefdier op de operatietafel zou overlijden. Erik, bedankt voor je vrijgevige houding ten aanzien van zoveel software.
De anaesthesie werd meestal begeleid door **Theo Groenland** en enkele malen door **Lammert Vos**. In de beginfase van de experimenten hebben **Cees Reuvers** en **Gerard Madern** geassisteerd. **Dieter Baumgartner** en **Erik Hesselink**, bedankt voor de vanzelfsprekende manier waarop jullie altijd insprongen wanneer dat nodig was.
Leon Kerkhofs, **Peter Schlejen** en **Ariane Cats** hebben veel van hun avonden in het laboratorium doorgebracht voor de postoperatieve zorg. Peter, bedankt voor je accurate administratie van de onderzoeksgegevens en voor je doorzettingsvermogen bij de arbeidsintensieve stollingsanalyse. Dat laatste geldt ook voor **Moniek de Maat**, **Maria Gomes** en **Harold Lampe** die allen een deel van de stollingsanalyse hebben verricht. **Robert Porte** en **Minke Bakker**, bedankt voor de lange avonden die jullie met mij hebben besteed aan de interpretatie van de bergen met stollingsgrafieken.
Jaap Kasbergen en **Roy Spruyt** stonden garant voor het tijdig leveren van de proefdieren en voor een uitstekende pre- en postoperatieve zorg. **Pim Schalkwijk** heeft alle laboratorium bepalingen verricht en **Amelie Bijma** heeft alle histocompatibiliteitstesten uitgevoerd.
De histologische preparaten zijn vervaardigd door **Rob Meijer**, **Marcel Vermeij** en **Olga Pelgrim**. Bij het beoordelen van de histologische preparaten heb ik veel hulp gehad van **Fiebo ten Kate**. **Kurt Dingemans** heeft de electronen-microscopie voor zijn rekening genomen, en **Wilma Frederiks** heeft met de hulp van **Nicole Posch** de enzym histochemie uitgevoerd en beoordeeld.
De heer **W.J. Kooreman** leverde kosteloos een grote hoeveelheid prostaglandine E_1.
Paul Schmitz, gaf waardevolle adviezen ten aanzien van de multiple regressie analyse en **Molly de Haan** corrigeerde de Engelse samenvatting. **Ingrid van den Bergh**, **Ton Dalm** en **Corinne Thiessen**, bedankt voor het razendsnel bij elkaar scharrelen van de meest exotische artikelen.
Mijn **collegae assistenten** van het Academisch Ziekenhuis Dijkzigt en van het Zuiderziekenhuis bedank ik hartelijk voor alle keren dat zij het werk overnamen dat ik in verband met dit proefschrift liet liggen.
Ton Hoofwijk en **Henk Oostvogel** bedank ik voor hun steun als paranimf. Ton, maat in Allcare, PATFILE is mijn kindje, maar jij hebt het groot gebracht.
Ariane, jouw hulp op het chirurgisch laboratorium was maar een beginnetje. Nu het proefschrift af is kan ik de rest van ons huwelijk aan jouw besteden.

CURRICULUM VITAE

28 september 1959	Geboren te Oranjestad (Aruba).
1971 – 1977	Atheneum–B, Revius Lyceum, Doorn.
1977 – 1984	Studie Geneeskunde, Rijks Universiteit Utrecht.
1984 – 1985	Arts–assistent afdeling chirurgie St. Elisabeth Ziekenhuis, Tilburg Hoofd: Dr Th.M.J.V. van Vroonhoven.
1985 – 1986	Arts–assistent afdeling chirurgie St. Maartens Gasthuis, Venlo Hoofd: Dr H.L. de Smet.
1986 – 1989	Arts–assistent in opleiding afdeling chirurgie Academisch Ziekenhuis Dijkzigt, Rotterdam. Opleiders: Prof. dr J. Jeekel en Prof. dr H.A. Bruining.
1989 tot heden	Arts–assistent in opleiding afdeling chirurgie Zuiderziekenhuis, Rotterdam. Opleiders: Dr G.A.A. Olthuis en dr M.K.M. Salu.

Photograph cover:

Salt and sand

May 1991, Dantes View, Death Valley, CA, USA

by Cees Blankensteijn, Wijk bij Duurstede.