STUDIES ON ACUTE HEPATIC INSUFFICIENCY

Onderzoekingen bij acute leverinsufficiëntie

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE
AAN DE ERASMUS UNIVERSITEIT ROTTERDAM
OP GEZAG VAN DE RECTOR MAGNIFICUS
PROF. DR. M. W. VAN HOF
EN VOLGENS BESLUIT VAN HET COLLEGE VAN DEKANEN.
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP
WOENSDAG 4 APRIL TE 15.45 UUR

DOOR

GERRIT HARMEN DE GROOT

GEBOREN TE VROOMSHOOP

krips repro meppel

1984

PROMOTOR: PROF. DR. M. FRENKEL

Het onderzoek werd mogelijk gemaakt door de steun van de stichting voor medisch wetenschappelijk onderzoek FUNGO en de druk van het proefschrift mede door de financiële steun van Smith, Kline & French en Diacare, Nederland.

voor mijn ouders
voor Mieke en Frederike

CONTENTS

CHAPTER I.	INTRODUCTION.	9
CHAPTER II.	A REPRODUCIBLE MODEL OF ACUTE HEPATIC FAILURE BY TRANSIENT ISCHEMIA IN THE PIG.	15
CHAPTER III.	OBJECTIVE MEASUREMENT OF HEPATIC ENCEPHALOPATHY IN PIGS BY MEANS OF SPECTRAL ANALYSIS AND EVOKED RESPONSES.	37
CHAPTER VI.	INCIDENCE OF ENDOTOXEMIA IN PIGS WITH ISCHEMIC HEPATIC NECROSIS TREATED BY HAEMODIALYSIS. PREVENTION OF ENDOTOXEMIA WITH LACTULOSE.	49
CHAPTER V.	COMPARISON OF LARGE-PORE MEMBRANE HAEMODIALYSIS AND CROSS-DIALYSIS IN ACUTE HEPATIC INSUFFICIENCY IN PIGS.	59
CHAPTER VI.	LARGE-PORE HAEMODIALYTIC PROCEDURES IN PIGS WITH ISCHEMIC HEPATIC NECROSIS; A RANDOMIZED STUDY.	77
CHAPTER VII.	STRATEGIES FOR HEPATIC SUPPORT IN ACUTE LIVER FAILURE. ROLE OF EXTRACORPORAL DEVICES VS AUXILIARY TRANSPLANTATION. A GENERAL DISCUSSION.	97
CHAPTER VIII.	SUMMARY AND CONCLUSIONS.	117
	SAMENVATTING EN CONCLUSIES.	121
	VERANTWOORDING.	124
	CURRICULUM VITAE.	126

CHAPTER I.

INTRODUCTION

Acute hepatic failure (AHF) is one of the most dramatic situations that a clinical physician can encounter. It is also one of the most frustating since death is the result in a large majority of the cases, despite all efforts of the medical and nursing staffs. Although a wide variety of experimental modalities has been devised to treat the syndrome, none has proven to be effective (1,2) and 80 to 90% of those with stage IV disease die (3,4). This prognosis is in marked contrast to that for renal failure, that has undergone a steady improvement in survival statistics in the past decades - largely as a result of rapidly improving artificial kidney devices (5,6). The continuing bleak prognosis for the patient with fulminant hepatic failure has stimulated investigation into the feasibility of developing some sort of artificial device which would take over the essential life-preserving functions of the liver. The basic therapeutic approach to AHF must be intensive care monitoring, treatment of complications, synthetic replacement of certain functions of the liver and detoxification, i.e. the removal of toxic substances which accumulate in fulminant hepatic failure (7).The rationale for detoxification is based on the observation that the accumulation of 'toxins' is related to the development of hepatic encephalopathy and cerebral edema (8), which influence survival (9).

Synthetic support of various functions of the liver includes:

1. Regulation of the glucose homeostasis; hypoglycaemia develops in 40-50% of the patients with severe liver damage (10,11,12). Inefficient degradation of insulin as well as deficiencies in gluconeogenesis has been suggested as the possible cause (13). Continuous administration of glucose usually prevents hypoglycaemia; however, frequent monitoring of blood glucose continues to be necessary.

2. Correction of clotting deficiencies; more than 50% of patients with

fulminant hepatic failure develop severe haemorrhage, usually in the upper gastro-intestinal tract but also in the nose, lungs or the retroperitoneal space. Fresh frozen plasma and blood are give to replace the missing clotting factors (9). Concentrated clotting factors should not be given because they enhance the process of disseminated intravascular coagulation. Vitamin K may be given but the effect on prothrombin time is usually insignificant. In addition to supplementing clotting factors, fresh frozen plasma may also help to restore the oncotic pressure and correct deficiencies of opsonins and complement factors (14).

Support of the detoxifying function of the liver

In acute hepatic failure many substances accumulate. Restoration of the 'internal milieu' is the main goal of therapy, resulting in reduction of hepatic encephalopathy, prevention of cerebral edema and enhancement of liver regeneration. The development of artificial hepatic support systems has proceeded empirically due to our ignorance of the toxins to be removed and the metabolic abnormalities to be corrected. A number of different approaches to the development of such systems has been proposed (7) and several have been brought into clinical use after only a minimum of 'in vitro' and animal testing.

What should a support system be able to do?

The substances that probably are involved in the pathogenesis of hepatic encephalopathy can be divided into three groups on the basis of their molecular size.

Firstly, three groups of small molecular water-soluble substances have been implicated in the pathogenesis of hepatic encephalopathy (8).

a. Ammonia, short-chain fatty acids and mercaptanes, acting either alone or synergistically (15,16).
b. Plasma amino acids. An imbalance of these substances will result in an increased brain uptake of free tryptophan, phenylalanine and tyrosine and interruption of the balance between inhibitory and excitatory neurotransmitters (17).
c. GABA. Increased brain GABA bound to an increased number of GABA receptors results in neural inhibition with subsequent encephalopathy (18,19,20). The origin of GABA is not exactly understood; some authors believe that it is produced in the colon (19,21).

Secondly, middle molecular substances with a molecular weight between 1000 and 5000 daltons, although as yet not identified, have been demonstrated in the serum (22,23,24) and brain (22) of comatose animals and men.

The third category of substances that could be involved in the pathogenesis of hepatic encephalopathy is those bound to proteins. Although specific protein-bound toxins have as yet not been identified, bilirubin and bile acids may serve as markers for this category.

An ideal hepatic support system should be capable of removing all three groups of molecular substances adequately.

Because of the complexity of the abnormal metabolic state one simple artificial device is unlikely to be able to normalize all disturbances of the 'internal milieu'. However the use of the available systems, for example haemodialytic procedures and haemoperfusion on charcoal or resins, may allow us to study the role of specific toxins in the pathogenesis of hepatic encephalopathy.

Studies on the treatment of acute hepatic failure.

Evaluation of the effect of hepatic support in animal experiments is only useful if the model can be compared with the human situations and if there is a reliable and objective method for measuring hepatic encephalopathy. A lack of both criteria has strongly hampered research in this field.

The main purpose of this thesis is:

1. To develop an animal model for acute hepatic failure by inducing transient ischaemia of the liver (Chapter II).
2. To measure hepatic encephalopathy by objective methods, such as spectral analysis of the EEG and evoked potentials (Chapter III).
3. To validate the cause of death by hepatic failure and to exclude the presence of endotoxaemia in pigs treated with oral lactulose and magnesiumsulfate (Chapter IV).
4. To study the effect of large-pore membrane haemodialysis, cross dialysis and haemofiltration on survival and the neurologic state in pigs with ischaemic hepatic necrosis and also to determine the removal of freely diffusable and protein-bound substances by various haemodialytic procedures. (Chapter V and VI).
5. To discuss the efficacy of the presently used artificial organs in acute hepatic failure with a view to the future (Chapter VII).

Literature.

1. Benhamou JP, Rueff B, Sicot C. Severe hepatic failure: a critical study of current therapy. In: Liver and Drugs. Editors: F.Orlandi and A.M. Jezequel, New York, Academic Press, 1972:213-228.
2. Berk PD, Popper H. Fulminant hepatic failure. Amer J Gastroent 1978; 69:349-400.
3. Sherlock S. Diseases of the liver and biliary system. 5th Edition. Blackwell, Oxford, 1975; 107-108.
4. Rakela J. A double-blinded, randomized trial of hydrocortisone in acute hepatic failure. Gastroenterology 1979;76:1297.
5. Johnson WJ, O'Kane HO, Woods JE, et al. Survival of patients with end-stage renal disease. Mayo Clin Proc 1973;48:18-23.
6. Maher JF, Nolph KD, Bryan CW. Prognosis of advanced chronic renal failure. Ann Intern Med 1974;81:43-47.
7. Berk PD, Martin JF, Scharschmidt BF, et al. Current status of artificial hepatic support systems. In: Progress in Liver Disease. Vol.5. Editors: H. Popper, F. Schaffner, New York, Grune & Stratton, 1976;398-417.
8. Zieve L. The mechanism of hepatic coma. Hepatology 1981;1:360-365.
9. Gimson AES, Ede R, Braude S et al. Fulminant hepatic failure and artificial liver support.Gastroenterologica Japonica 1982;17:144-176.
10. Samson RI, Trey C, Tumme AH et al. Fulminant hepatitis with recurrent hypoglycaemia and haemorrhage. Gastroenterology 1967;53:291-300.
11. Felig P, Brown V, Levine RA et al. Glucose homeostasis in viral hepatitis. N Eng J Med 1970;283:1436-1440.
12. Saunders SJ, Hickman R, McDonald R et al. The treatment of acute liver failure. Progress in Liver Disease. Vol.5. Editors: H. Popper, F.Schaffner, New York, Grune & Stratton, 1976;333-343.
13. Gitler RE. Spontaneous hypoglycaemia. N Y J Med 1962;62:236-250.
14. Wijke RJ, Rajkovic IA, Eddleston ALWF. Defective opsonisation and complement deficiency in serum from patients with FHF. GUT 1980;21:643-649.
15. Gabuzda GJ. Hepatic coma: clinical considerations, pathogenesis, and management. In: Dock W, Snapper I, eds. Advances in internal medicine. Chicago Year Book 1962;11:11-73.

16. Zieve L, Doizaki WM. Brain and blood methanethiol and ammonia concentrations in experimental hepatic coma and coma due to injections of various combinations of these substances. Gastroenterology 1980;79:1070.
17. James JH, Ziparo V, Jeppson B, et al. Hyperammonemia, plasma amino acid imbalance and blood-brain amino acid transport: a unified theory of portal systemic encephalopathy. Lancet 1979;2:772-775.
18. Schafer DF, Thakur AK, Jones EA. Increased gamma-aminobutyric acid receptors associated with acute hepatic encephalopathy in rabbits. Clin Res 1980;28:485.
19. Schafer DF, Thakur AK, Jones EA. Acute hepatic coma and inhibitory neurotransmission: increase of gamma-aminobutyric acid to neural membranes. Gastroenterology 1980;79:1123.
20. Baraldi M, Zeneroli ML. Experimental hepatic encephalopathy: changes in the binding of gamma-aminobutyric acid. Science 1982;216:427-429.
21. Ferenci P, Schafer DF, Kleinberger G. Serum levels of gamma-aminobutyric acid-like activity in acute and chronic hepatocellular disease. Lancet 1983;ii:811-814.
22. Faguer P, Delorme ML, Cueille G et al. Demonstration of middle molecules in plasma and brain during experimental acute hepatic encephalopathy. ASAIO J 1980;3:153.
23. Chang TMS. Hemoperfusion, exchange transfusion, cross circulation, liver perfusion, hormones, and immobilized enzymes. In: Artificial liver support. Editors: Brunner G, Schmidt FW (eds). Springer-Verlag, Berlin Heidelberg, New York 1981, p.126.
24. Leber HW, Klausmann J, Goubeaud G et al. Middle molecules in the serum of patients and rats with liver failure: influence of sorbent haemoperfusion. In Artificial liver support. Editors: Brunner G, Schmidt FW (eds). Springer-Verlag, Berlin Heidelberg, New York 1981, p.96-102.

CHAPTER II

A REPRODUCIBLE MODEL OF ACUTE HEPATIC FAILURE BY TRANSIENT ISCHEMIA IN THE PIG.

Gerrit H. de Groot[1], Cees B. Reuvers[2], Solko W. Schalm[1], Anton L. Boks[1], Onno T. Terpstra[2], Hans Jeekel[2], Fibo W.J. ten Kate[3] and Jacques Bruinvels[4].
Departments of Internal Medicine II[1] and Surgery[2], Pathology[3] and Pharmacology[4], University Hospital Dijkzigt, Erasmus University, Rotterdam

Supported by Foundation for Medical Research FUNGO, The Netherlands.

Submitted for publication.

Abstract

A model of transient acute hepatic failure has been developed in the pig. Three days after a functional end-to-side portocaval shunt was introduced, 15 ambulant animals underwent total liver ischemia for 4 or 6 hours by the closure of a mechanical clamp surrounding the hepatic artery. Four of the 8 animals subjected to 4 hours of ischemia survived. All but one of the animals undergoing 6 hours of hepatic ischemia developed grade 4 encephalopathy after 24 to 30 hours and died within 50 hours. Quantitative estimation of liver cell necrosis revealed less than 40% necrosis in the survivors, and approximately 62% (range 49-75%) in animals who died of hepatic coma. As far as the putative toxins are concerned, significant differences were found between animals undergoing 4 and those undergoing 6 hours of ischemia, especially in the plasma ammonia levels and the plasma ratio's for tyrosine and phenylalanine. Plasma arginine levels had fallen to zero in both groups at 24 hours and only rose to pre-ischemic values in animals who survived.

This large animal model fulfills the accepted criteria of potential reversibility, reproducibility and death due to hepatic failure.

Introduction

To evaluate new therapies for human fulminant hepatic failure a suitable animal model is urgently needed. Surgical models such as hepatectomy (1) or a portocaval shunt with permanent ligation of the hepatic artery in one or two stages (2,3) are not ideal because they lack both potential reversibility (4) and the release of products from damaged liver cells into the circulation. To overcome these problems Misra (5) and Fisher (6) created a model of reversible hepatic ischemia in ambulant conscious dogs and pigs, respectively. Sixty minutes of hepatic ischemia were always fatal to dogs. For pigs, the minimum period of hepatic ischemia required to produce death due to hepatic coma could not be estimated; according to other investigators the period of hepatic ischemia tolerated by pigs varied between 35 and 180 minutes (7-10). Hepatic encephalopathy however could not be induced in these studies, since the animals either survived or died without a definite period of clinically manifest neurological abnormalities.

The aim of our investigation was to develop a model of acute hepatic failure by inducing temporary hepatic ischemia in fully ambulant pigs, and to describe the clinical, biochemical, hemodynamic, histological and electrophysiological features of this model.

Material and Methods

Preparatory surgery.

Fifteen healthy White pigs, weighing 28-33 kg, were used. One and two days before surgery, the bowel was cleansed by the oral administration of 25 g of magnesium sulfate and 150 ml of lactulose. Anesthesia was induced with an intramuscular injection of ketamine chloride (35 mg/kg) The animal was intubated and connected to a BA-4 Anesthesia Ventilator; anesthesia was maintained with a mixture of NO_2-O_2 and Ethrane^R. The anesthetized pig was placed on the operating table in a supine position. A Scribner shunt was inserted between the carotid artery and external jugular vein for pressure monitoring and blood sampling. The internal jugular vein was cannulated with

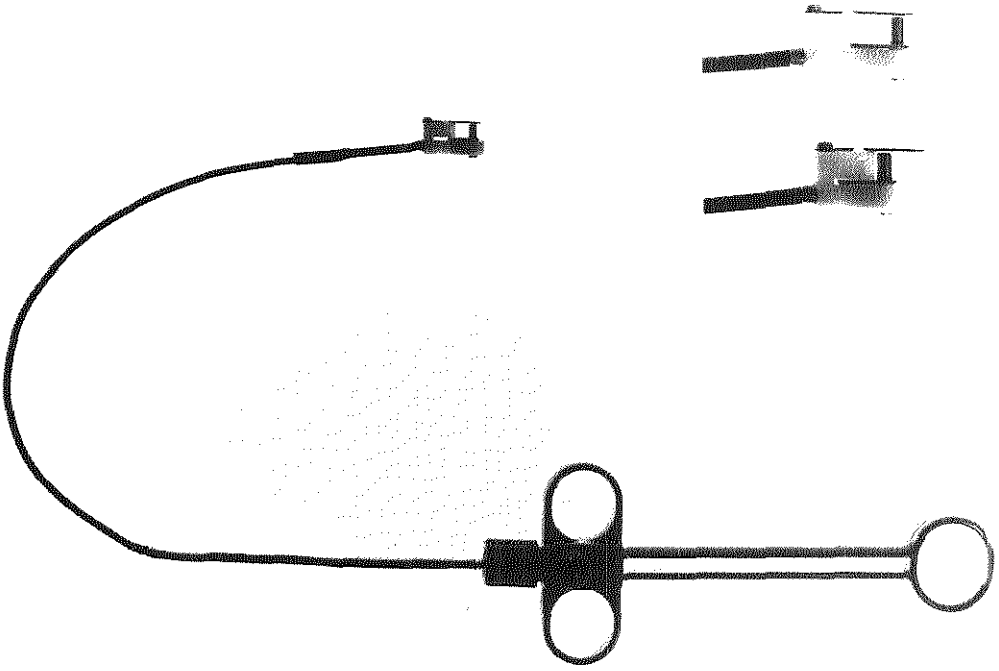

Fig 1. View of the mechanical clamp used to occlude the hepatic artery of fully alert pigs.

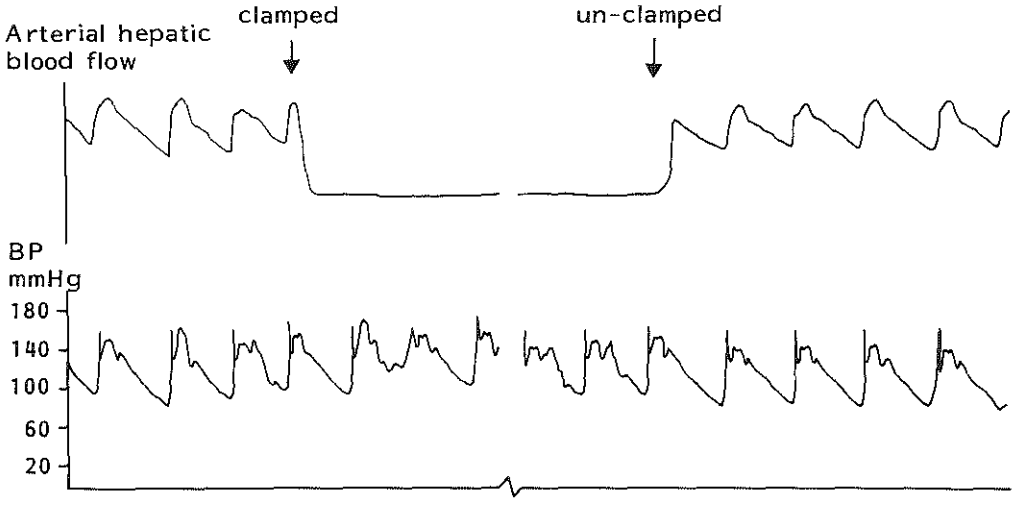

Fig 2. Blood flow curve of the hepatic artery and systemic blood pressure before, during and after clamping of the hepatic artery.

a polyethylene cannula for infusion of intravenous fluids. After opening the abdomen with a long midline incision the liver was freed by dissecting the triangular ligaments, the falciform ligament and all peritoneal attachments of the liver. All structures in the hepatoduodenal ligament except the portal vein, the hepatic artery and the common duct were divided. Blood vessels running along the vena cava into the liver at the level of the diafragm were interrupted by diathermia.

A side-to-side portocaval shunt was made, followed by ligation and transection of the portal vein close to the hilum to create a functional end-to-side shunt. A specially constructed vessel occluder (fig.1) and a perivascular electromagnetic blood flow sensor (Skalar, Delft, the Netherlands) were positioned around the isolated hepatic artery; they were anchored to the abdominal wall and the leads were guided through the skin via separate incisions. The occluder and flow probe were tested during surgery by tightening the occluder, which resulted in a total flattening of the blood flow curve on the oscilloscope (fig.2).

The common bile duct was opened and a silicon tubing was inserted. This drain was firmly attached by two 2-0 silk ligatures around the proximal and distal ends of the drain, thus preventing blood flow through the wall of the common bile duct to the liver. Abdominal closure was performed in two layers.

Subsequently five silver electrodes (diameter:ϕ1 mm) were positioned upon the dura, anchored in the skull with acrylic bone cement and channeled percutaneously; two electrodes were placed above the frontal cortex, one in the vertex and two above the occipital cortex. With this technique artefact-free registration of the electroencephalograms was possible. After discontinuation of anesthesia the animals were kept on the operating table until they were awake, breathing adequately and restoring their body temperature.

Throughout the surgical procedure 0.9% NaCl was administered; just before construction of the portocaval anastomosis an additional dose of 400 ml of Haemacel was given intravenously. At the beginning of surgery and immediately afterwards ampicilline (0.5 gr) and kanamycin (0.5 gr) were injected intravenously.

Induction of ischemic necrosis.

Three days after construction of the portocaval shunt the normothermic animals, fully awake, were fixed on a table with two cotton sheets. The animals accepted this procedure. Ischemic hepatic necrosis was induced by

tightening the clamp around the hepatic artery; arrest of hepatic blood flow was confirmed by total flattening of the flow curve on the oscilloscope (fig.2). Arterial occlusion was maintained for 4 and 6 hours, respectively. To validate the total devascularization of the liver, two animals underwent aortography and selective angiography of the hepatic artery. After unlocking the clamp, restoration of blood flow through the hepatic artery was confirmed by continuous registration of the electromagnetic flow measurements.

All animals received 35 ml of 8.4% sodium bicarbonate within 15 minutes of declamping, followed by a continuous infusion of glucose (12 g/kg/24 hrs). Penicillin G (9 mega U/ 24 hrs), kanamycin (3g/24 hrs), potassium and phosphate (37 mmol/ 24 hrs and 45 mmol/24 hrs, respectively) were added to the glucose. After the ischemic period, the animals were placed in special cages in which they could move around without disturbing their continuous infusion. Heart rate and mean arterial blood pressure, recorded with an electromanometric transducer, were registered continuously. Temperature was measured with a tele-thermometer.

Neurologic assessment.

The behavior of the animals after temporary ischemia of the liver was checked frequently. Standard auditory and pain stimuli were administered regularly and the responses were graded as follows: 0= absent; 1= dubious; 2= present. Spontaneous grunting and muscular rigidity were also noted. The duration of survival was defined as the period between the induction of hepatic ischaemia and the time of death.

Electroencephalograms were made before induction of hepatic necrosis and repeated at 24,30,48,54 and 72 hrs. Four bipolar tracings (left and right fronto-occipital, fronto-frontal and occipito-occipital leads) were recorded on a Gogh apparatus (Ahrend van Gogh, Amsterdam, the Netherlands). The EEG recordings were analyzed independently by an electroneurologist; the 5-grade classification described by Opolon (11) was used.

Biochemical measurements.

Blood samples were taken before and 24,30,48,54 and 72 hrs after induction of ischemic hepatic necrosis. In addition blood for acid-base status and coagulation studies was also drawn 0.5 hr and 1-3 hrs after release of the occlusive clamp. Blood glucose, sodium, potassium, urea, pH, pO2 and pCO2, SGOT, bilirubin, bile acids, platelets and clotting factors (fibrinogen, prothrombin time, activated partial thromboplastin time) were measured by

standard laboratory techniques. Blood samples were cultured in 60 ml of trypticase soy broth at 37° and observed for 2-3 days.

Ammonia was measured by an enzymatic method (12). Plasma amino acid profiles were determined with a LKB-4400 amino acid analyzer (LKB Biochrom. Ltd, Cambridge, England) in plasma supernatant which had been rendered protein free by treatment with sulfosalicylic acid 75% w/v. The results for the neutral amino acids threonine, valine, leucine, isoleucine, tyrosine, phenylalanine, tryptophan, methionine and histidine are not expressed as simple concentrations but as plasma ratio's. The individual amino-acid ratio (for instance threonine) can be calculated as follows: THR/VAL + LEU + ILE + TYR + PHE + TRY + MET + HIS + THR. These neutral amino acids are transported by the same transport system in the blood-brain barrier (13). The ratio of the individual amino acids will reflect the influx of amino-acids into the brain.

Morphology.

Postmortem examinations were performed in all cases as soon as the animal died. After macroscopic inspection of the heart, lungs, kidney, stomach and portocaval anastomosis, the liver was removed. Two one cm thick transverse slices from the upper and lower part of the liver were fixed in 10% formaldehyde. After fixation, 7-u sections of each complete transversal liver slide were stained with hematoxylin-eosin, PAS or gallocyanine. The degree of hepatic necrosis in each slide was estimated by point analysis (fig.3a,b); for this purpose 625 areas chosen at random were assessed for liver cell necrosis (defined as apparent disappearance of hepatocytes or eosinophilic condensation in the cytoplasm with nuclear pyknosis).

Statistical analysis.

Data are expressed as mean values ± SD. For unpaired samples with a normal distribution of data points, the Student's t test was used, while the Wilcoxon rank sum test was used in the event of a skewed data profile. Differences were assumed to be statistically significant when the p values were less than 0.05.

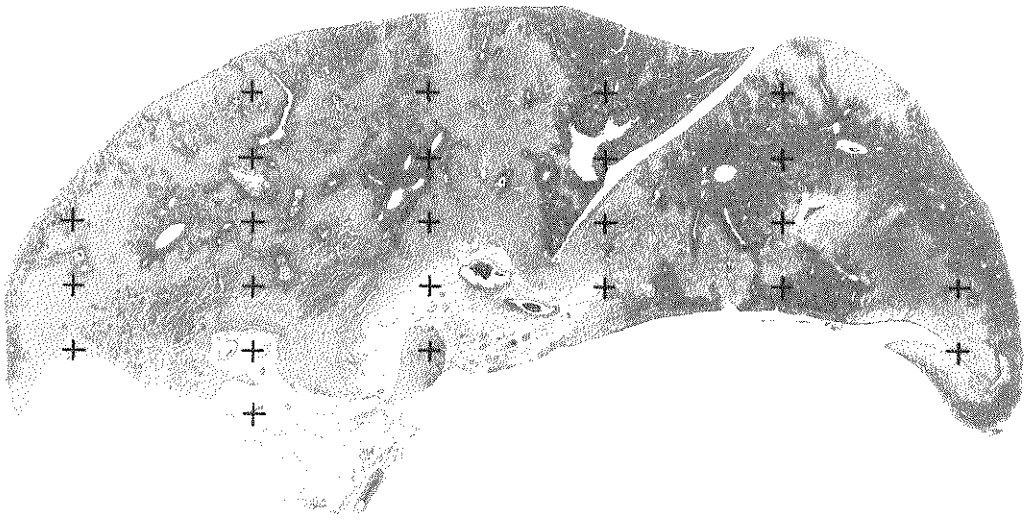

Fig 3a.A transverse cut of the liver (real size) with 25 points chosen at random for analysis of hepatic necrosis.

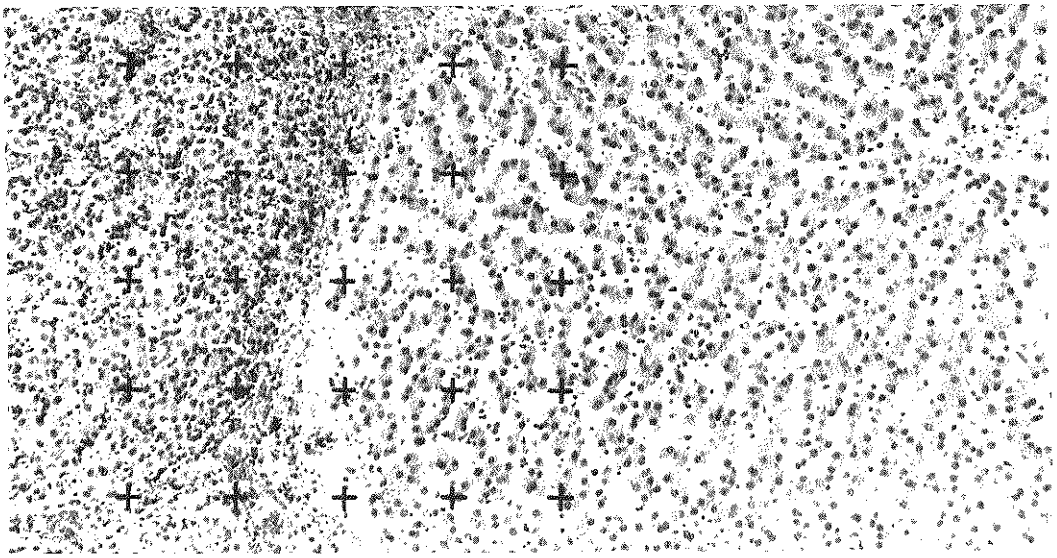

Fig.3b.A magnification (objective 10x) of one area-point which again is divided into 25 points for analysis.

Results

Adequacy of the ischemic procedure.

For all animals, there was a total flattening of the blood flow curve on the oscilloscope during the ischemic period. Aortography and selective angiography of the liver of two animals showed complete devascularization of the liver. After release of the clamp, flow through the hepatic artery was restored in all animals as demonstrated by flow measurements (fig 2).

Survival.

After 4 hours of ischemia, four out of 8 pigs survived (fig. 4). Two animals died soon after hepatic ischemia due to technical complications (broken intravenous tubing and bile leakage from the tube); two other animals, that died 26 and 51 hours after ischemia, were found to have a positive blood culture with E.coli. After 6 hours of ischemia, 6 of the 7 animals died within 50 hours; one animal survived for 72 hours with a grade 4 encephalopathy. In four of the 6 animals death was due to hepatic coma; in the 2 remaining animals death was precipitated by a bleeding gastric ulcer.

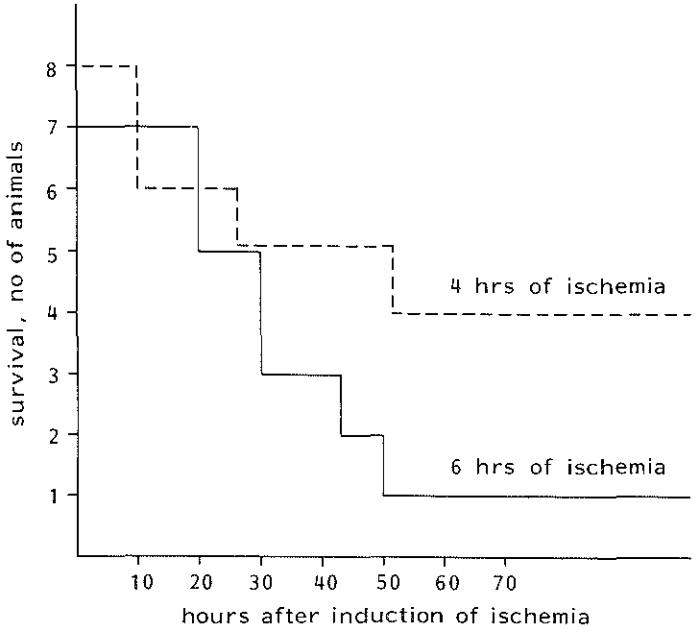

Fig 4. Survival after induction of transient liver ischemia (4 hrs or 6 hrs of ischemia) in normothermic pigs.

Neurological assessment.

The surviving animals undergoing four hours of ischaemia did not show marked abnormalities in behavior. Immediately after revascularization of the liver most pigs were ambulant and alert, although some appeared excited.The first abnormality observed in animals that ultimately died was usually an ataxic gait and impaired balance. The animals swayed from one side to the other; this was followed by drowsiness lasting several hours. Within 28 hours, pain sensations and spontaneous grunting had decreased markedly. After loss of sensation coma developed between 24 and 30 hours after hepatic ischaemia and was accompanied by muscular twitching of the neck and limbs, and later rigidity. Some hours before death, tachycardia and hyperventilation were noted. Terminally, there was gasping with cyanosis, vomiting and hypotension.

The courses of EEG grades for animals undergoing 4 and 6 hour periods of hepatic ischemia are shown in figure 5. The EEG grades became more abnormal in both groups after 24 hours. In general, the EEG changes deteriorated very slowly in '4-hour pigs'; only one animal had reached grade 4 encephalopathy at 48 hours. 'Six-hour pigs' showed a rapid deterioration of the EEG to grade 4 encephalopathy between 24 and 30 hours after the ischemic period.

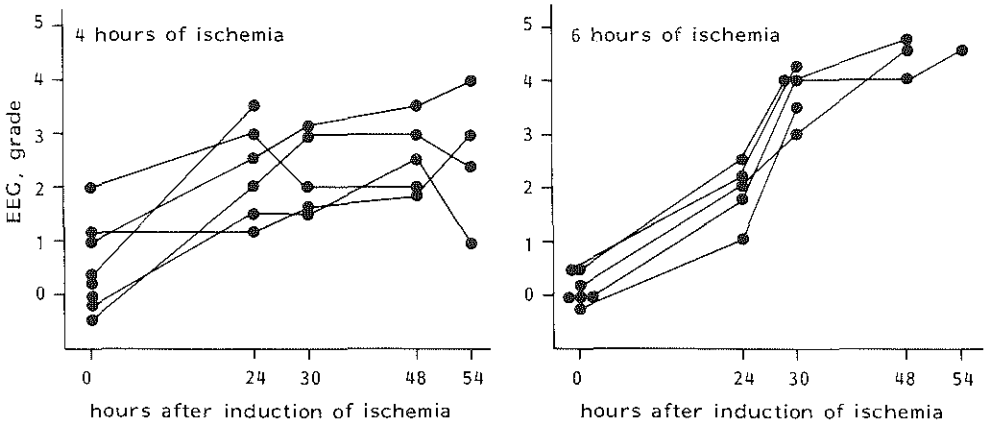

Fig.5. The course of EEG grades in pigs after 4 and 6 hours of transient ischemia of the liver. The 6-hour animal, that survived for 43 hours, has been included in the 48 hour group.

General and biochemical measurements.

Heart rate and mean systemic blood pressure remained fairly constant, except during a short period immediately after revascularization of the liver (mean arterial blood pressure decreased by 20 mm Hg, and there was a mean increase in the heart rate of 18 beats per minute). Hypothermia did not develop during the experiments. Metabolic acidosis was observed after revascularization, but was easily corrected by administration of sodium bicarbonate (fig 6). The levels of plasma glucose, potassium and sodium remained within the normal range, also after release of the clamp. Plasma SGOT levels reached a maximum at 24 hours; '4-hour pigs' had lower levels (2073 ± 817 IU/1) at 24 hours than '6-hour animals' (3259 ± 1600 IU/1) ($p > 0.05$).

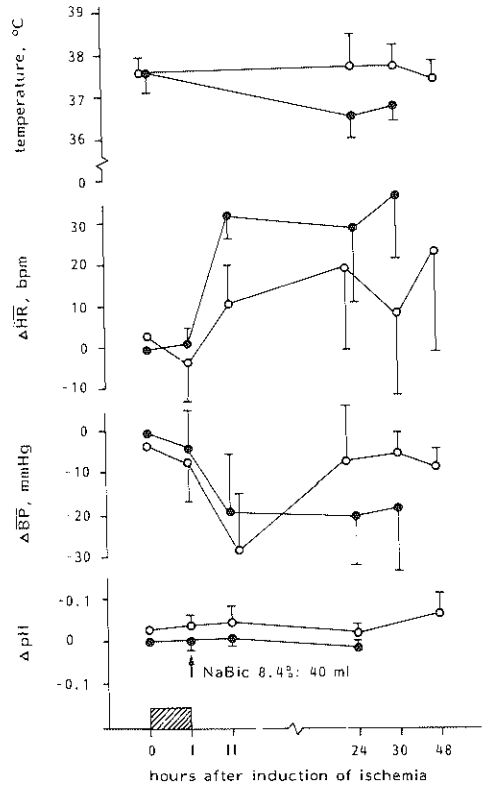

Fig.6. Effect on temperature, heart rate (HR), blood pressure (BP) and pH (mean ± SD) of 4 hrs of liver ischemia (o——o) and 6 hrs of liver ischemia (●——●).
I: just before revascularization of the liver.
II: 30 minutes after revascularization of the liver.

Coagulation factors (fig.7).

A remarkable decrease in platelets was observed after the revascularization of the liver; the lowest value (66 x 10^9/l) was recorded three hours after revascularization . Platelet concentration increased gradually in 4-hour pigs but remained low in animals that underwent 6 hours of ischemia. The same course was observed for coagulation factors (prothrombin time, activated partial thromboplastin time and fibrinogen level).

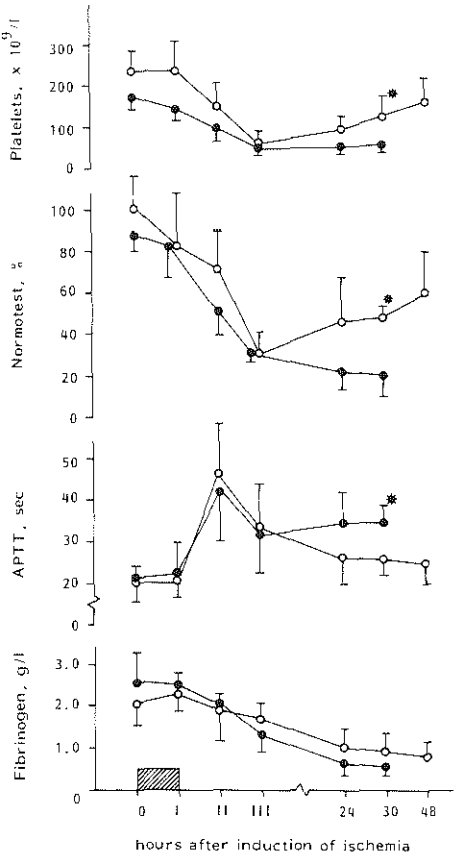

Fig 7. Platelet counts, normotest and APTT and fibrinogen levels (mean ± SD) after 4 hrs of liver ischemia (o——o) and 6 hrs of liver ischemia (● ●).

I: just before revascularization of the liver.

II: 30 minutes after revascularization of the liver.

III: 3 hours after revascularization of the liver.

* $p < 0.05$, comparison between '4 hour animals' and '6 hour animals'.

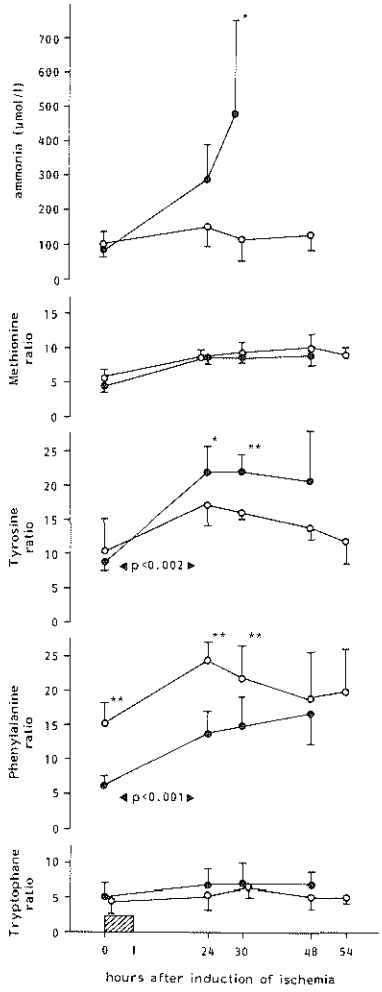

Fig 8. Plasma ammonia concentrations, and methionine, tyrosine, phenylalanine and tryptophane ratio's (mean ± SD) in pigs subjected to 4 hours of liver ischemia (o——o) and with 6 hours of liver ischemia (●——●).

* $p < 0.05$, ** $p < 0.01$, comparison between '4 hour animals' and '6 hour animals'.

Putative toxins (fig.8).

Plasma ammonia was only moderately elevated at 24 hrs (162 ± 86 umol/l) in '4-hour pigs', in contrast to the levels found in animals undergoing 6 hours of ischemia: 283 ± 113 umol/l at 24 hours.

The plasma ratio's for leucine, isoleucine and valine decreased to a minimum level at 24 hrs after the ischemic period, without any difference between the 4 and 6-hour groups (Leucine from 20 to 12; Isoleucine from 15 to 8 and valine from 32 to 20). The plasma ratio's for methionine, tyrosine and phenylalanine increased in both groups. In the 4-hour group, the plasma ratios for tyrosine and phenylalanine normalized to pre-ischemic values after 48 hours, while the values for the 6-hour group remained significantly higher at 30 and 48 hours. Significant differences in the ratio for trypthophan were not found. With this relatively insensitive method, GABA levels could not be detected in plasma except in 2 animals (1.6-8.0 nmol/l).

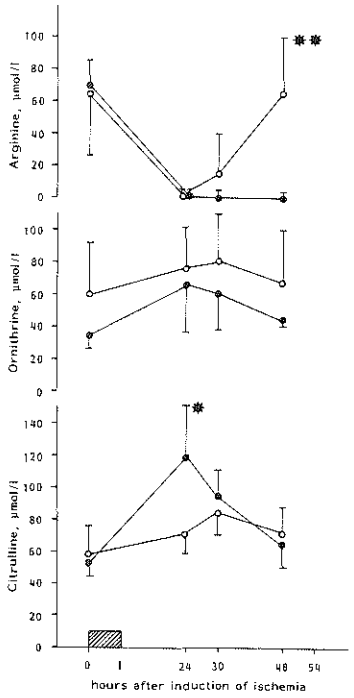

Fig 9. Plasma arginine, ornithine and citrulline concentrations (mean ± SD) in pigs after 4 hours of liver ischemia (o——o) and 6 hours of liver ischemia (●——●).
* $p < 0.05$, ** $p < 0.001$, comparison between '4 hour animals' and '6 hour animals'.

Urea cycle amino acids (fig. 9).

In both groups serum arginine levels had dropped to zero 24 hrs after the ischemic period. The plasma arginine levels gradually returned to the initial values in all but one of the 4-hour animals, while remaining zero in 6 of the 7 animals that underwent 6 hours of ischemia. The plasma ornithine and citrulline levels increased in both groups but the rise was only significant in 6-hour animals.

Histology (table I)

In 4-hour pigs a mean of 42% of liver cells showed total necrosis. In those who died, more than 50% of the liver cells were necrotic, and in those who survived less than 40%. Six hours of ischemia resulted in a mean necrosis of 62% (range 49-75%). Necrosis of 50% or more resulted ultimately in hepatic coma in 9 out of 13 animals. It should be mentioned that beyond the necrotic areas large fields of degenerated hepatocytes were always seen; these cells were not included in the quantitative assessment of liver cell necrosis.

TABLE I. Quantitative assessment of liver necrosis by point analysis of whole liver slices.

	4 hrs of liver ischemia		6 hrs of liver ischemia	
	survival(hrs)	% necrosis	survival(hrs)	% necrosis
animal no 1	72	15	20	70
2	26	68	20	66
3	10	67	29	49
4	72	34	72	50
5	72	36	50	50
6	72	34	43	75
7	6	+	30	69
8	51	+		

+ no histology available.

Discussion

Our experiments show that ischemia of the liver in fully ambulant normothermic pigs was often tolerated for 4 hours, but that 6 hours of hepatic ischemia was usually followed by hepatic coma and death. Previous studies (6) had failed to identify the minimum ischemic period required to produce hepatic coma in non-anesthetized pigs mainly because of poor reproducibility. Our results, however, confirm several recent reports (9,10) that the normothermic liver appears to be more resistant to ischemia than previously appreciated.

Except for the duration of transient hepatic ischemia other factors may influence the effect of hepatic ischemia on survival. Anesthesia with its variations in duration and depth and the variable metabolic disturbances associated with surgery may modulate the extent of liver damage and thereby the final outcome (5). Therefore, in contrast to most previous studies (7-10) we induced transient hepatic ischemia in the pig after the effects of anesthesia and surgery had disappeared.

The time interval between initial surgery and the induction of liver ischemia is said to influence the extent of liver cell necrosis due to the formation of collaterals (6,14). Since we had nearly always observed encephalopathy and death due to massive necrosis of the liver in earlier experiments with non-surgical induction of permanent hepatic ischemia (15), we have continued to use a time interval of three days between initial surgery and the non-surgical induction of hepatic ischemia. A potential source of collateral circulation in a model based on transient ischemia is the wall of the common bile duct. This structure contains a network of blood vessels which can supply an appreciable amount of blood to the liver. Therefore a short piece of tubing was placed in the common bile duct and the blood flow along the common duct was interrupted by two ligatures around the tube ends.

Another factor that could affect liver cell necrosis and thus the duration of tolerable liver ischemia is the putative toxins that appear in the anhepatic state. Extensive bowel cleansing before induction of hepatic ischemia and an adequate supply of calories afterwards were included in our protocol in an attempt to minimize the disturbances of the 'internal milieu'.

Is this an adequate model of acute hepatic failure? The requirements for a satisfactory animal model of acute hepatic failure, as compiled by Terblanche (4), include 1. potential reversibility, 2. reproducibility, 3. death due to hepatic coma after elapse of a time period sufficiently long to allow hepatic support procedures to be instituted, 4. the use of a large animal, 5. induction of liver necrosis without biohazard.

1.Reversibility. Since hepatic circulation is restored in our model (albeit only through the hepatic artery), the potential for recovery and regeneration is present. Histologically 6 hours of hepatic ischemia resulted in necrosis less than 75% of the liver cells. None of the animals had a totally (90-100%) necrotic liver. Therefore, at least 25% of the liver tissue remained available for recovery and possibly regeneration, assuming that the majority of cells in various stages of degeneration retain the potential of recovery in a normal ' internal milieu' (17,18).

2.Reproducibility. All animals subjected to 6 hours of ischemia developed severe encephalopathy (EEG grade 4) within 30 hours and died within 20 to 50 hours, except for one animal that survived for 72 hours. The histological data showed necrosis of 50-75% of the liver and all biochemical measurements, including analysis of putative toxins were fairly uniform. The variations in observations which are inherent to any biological experiment appear less prominent in our model than in most models for drug-induced acute hepatic failure (19,20).

3. Death from liver failure. Within 30 hours all animals that underwent 6 hours of hepatic ischemia developed severe encephalopathy (EEG grade 4) which was followed by death. In two animals liver failure was complicated by gastric hemorrhage that resulted in early death. With respect to this complication, stress induced by insufficient freedom of movement might be of pathogenetic importance in this animal species. Endotoxemia and bacteremia were excluded as non-hepatic causes of death in our earlier studies (16). The time between induction of ischemia and the development of encephalopathy and death is about 24 and 48 hours respectively; such a period is sufficiently long for introduction of an experimental treatment and evaluation of its effects.

4. Other requirements for an appropriate animal model are the use of a large animal and minimal hazards to the personnel. In our model highly inbred pigs were used. The model of hepatic ischemic necrosis does not require the use of dangerous toxic substances.

We think therefore that our animal model fulfills all the criteria proposed (4) for an appropriate animal model of fulminant hepatic failure.

Several other interesting observations with regard to this animal model were made.

1. Clinical neurological assessment by means of semi-quantitative measurements was of restricted diagnostic value and distinguishes only non-coma from coma. In contrast EEG assessment identified all grades of encephalopathy in pigs with ischemic hepatic necrosis. Automated EEG analysis showed that objective measurement of encephalopathy in pigs is feasible (data will be published elsewhere).

2. Hepatic ischemia for 6 hours did not in itself induce marked abnormalities in coagulation tests. However, as soon as revascularization of the ischemic liver was established severe disturbances developed. A marked drop in platelet count and in the levels of fibrinogen and other clotting factors was observed, suggesting intravascular coagulation. Exposure of the blood to damaged sinusoidal cells within the ischemic liver seems a likely explanation for the observed findings (21). No reduction in platelet counts was observed in earlier experiments in pigs with permanent ischemia of the liver who showed a decrease in the levels of the clotting factors to 20% at 24 hours (15,22,31).

3. Tyrosine and phenylalanine ratio's clearly increased in our model. Elevation of the tyrosine ratio was greater for the 6-hour animals and therefore seems to be related to the degree of hepatic insufficiency (23-27). Correlations between the degree of liver cell necrosis and other amino acids ratio's were not found. Assuming that disturbances in the transport of amino acids across the blood-brain barrier are best expressed by plasma ratio's, tyrosine appears to be the major abnormality of neutral amino acids in hepatic encephalopathy in the pig. These observations contrast with the findings in rats and dogs of a concurrent rise of tyrosine, phenylalanine and tryptophan.

4. Associated with the liver failure was a decrease in plasma arginine levels of more than 90%. In animals who survived the plasma arginine levels normalized while they remained zero in those who died. Arginine is required for effective utilization of ammonia in the urea cycle (28). A correlation between the persistent absence of plasma arginine and the rapid rise in ammonia may be entertained. However, the observations (29,30) that arginase is released from the necrotic liver cells into the plasma

compartment, and induces conversion of plasma arginine into ornithine and urea (a rise in plasma ornithine levels was indeed observed, fig.10), emphasizes the need for measurements of intracellular concentration of arginine before inferences about the activity of the urea cycle can be made.

In conclusion we believe that our animal model of acute hepatic failure is comparable to the human condition of acute hepatic failure. Since the model is reversible as well as reproducible and does not constitute a biological hazard, it can be used for studies of the pathogenesis and complications of acute liver failure. Moreover, since our model utilizes a large animal with a life expectancy of about 48 hours, testing of hepatic support systems or assessment of auxiliary liver transplantation procedures is possible.

Literature

1. Giges B, Demn HL, Sborov VM, et al. Experimental hepatic coma. Surg Gynec Obstet 1953;97:763-768.
2. Tonnesen K. Total devascularization of the liver. An experimental model of acute liver failure. Scand J Gastroent 1976;11 (suppl 37):23-26.
3. Rappaport AM, Malcolm PH D, MacDonald H, et al. Hepatic coma following ischemia of the liver. Surg Gynec Obstet 1953;97:748-762.
4. Terblanche J, Hickman R, Miller D, et al. Animal experience with support systems: are they appropriate animal models of fulminant hepatic necrosis? In Artificial Liver Support. Edited by Williams R, Murray-Lyon I. London, Pitman, 1975;p 163-172.
5. Misra MK, P'eng FK, Sayhoun A, et al. Acute hepatic coma: a canine model. Surgery 1972;72:634-642.
6. Fischer M, Stötter L, Schmahl W, et al. Acute liver failure due to temporary hepatic ischemia in the pig. Acta Hepato-Gastroenterol 1976; 23:241-249.
7. Battersby C, Hickman R, Saunders SJ, et al. Liver function in the pig: 1. The effects of 30 minutes normothermic ischemia. Br J Surg 1974;61:27-32.
8. Fredlund PE, Ockerman PA, Vang JD. Acidosis and increased plasma levels of B-D glucosidase and B-D galactosidase after hepatic inflow occlusion in the pig. Acta Clin Scand 1974;140:234.
9. Nordlinger B, Douvin D, Javaudin L, et al. An experimental study of survival after two hours of normothermic hepatic ischemia. Surg Gynec Obstet 1980; 150:859-864.
10. Harris KA, Wallace AC, Wall WJ. Tolerance of the liver to ischemia in the pig. J Surg Res 1982;33:524-530.
11. Opolon P, Lavalland MC, Huguet CL, et al. Hemodialysis versus cross-dialysis in experimental hepatic coma. Surg Gynec Obstet 1976;142: 845-854.
12. da Fonseca, Wollheim. Direkte plasma-ammoniak bestimmung ohne enteiweissung. Z Klin Chem Biochem 1973;11:426-431.
13. Pardridge WM, Oldendorf WH. Transport of metabolic substrates through the blood-brain barrier. J Neurochem 1977;28:5-12.
14. Joyeuse RB, Ivanisevic WP, Longmire Jr JV. The treatment of experimental hepatic coma by parabiotic cross circulation. Surg Gynec Obstet 1963;117:129-134.

15. de Groot GH, Schalm SW, Schicht I, et al. Comparison of large-pore membrane hemodialysis and cross dialysis in acute hepatic insufficiency in pigs. Eur J Clin Invest 1983;13:65-71.
16. de Groot GH, Schalm SW, Batavier P, et al. Endotoxemia in pigs with ischemic hepatic necrosis treated by lactulose and hemodialytic procedures. Hepatogastroenterology 1983,30:240-242.
17. James J, Myagkaya GL. De celdood; nieuwe inzichten in een oud probleem. Ned T Gen 1983;35:1572-1578.
18. Frederiks WM, James J, Bosch KS, et al. A model for provoking ischemic necrosis in rat liver parenchyma and its quantitative analysis . Exp Pathol 1982;22:245-252.
19. van Leenhoff JW, Hickman R, Saunders SJ. Massive liver cell necrosis induced in the pig with carbon tetrachloride. S Afr Med J 1974;48: 1201-1204.
20. Miller DJ, Hickman R, Fratter R. An animal model of fulminant hepatic failure: a feasibility study. Gastroenterology 1976;71:109-113.
21. Dinbar A, Rangel DM, Fonkalsrud EW. Effects of hepatic ischemia on coagulation in primates. Application to liver transplantation. Surgery 1970;68:269-276.
22. Tonnesen K. Experimental liver failure. Acta Clin Scand 1977;143:271-277.
23. Mazziotti A, Bernardi M, Antonini L,et al. Plasma amino acid patterns in experimental acute hepatic failure: comparison between hepatectomy and liver devascularization in pigs. Surgery 1981;90:527-534.
24. Wustrow Th, van Hoorn-Hickman R, van Hoorn WA, et al. Acute hepatic ischemia in the pig- the changes in plasma hormones, amino acids and brain biochemistry. Hepato-Gastroenterol 1981;28:143-146.
25. Chase RA, Davies M, Trewby PN et al. Plasma amino acid profiles in patients with fulminant hepatic failure treated by repeated polyacrilonitrile membrane hemodialysis. Gastroenterology 1978;75: 1033-1040.
26. Munro HN, Fernstrom JO, Wurtman RJ. Insulin plasma amino acid imbalance and hepatic coma. Lancet 1975;1:722-724.
27. Rosen HM, Yoshimura N, Aguivre A et al. Plasma amino acids in patients with acute hepatic failure. Gastroenterology 1977;72:483-487.
28. Zieve L. Amino acids in liver failure. Gastroenterology 1979;76:219-221.

29. Cacciatore L, Antoniello S, Valentino B et al. Arginase activity, arginine and ornithine of plasma in experimental liver damage. Enzyme 1974;17:269-275.
30. Vijaya S, Nagarajan B. Arginine metabolism in rat liver after hepatic damage. Biochem Med 1982;27:86-94.
31 de Groot GH, Schalm SW, Schicht I, et al. Large-pore haemodialytic procedures in pigs with ischaemic hepatic necrosis; a randomized study. Hepato-Gastroenterology 1984;no.4-5:in press.

CHAPTER III.

OBJECTIVE MEASUREMENT OF HEPATIC ENCEPHALOPATHY IN PIGS BY MEANS OF SPECTRAL ANALYSIS AND EVOKED RESPONSES.

Gerrit H. de Groot, M.D., Solko W. Schalm, M.D., Marinus de Vlieger, M.D., Carin C.D. van der Rijt.
Departments of Internal Medicine II and Electroneurology, Erasmus University, Rotterdam.

Supported by the Foundation for Medical Research FUNGO, the Netherlands.

Submitted for publication.

Abstract

Objective measurement of hepatic encephalopathy by means of spectral analysis of the EEG, visual evoked potentials (VEP) and brain stem auditory evoked potentials (BAEP) was studied in pigs with ischemic hepatic necrosis. The mean dominant frequency (MDF) and the relative power of the delta frequency band (% power) showed significant changes with increasing encephalographic grades of encephalopathy: MDF dropped from 7.0 ± 0.5 Hz (grade 0) to 2.7 ± 0.3 Hz (grade 3 encephalopathy) and % power increased from 52 ± 7% (grade 0) to 83 ± 6% (grade 3).

The patterns of the VEP in pigs corresponded to those of the human VEP. However, significant differences in either latency time of the peaks or the peak amplitude with increasing stages of hepatic encephalopathy could not be found. The BAEP registered for pigs were reproducible but also were not as useful as spectral analysis for grading hepatic encephalopathy.

Running title: Spectral analysis and evoked responses in hepatic coma.

Introduction

Pigs are often used to study the pathogenesis or treatment of hepatic encephalopathy, because of their physiological similarity with the human being. Reliable methods for the objective measurement of encephalopathy in pigs are non-existent. Clinical neurological assessment of the grade of encephalopathy in pigs is subjective and unreliable, since it yields only a distinction between coma and non-coma. Conventional EEG readings have been proposed as a method for grading hepatic encephalopathy (1,2,3,4), but interpretation of the results remains subjective and therefore dependent on the experience and consistency of the electroneurologist.

The introduction of quantitative analysis of the EEG offered the possibility of measuring hepatic encephalopathy objectively (5,6). By this method the EEG can be expressed in terms of the mean dominant frequency (M.D.F.) and the wave amplitude as a function of the frequency (power spectrum). Recently evoked potentials were introduced as another objective test for hepatic encephalopathy (7-11).

The present work was carried out to determine the reliability of quantitative EEG analysis and evoked potentials as a means of grading pigs with hepatic encephalopathy.

Material and Methods

Surgical preparation. Fifteen large White pigs, weighing 28 to 33 kg, were used. Pigs underwent laparotomy for the introduction of a functional end-to-side portocaval shunt. All ligaments and peritoneal attachments to the liver were cut or coagulated. Subsequently a clamp was placed around the isolated hepatic artery. Five silver electrodes (ϕ 1 mm) were inserted through the skull onto the dura for the recording of the EEG and evoked potentials: two over the frontal cortex, one in the vertex and two over the occipital cortex. The electrodes were anchored with sterile acrylic bone cement (Surgical Simplex, Howmedica, London,England) and channeled percutaneously. Three days after construction of the portocaval shunt acute hepatic necrosis was induced by temporarily tightening the clamp to close off the hepatic artery for 4 or 6 hours. Anesthesia was not used in this phase, since it may interfere with subsequent neurological assessments. Glucose (12 g/kg/24 hrs), penicillin G (9 megaU/24 hrs) and kanamycin (3 g/24 hrs) were

infused continuously. The environmental temperature was maintained at 25°C to ensure normothermia of the pigs with hepatic necrosis.

Recording procedure. During the study periods the animals were placed in a dim room on their left side and covered with a sheet to ensure adequate relaxation. EEG recordings were taken before induction of hepatic necrosis and 24, 30, 48, 52 and 72 hrs afterwards. It should be noted that pigs as soon as they tend to fall asleep have an 'abnormal' slow EEG. Therefore in order to obtain a meaningful EEG they had to be aroused each time. The potentials in four bipolar leads (left and right fronto-occipital, frontofrontal and occipito-occipital) were registered by an EEG apparatus (Ahrend v.Gogh, Amsterdam, Holland). During 3 'epochs' of 100 seconds the signals of the fronto-occipital leads were stored simultaneously on a magnetic tape (Analog 7, Philips, Eindhoven, Holland) for (quantitative) spectral analysis. The EEG channels were filtered with a band pass filter between 0.5 Hz and 70 Hz. The stored data were fed into a computer (PDP 11/34) at a sampling rate of 51.2 Hz and a sensitivity of 11 bits/5V. Each epoch of 100 seconds was divided into 10 periods. The power spectrum (the sum of wave amplitudes as a function of each frequency or frequency band) was calculated for each period of 10 seconds using the Fast Fourier Transformation, and the mean power spectrum for each 100-second epoch was obtained. The frequency resolution was 0.1 Hz. To minimize the effect of artefacts, only the range of 1.0-25.6 Hz was used to calculate the mean spectrum which was then analyzed (fig.1).

The parameters calculated were the mean dominant frequency and the relative power of the delta, theta and alpha frequency range (12). The mean dominant frequency (MDF) was defined as:

$$MDF = \sum_{i=1}^{n} fi/Si \Big/ \sum_{i=1}^{n} Si$$

(fi= frequency i, Si=power of frequency i, n= the number of frequencies).
The delta activity was defined as the activity of the frequencies 1.0-3.5 HZ, the theta activity that of the frequencies 3.5-8.0 Hz, the alpha activity that of the frequencies 8.0-13.0 Hz, and the beta activity that of the frequencies 13.0-25.6 Hz. The 3 epochs of 100 seconds were averaged. Spectra that showed high muscle activity in the beta frequency band or asymmetry between the two fronto-occipital leads were not included in the calculations.

The EEG recordings were analyzed independently by an experienced electroneurologist; the 5 grade classification system described by Opolon (2,4) was used.

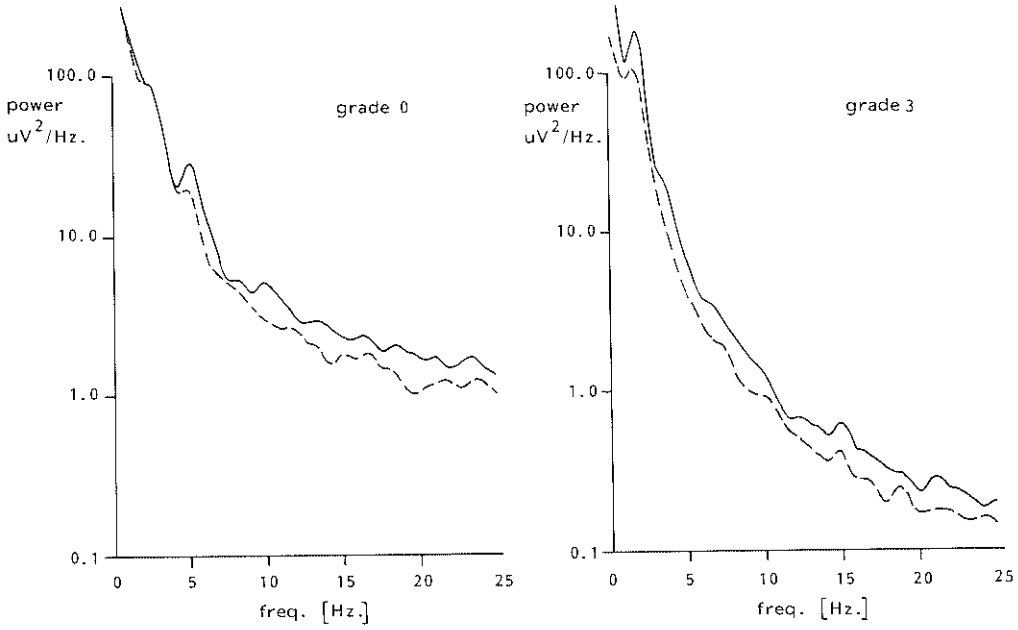

fig.1a,b. A visual representation of the EEG power spectrum (sum of all amplitudes as a function of the frequency) for normal pigs (a) and for pigs with hepatic encephalopathy (b).

Visual evoked potentials (VEP). A photo stimulator with a xenon flashlight provided stimuli with a flash intensity of 1 J and a stimulation frequency of 1 Hz. The experiments were performed in a darkened room with the flashlight at a constant distance of 70 cm from the right eye. The EEG, monitored on an oscilloscope and on the EEG recorder, was stored on the magnetic tape during a 300-second artefact-free period. For the two fronto-occipital leads VEP's were obtained by averaging the response of 100 stimuli. Only the responses during the first 400 msec were used (13).

Brain stem evoked potentials (BAEP). Responses from electrodes at the vertex and ear lobes were recorded. Ten clicks/sec were generated; the intensity of each click was 70 dB and it lasted 0.2 msec. Clicks were presented to the animal via an earphone. The average response in the 10-msec interval after the stimulus (n=2000) was calculated by computer (PDP 11/34). Each animal was subjected to two mono-aural tests in a 5 minute period.

Data evaluation. Analysis of the VEP in normal pigs showed 7 waves (fig.2a). Peaks I,III,V and VII were positive, whereas peaks II,IV and VI were negative. All peaks occurred within 150 msec after visual stimulation.

Analysis of the BAEP in pigs showed 5-6 positive waves within the first 6 msec (fig.3a).

All waves found for normal animals were compared with the waves found for animals with hepatic encephalopathy. Moreover each animal was used as its own control during the development of encephalopathy. The latency time was defined as the time (msec) between onset of the stimulus and the maximal amplitude of each peak. Amplitudes between two consecutive positive and negative peaks were measured.

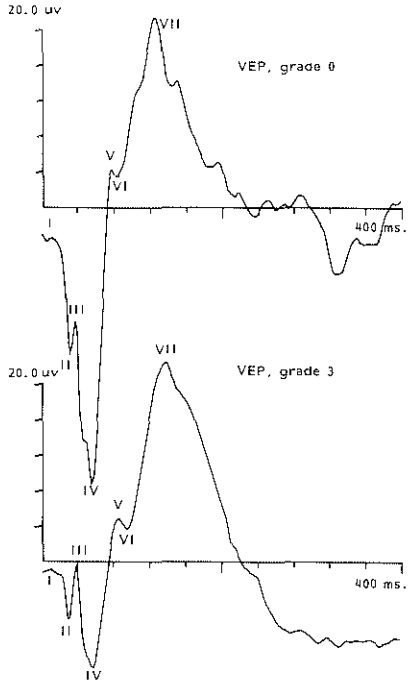

fig.2a. VEP of a normal pig.

b. VEP of a pig with grade 3 hepatic encephalopathy.

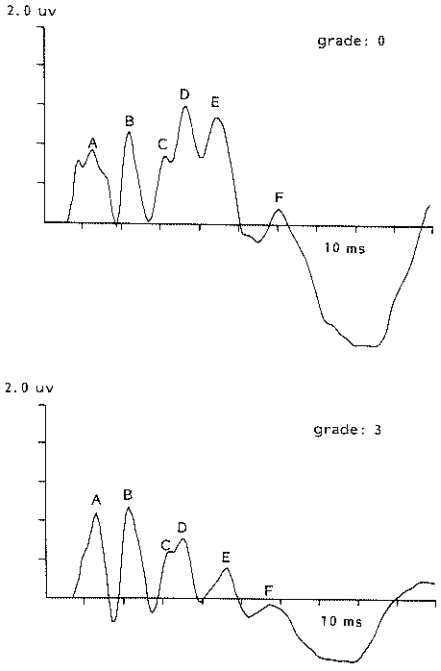

fig.3a. BAEP of a normal pig.

b. BAEP of a pig with grade 3 hepatic encephalopathy.

Results

Survival.

Fifty percent of the pigs undergoing four hours of ischemia of the liver survived. Two animals died from complications (air embolism, peritonitis) and 2 of hepatic coma. Six hours of ischemia was followed by hepatic encephalopathy in all animals. Four of the 7 animals died of hepatic coma; one animal in hepatic coma, grade 4, was sacrificed after 72 hours and two other animals had concurrent gastric hemorrhage.

EEG and spectral analysis (fig.4,5).

The EEG, semiquantified into grades 1-5, gradually deteriorated after induction of ischemia. Animals subjected to 4 hours of ischemia developed mild encephalopathic EEG changes whereas 6 hours of ischemia produced severe

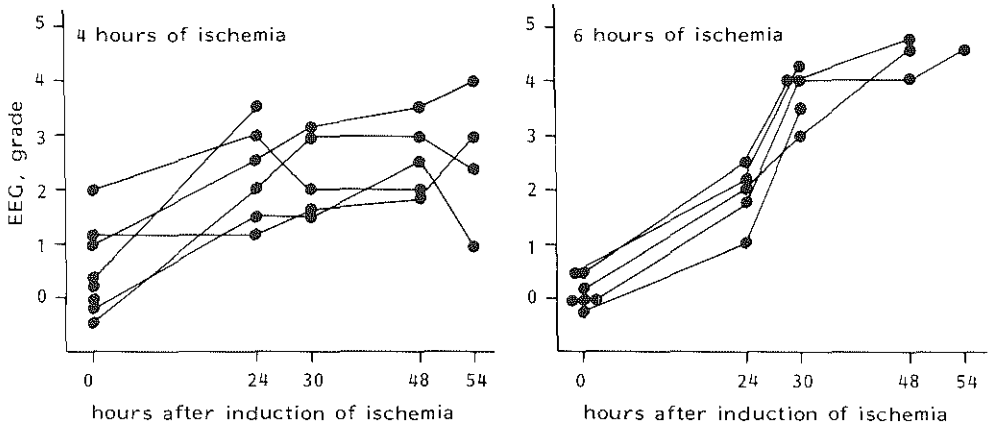

fig.4. The course of the EEG grades for pigs that have undergone 4 hours and 6 hours of ischemia of the liver.

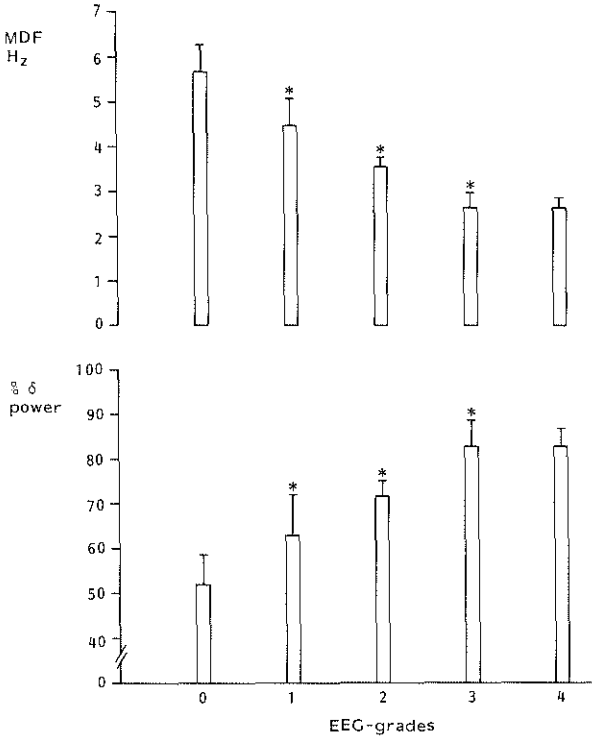

fig.5. Spectral analysis data for pigs with various EEG grades of encephalopathy, expressed as the mean dominant frequency (MDF) (mean ± SD) and the relative percentage of the delta power (mean ± SD). The differences between grades 0,1,2 and 3 were significant ($p < 0.01$).

encephalopathic EEG changes within 30 hrs.

The mean dominant frequency dropped significantly with each grade of encephalopathy up to grade 3. No significant frequency differences were observed between grade 3 and grade 4 encephalopathy.

The relative power of the delta activity was 52 ± 7% in grade 0, and this increased significantly with each grade of encephalopathy up to grade 3 (83 ± 6%). Delta activities for grades 3 and 4 did not differ significantly. An example of the power spectra for grade 0 and grade 3 encephalopathy is given in fig.1.

VEP (table I).

The latency time for all peaks was measured during several grades of encephalopathy. The overall results did not show significant differences in peak latency or changes in amplitude between grade 0 and grade 4 encephalopathy. However, in some animals there was an increase in latency time of peak VII and an decrease in amplitude between peak IV and V during encephalopathy (fig.2b).

TABLE 1. VEP peak latency times (msec, mean ±SD) in relation to the grade of encephalopathy.

	peaks						
	I	II	III	IV	V	VI	VII
encepha-lopathy grade 0	33 ± 8	40 ± 5	54 ± 6	82 ± 11	107 ± 18	133 ± 25	154 ± 17
1	31 ± 2	36 ± 5	52 ± 9	82 ± 19	101 ± 15	116 ± 16	151 ± 10
2	24	35 ± 5	49 ± 7	76 ± 14	103 ± 12	125 ± 12	143 ± 15
3	24	38	54 ± 6	89 ± 17	122 ± 27	131	160
4	++	36 ± 4	48 ± 9	++	++	++	153 ± 34

++ no definite peak discernable.

BAEP (table II).

As a rule the five positive peaks could be identified during all grades of encephalopathy. The latency times and amplitudes of the five peaks did not change significantly with the grade of encephalopathy (fig.3b).

TABLE II. BAEP peak latency times (msec, mean ±SD) in relation to the grade of encephalopathy.

	peaks					
	A	B	C	D	E	F
encephalopathy grade 0	1.4 ± 0.1	2.2 ± 0.2	2.8 ± 0.2	3.5 ± 0.2	4.4 ± 0.2	5.6 ± 0.3
1	1.4 ± 0.1	2.2 ± 0.2	2.6	3.5 ± 0.1	4.2 ± 0.3	5.5 ± 0.3
2	1.3 ± 0.1	2.0 ± 0.2	2.6 ± 0.1	3.4 ± 0.3	4.2 ± 0.3	5.6 ± 0.3
3	1.5 ± 0.2	2.2 ± 0.1	2.6 ± 0.2	3.4 ± 0.1	4.4 ± 0.2	5.9 ± 0.5
4	1.4 ± 0.1	2.0 ± 0.2	2.5 ± 0.1	3.4 ± 0.2	4.1 ± 0.3	5.5 ± 0.4

Discussion

Since clinical grading of hepatic encephalopathy in pigs is subjective and difficult, Opolon et al (4) introduced 5 grades of encephalopathy based on conventional EEG recordings. However, the interpretation of the EEG remains subjective and dependent on the expertise of the observer. Our study showed that objective measurement of hepatic encephalopathy in pigs is possible by means of spectral analysis. The EEG activity, represented by the mean dominant frequency, exhibited a fairly close correlation with the grade of encephalopathy. The relative power of the delta band expressed as a percentage of the total power of the alpha, beta, theta and delta bands, increased significantly with deterioration of the EEG, reflecting an increase in slow wave activity. Although the mean values of MDF and % delta power were significantly different for each grade of encephalopathy, individual measurements showed some overlapping. Therefore both parameters should be used together to classify the grade of encephalopathy, although our results indicate that a reliable distinction between grades 3 and 4 encephalopathy was not possible.

VEP's in pigs have the seven main components present in man (13). However, the reproducibility of the latency times and the amplitudes of two consecutive positive and negative peaks for each grade of encephalopathy was

rather poor. In some individual animals we observed a decrease in amplitudes of two consequence positive and negative peaks and an increase in the latency time of peak VII. That VEP constitutes a sensitive procedure to measure hepatic encephalopathy in pigs could not be confirmed, in contrast to the optimistic results obtained with rats and rabbits (7,8).

The BAEP's were measured to obtain information about the conduction time in the brain stem during hepatic encephalopathy. In contrast to VEP's BAEP's were highly reproducible. Five well-defined peaks could be obtained even in coma (grade 4). However, significant changes in the latency times of any of the peaks were not detected during progression of the hepatic encephalopathy.

In conclusion, objective measurement of hepatic encephalopathy in pigs by means of spectral analysis of the EEG is possible. Evoked potentials, however, are not sufficiently sensitive to follow the course of hepatic encephalopathy in pigs.

Literature

1. Parsons-Smith BG, Summerskill WHJ, Dawson AM, et al. The electroencephalogram in liver disease. Lancet 1957;2:867-871.
2. Opolon P, Rapin JR, Huguet C, et al. Hepatic failure coma treated by polyacrilonitrile membrane hemodialysis. Trans Am Soc Artif Intern Organs 1976;XXII:701-711.
3. Conn HO, Lieberthal MM. The hepatic coma syndromes and lactulose. Baltimore: The Williams ans Wilkins company, 1979;p.20-28.
4. Opolon P, Lavallard MC, Huguet CL, et al. Hemodialysis vs. cross-dialysis in experimental coma. Surg Gynec Obstet 1976;142:845-854.
5. Hawkes CH, Macpherson AJS,Pryor H, et al. The value of EEG frequency analysis in hepatic encephalopathy. J Roy Coll Surg Edinb 1970; 15:151-156.
6. Yaar J, Shapiro MB, Pottala EW. Spectral analysis of the EEG in hepatic encephalopathy treated with levodopa. Electroenceph Clin Neurophysiol 1981;52:617-625.
7. Zeneroli MC, Penne A, Parrinello G. Comparative evaluation of visual evoked potentials in experimental hepatic encephalopathy and in pharmacologically induced coma-like states in rat. Life Sciences 1981;28:1507-1515.
8. Schafer DF, Brody LE, Jones EA. Visual evoked potentials: an objective measurement of hepatic encephalopathy in the rabbit. Gastroenterology 1981;77:A38.
9. Zeneroli ML, Cremonini C, Pinelli G. Visual evoked potentials: a tool to discuminate preclinical and clinical stages of hepatic encephalopathy. Hepatology 1982;Vol.2: abstract no.10.-
10. Starr A, Achor LJ. Auditory brainstem responses in neurological disease. Arch Neurol 1975;32:761-768.
11. Jewett DL, Romans MN, Williston JS. Human auditory evoked potentials. Science 1970;167:1517-1518.
12. Holm E, Thiele H, Wolpert EM, et al. Neurologische und psychiatrische Symptome bei akuten und chronische Leberkrankungen. Therapiewoche 1980;30/28:4790-4806.
13. de Vlieger M, Sadikoglu S, van Eijndhoven JHM, et al. Visual evoked potentials, auditory evoked potentials and EEG in shunted hydrocephalic children. Neuropediatrics 1981;12:55-56.

CHAPTER IV.

INCIDENCE OF ENDOTOXEMIA IN PIGS WITH ISCHEMIC HEPATIC NECROSIS TREATED BY HEMODIALYSIS.

Prevention of endotoxemia with lactulose.

G.H. de Groot[1], S.W. Schalm[1], P. Batavier[2], H.C.M. Maas[3], I. Schicht[2].
Department of Internal Medicine II[1] and Medical Microbiology[3], University Hospital Dijkzigt, Rotterdam, and Department of Nephrology[2], University Hospital Leiden.

A grant-in-aid for this study was received from Byk, Holland.

Hepato-Gastroenterology, 1983;30:240-242.

ABSTRACT

The incidences of endotoxemia and bacteremia were evaluated in 30 pigs with ischemic hepatic necrosis that were treated by hemodialytic procedures. Prior to induction of hepatic ischemia, ten pigs underwent bowel cleansing by means of an oral dose of magnesium sulfate and 20 received a combination of magnesium sulfate and lactulose.

Endotoxemia and bacteremia seldom occurred during the development of hepatic encephalopathy, but the incidence of both increased markedly shortly before death. Pigs pretreated with magnesium sulfate and lactulose however did not develop preterminal endotoxemia. A significant relation between endotoxemia or bacteremia and survival was not found, irrespective of pretreatment with lactulose. Of the positive limulus tests, 67% were accompanied by a positive blood culture, while 42% of all positive blood cultures were associated with a positive limulus test. Dialysis with dialysates contaminated with endotoxins did not increase the risk of endotoxemia.

It is concluded that in an animal model of ischemic hepatic necrosis (1) endotoxemia and bacteremia appear mainly in the preterminal stage but do not influence the duration of survival significantly;(2) lactulose prevents endotoxemia and (3) dialytic procedures do not increase the risk of endotoxemia and bacteremia.

Key-words: Endotoxemia and bacteremia - Ischemic hepatic necrosis - Hemodialytic treatment - Prevention with lactulose.

Introduction

Endotoxemia is said to be a common feature of acute hepatic falure and has been suggested to be a major cause of death for anhepatic animals (1,2). Endotoxemia has therefore to be excluded as an interfering event in animal model used for the study of hepatic encephalopathy. We used the model of ischemic hepatic necrosis for our studies on the efficacy of hemodialytic procedures in acute liver failure (3). To validate our model we studied the incidence of endotoxemia and bacteremia during the development of encephalopathy, and in particular the effect of hemodialysis, which might increase the risk of endotoxemia and bacteremia because of contaminated bath water (4,5). Since it has been suggested that lactulose may have an endotoxin-reducing effect in liver disease (6,7) we followed two bowel cleansing protocols to evaluate the efficacy of lactulose in the prevention of endotoxemia in our animals.

Methods

Animals: In 30 large White pigs, 8-10 weeks old and weighting 25-30 kg, a portocaval shunt was constructed and a loose silicone loop placed around the hepatic artery and common bile duct. Two days later, one day before the induction of ischemic hepatic necrosis by tightening the silicone loop, bowel cleansing was performed. The first 10 animals received 30 g of magnesium sulfate via a gastric tube, the last 20 animals were given 150 ml of lactulose in addition to the magnesium sulfate. Immediately after induction of ischemic hepatic necrosis all animals received glucose (9kg/24 hrs), penicillin G (9 M units/24 hrs) and kanamycin (3 g/24 hrs), intravenously. Hemodialytic procedures were started 18 hrs later, as described previously (9). Twelve animals underwent AN69 hemodialysis with a polyacrylonitrile (AN69) membrane (Rhone Poulenc, Paris, France), 4 cross-hemodialysis, 5 hemofiltration with reinfusion of an electrolyte solution and 5 hemofiltration with reinfusion of autologous ultrafiltrate. Four animals did not undergo any extracorporeal procedure.

Measurements. The duration of survival was defined as the period between the induction of ischemic hepatic necrosis and the time of death.

Blood samples for bacterial cultures and the endotoxin assays were taken before induction of ischemic hepatic necrosis, 14 and 24 hrs later and just before death. Blood was drawn under sterile conditions from the arterial end of the Scribner shunt. Dialysate was collected for bacterial cultures and the endotoxin assays at the end of each dialysis period.

Endotoxin assay. Glass tubes with covers (Kimax) were cleansed by rinsing with distilled, pyrogen-free water and 96% w/v alcohol. After autoclaving at 180°C for 2 hrs the covers were placed on the glass tubes and both were autoclaved at 180°C for 3 hrs. Four ml of blood were collected in a Kimax tube containing 100 U of heparin. After centrifugation at 800 g for 10 minutes, 1 ml plasma was transferred to another Kimax tube and stored at -25°C until assayed. After thawing, 0.1 ml of plasma was diluted 10 times with 0.9% NaCl. The mixture was boiled for 2 minutes and than allowed to cool to room temperature. In the test procedure, equal volumes (0.1 ml) of the solutions to be tested and limulus lysate (Pyrogent; Mallinckrodt/Byk, Netherlands) were mixed in sterile, pyrogen-free 10 mm x 75 mm glass test tubes, and incubated in a water bath at 37°C for 24 hours. The formation of a firm gel, which could be inverted twice without breaking, was considered a positive test. This assay has a detection limit of 0.060 ng of endotoxin per ml of plasma or dialysate. Positive and negative controls were run with each test. Plasma of a healthy volunteer with 0.12 ng of endotoxin per ml was used as the positive control, pyrogen free water and 0.9% NaCl were used as negative controle.

Bacteriology. Blood samples were cultured in 60 ml of Trypticase soy broth at 37°C and observed for two days. In the event of bacterial growth, a subdivision was made for the culture of aerobic bacteria. Dialysate was cultured in Brewer's thioglycolate medium and on McConkey agar; after an incubation period of 24 hours subcultures were made.

Results

Endotoxins. Two of the 30 animals (7%) had a positive limulus test before

induction of ischemic hepatic necrosis. At 14 hrs, 4 animals had a positive test, endotoxemia persisted in these animals until death. At 24 hrs, the incidence of endotoxemia did not increase. However, 6 of 16 animals (38%) developed endotoxemia preterminally (Table 1). A association between survival time and endotoxemia was not observed. Animals with endotoxemia did not die sooner than animals without a positive limulus test (Table 2).

Blood cultures. Three of the 30 animals (10%) had positive E.coli cultures before induction of ischemic hepatic necrosis; the cultures remained positive during the 'anhepatic' state. At 24 hrs, a slight increase in the number of positive blood cultures was observed. However, preterminally and coincident with the increased incidence of endotoxemia, there was a marked increase in the number of positive blood cultures to 59% of all animals. E.coli, Enterobacter and Klebsiella were the bacteria most frequently isolated (Table 1). An association between survival time and positive blood cultures could not be detected (Table 2).

Endotoxemia and bacteremia. Combined analysis of all blood cultures (n=74) and endotoxin (n=74) showed that of the 12 animals with a positive limulus test, 8 also had a positive blood culture (67%).

Negative blood cultures (n=55) were associated with a positive limulus test in only 4 cases (7%). Nineteen positive blood cultures were accompanied by 8 positive limulus test (42%) and 11 negative limulus tests (58%). A negative endotoxin assay did not exclude bacterial invasion, since 11 of the 62 samples (18%) with a negative limulus test showed bacterial growth.

Effect of hemodialysis on endotoxemia. In nine cases, all animals undergoing closed-circuit hemodialysis, the dialysate contained both bacteria and endotoxins. The predominant microbe in the dialysate was identified as Pseudomonas aeruginosa, except in one case in which E.coli was the main bacterium. The endotoxin concentration in all dialysates exceeded 0.5 ng/ml. All blood cultures and limulus tests were negative before dialysis (Table 3). At the end of the procedure one animal had a positive blood culture (e.coli) accompanied by a positive blood culture (e.coli) accompanied by a positive limulus test.

TABLE 1.

Incidence of endotoxemia and bacteremia in ischemic hepatic necrosis.

	Endotoxin			Blood culture			Endotoxin + blood culture		
hrs of ischemia	N	pos	%	N	pos	%	N	pos	%
0	30	2	7	30	3	10	30	0	0
14	28	4	14	27	6	22	27	2	7
preterminal (32-64)	16	6	38	17	10	59	16	6	38

TABLE 2.

Effect of endotoxemia and bacteremia on survival of pigs with ischemic hepatic necrosis treated by hemodialytic procedures.

		no of animals	survival time* (hrs)
Endotoxin	pos	6	47.7 ± 12.0
	neg	15	48.6 ± 13.1
Blood culture	pos	12	49.3 ± 13.0
	neg	9	45.8 ± 9.0

* : survival time is expressed as mean ± 1 S.D.
: 12 animals with hemodialysis, 4 with cross dialysis, and 5 with hemofiltration with reinfusion of an electrolyte solution.

TABLE 3.

Bacteriological findings before and after hemodialysis of pigs with ischemic hepatic necrosis.

	endotoxin		culture	
	pos	neg	pos	neg
Blood (n=9)				
before dialysis	0	9	0	9
after dialysis	1	8	1	8
Dialysate (n=9)				
after dialysis	9	0	9	0

Effect of bowel cleansing. Bowel cleansing with lactulose and magnesium sulfate markedly affected the incidence of endotoxemia (Table 4). Out of a total of 48 blood cultures and endotoxin assays in the lactulose group, 7 and 2 samples, respectively, were positive in the non-lactulose group of 26 blood cultures and endotoxin assays 12 and 10, respectively, were positive. Of the animals that did not receive lactulose, 75% had a positive limulus test preterminally, while none of the animals pretreated with lactulose had a positive limulus test at that time. The survival time of animals treated with lactulose was 51 ± 11 hrs which does not differ significantly from that of animals without lactulose pretreatment (45 ± 12 hrs).

TABLE 4.
Incidence of endotoxemia and bacteremia in relation to bowel cleansing for pigs with ischemic hepatic necrosis.

	$MgSO_4$, no Lactulose N=10			Lactulose + $MgSO_4$ N=20		
	time after ischemia			time after ischemia		
	0	14	preterminal	0	14	preterminal
pos.blood culture	1	3	8	2	3	2
pos.endotoxin	1	3	6*	1	1	0

* only 8 of the 10 preterminal blood samples were available.

Discussion

Our results confirm the high incidence of bacteremia (60%) in acute hepatic failure described by others (1), but the incidence of endotoxemia was lower (38%) than that described by Wilkinson (8) in humans, and by Grün and Liehr (9) in rats with a galactosamine-induced hepatitis. In general

endotoxemia and bacteremia developed in our model preterminally; the incidence did not increase markedly during the development of hepatic encephalopathy . Gans et al (1,2) proposed a direct effect of bacteremia and endotoxemia on the survival of anhepatic dogs; in our model of ischemic hepatic necrosis we found no evidence for an effect of endotoxemia on survival. In fact, we found no clinical difference between endotoxin positive and endotoxin-negative animals. During ischemic hepatic necrosis all animals developed a similar progressive and ultimately severe encephalopathy with a flat EEG shortly before death.

A pronounced correlation between a positive limulus test and a positive blood culture of endotoxin-related microbes was found. A positive limulus test and a negative blood culture was only seen sporadically (7%). From our data we conclude that a blood culture remains the most simple and sensitive method for detection of bacteria or bacterial products in acute experimental liver disease.

Our data suggest that an AN69 polyacrylonitrile membrane is not permeable to either bacteria or endotoxins. Eight of the 9 animals had a negative limulus test after dialysis. The one positive endotoxin test can be explained by the positive blood culture containing E.coli. These data confirm the observations of Bernick (10) who demonstrated the absence of endotoxins in plasma after dialysis with contaminated dialysate. The conflicting data presented by Tobin (11) and Raij (4) are based upon their experience with Kill and other non-disposable dialysers with design defects; this, together with faulty sterilization, can result in direct contamination of the blood compartments with endotoxins. AN69 hemodialysis, however, seems to be safe, since it does not increase the risk of endotoxemia.

The interesting effect of lactulose plus magnesium sulfate on the incidence of bacteremia and endotoxemia can be explained by the bowel cleansing effect of these drugs which results in a diminished number of endotoxin-producing gut bacteria. Liehr et al.(7) proposed a direct anti-endotoxin effect of lactulose. The effect of lactulose on endotoxemia in men with liver disease has been described by Magliulo (6) and Scevola (12). The latter obtained positive limulus tests for 9 patients with acute hepatitis, 8 with cirrhosis, 1 with hepatic coma and 1 with chronic persistent hepatitis; after treatment with lactulose and paromomycin 18 of the 19 patients had a negative endotoxin assay. Our experimental results confirm these clinical observations. The implications of this finding in particular on survival and

other endotoxin-related complications, however, remain unclear.Further investigations into the use of lactulose to reduce endotoxin mediated symptoms in hepatic failure are warranted.

References

1. Gans H, Mori K, Lindsey E et al. Septicemia as a manifestation of acute liver failure. Surg Gynecol Obstet 1971;132:783-790.
2. Gans H. Effect of ligation of the hepatic artery or occlusion of the portal vein on the development of endotoxemia. Surg Gynecol Obstet 1974;139:689-692.
3. Groot GH de, Schalm SW, Schicht I et al. Comparison of large pore membrane hemodialysis and cross-dialysis in acute hepatic insufficiency in pigs. Eur J Clin Invest 1983;13:65-71.
4. Raij L, Shapiro FL, Michael AF. Endotoxemia in febrile reactions during hemodialysis. Kidney Int 1973;4:57-60.
5. Hindman SH, Favero MS, Carson LA et al. Pyrogenic reactions during hemodialysis caused by extramural endotoxin. Lancet 1975;2:732-734.
6. Magliulo EA, Dietz A, Torne V et al. Endotoxin spillover in viral hepatitis. Infect 1979;7:155-156.
7. Liehr H, Englidh G, Rasenack U. Lactulose - A drug with anti-endotoxin effect. Hepato-Gastroenterology 1980;27:356-360.
8. Wilkinson SP, Arroyo V, Gazzard BG et al. Relation of renal impairment and haemorrhagic diathesis to endotoxemia in fulminant hepatic failure. Lancet 1974;I:521-524.
9. Grün M. Liehr H, Rasenack U. Significance of endotoxemia in experimental 'Galactosamine-Hepatitis' in the rat. Hepato-Gastroenterology 1976;23:64-81.
10. Bernick JJ, Port FK. Absence of bacteremia and endotoxemia despite contaminated dialysate. Clin Nefrology 1980;14:13-17.
11. Tobin BM, Ralston AJ, Jones DM. Dialyzer gaskets. Br Med J 1970;3:226.
12. Scevola D, Magliulo E, Trpin L et al. Control of endotoxemia in liver disease by lactulose and paramomycin. Boll Ist Sieroter Milan 1979;58:242-247.

CHAPTER V.

COMPARISON OF LARGE-PORE MEMBRANE HAEMODIALYSIS AND CROSS-DIALYSIS IN ACUTE HEPATIC INSUFFICIENCY IN PIGS.

Gerrit H. de Groot, Solko W. Schalm, Ike Schicht, Patricia Batavier, Minus W.C. de Jonge, Jaap Lens and Johannes L. Terpstra.
Department of Internal Medicine II, University Hospital Dijkzigt, Rotterdam and Departments of Internal Medicine, Nephrology and Surgery, University Hospital, Leyden, the Netherlands.

Eur J Clin Invest 1983;13:65-71.

Abstract

We studied the duration of survival and the removal of putative toxins in forty pigs with ischaemic hepatic necrosis, undergoing haemodialysis or cross-dialysis with a large-pore membrane. Ischaemic hepatic necrosis was induced in conscious animals by tightening a loop around the hepatic artery 3 days after construction of a portocaval shunt. Pigs treated by a dialysis procedure survived significantly longer (45.2±11.9h) than controls (26.3±5.4h). There was no difference between haemodialysis and cross-dialysis.

Blood ammonia initially dropped significantly ($p < 0.05$) more during haemodialysis (560±107 → 210±51 umol/l) than during cross-dialysis (596±131 → 398±81 umol/l) but it subsequently increased beyond initial values despite efficient removal during continuous dialysis. Removal of ammonia was greater during cross-dialysis than during haemodialysis, but haemodialysis was more effective in the removal of the ammonia precursors glutamine and urea.

We conclude that dialysis procedures can prolong survival in pigs with ischaemic hepatic necrosis. The removal of ammonia-precursors is more effective in the prevention of hyperammonaemia than the removal of ammonia itself. Since dialysis cannot prevent progressive hyperammonaemia, control of excessive toxin production seems mandatory for effective hepatic support.

Key words: acute hepatic insufficiency, haemodialysis, cross-dialysis, ammonia.

Introduction

Various extracorporal liver-support systems have been used in acute hepatic failure. However, exchange blood transfusions, cross-circulation, total body washout, charcoal-haemoperfusion, haemodialysis and cross-haemodialysis are in general not considered acceptable because of the side-effects and/or insufficient efficacy in relation to the complexity of the procedure.

Recently, haemodialysis with a large-pore polyacrylonitrile (AN-69) membrane was described as a relatively simple procedure with promising efficacy. Reversal of electroencephalographic abnormalities was observed in animal experiments (1), and over 50% of the patients with acute hepatic failure and grade IV coma regained consciousness (2), but the effect on survival is as yet uncertain (1-3).

The aim of our investigation was to study the effect of AN-69 membrane dialysis on the survival of pigs with ischaemic hepatic necrosis. In addition, we studied the capacity of large-pore membrane dialysis techniques to remove free- and protein-bound toxins, since a limited toxin removal capacity could have been the major cause of the failure of previous hepatic support systems. Thirdly, in order to test the hypothesis that deficiencies of liver-dependent factors play a role in hepatic failure, we compared haemodialysis with cross-dialysis: in haemodialysis toxins are removed, while in cross-dialysis it is possible that -in addition to toxin removal- deficient factors will be transferred from the normal animal to the anhepatic animal.

Material and methods.

Animals

Forty Large White pigs, 8-10 weeks old and weighting 25-30 kg, were used. Anaesthesia was induced by intramuscular injections of ketamine chloride 20 mg/kg and after endotracheal intubation maintained with Fluothane 0.6-0.8 vol% and N_2O/O_2 (2:1).

Pigs underwent laparotomy for construction of a functional end-to-side portocaval shunt. All ligaments and peritoneal attachments to the liver were

cut or coagulated. Subsequently, a loose silicon loop was placed around the isolated hepatic artery and the common bile duct, and all other structures in the hepato-duodenal ligament were severed. A Scribner shunt was inserted, connecting the carotid artery to the jugular vein.

Three days after the construction of the portocaval shunt and 1 day after cleansing of the bowel with 25 g of magnesium sulphate, acute ischaemic hepatic necrosis was induced by tightening the loop around the hepatic artery. Anaesthesia was not used in this phase, since it may interfere with the subsequent neurological assessments. Pigs were placed in restraining cages, in order to avoid unnecessary suffering of the animals. Glucose (12 g/kg/24h), penicillin G (9 mega-U/24h) and kanamycin (3 g/24h) were infused continuously. The environmental temperature was maintained at 25°C to ensure normothermia in the pigs with ischaemic hepatic necrosis.

Experimental design

For the first series of experiments dialysis procedures were performed once for a 6 hr period, starting 18 h after ischaemic hepatic necrosis was induced. Each week three pigs were prepared: two animals with an open Scribner shunt were selected by an independent laboratory technician for either haemodialysis or cross-dialysis, while the third animal served as a control. Finally the haemodialysis group comprised six animals, the cross-dialysis group six animals and control group seven animals (two animals died shortly after surgery due to technical complications).

For the second series of experiments, dialysis procedures were continued 24 h a day for as long as the pig remained alive, up to a maximum of 60 h after ischaemic hepatic necrosis was induced. At the end of this series, the continuous haemodialysis group, the cross-dialysis group and the control group each comprised seven animals.

Haemodialysis (fig.1a).

Pigs undergoing haemodialysis were connected to a disposable AN-69 haemodialyser (Rhone Poulenc, Paris, France) primed with 300 ml of 0.9% NaCl. Blood from the arteriovenous Scribner shunt was pumped through the haemodialyser at the rate of approximately 150 ml/min, as measured by an electromagnetic flowmeter. Initially, only four of the sixteen compartments of the haemodialyser were used (to prevent circulatory collapse); every 10 min additional compartments were opened until complete utilization of all

compartments was achieved within 1-2 h. A closed circuit 751 Rhodial Apparatus (Rhone Poulenc, Paris, France) was used as dialyser. The dialysate flow was maintained at 500 ml/min, and the temperature at 38°C. The composition of the dialysate was Na^+ 140 mmol/l, K^+ 3 mmol/l, Mg^+ 0.75 mmol/l, Ca^{++} 1.5 mmol/l, Cl^- 113 mmol/l, acetate$^-$ 35 mmol/l, and glucose 4 mmol/l. During continuous haemodialysis, the dialysate was changed every 6 hrs. Anticoagulation was achieved by systemic administration of heparin; after a priming dose of 3000 IU, heparin was continuously infused at the rate of approximately 1000 IU/h in order to obtain a two-fold prolongation of the recalcification time.

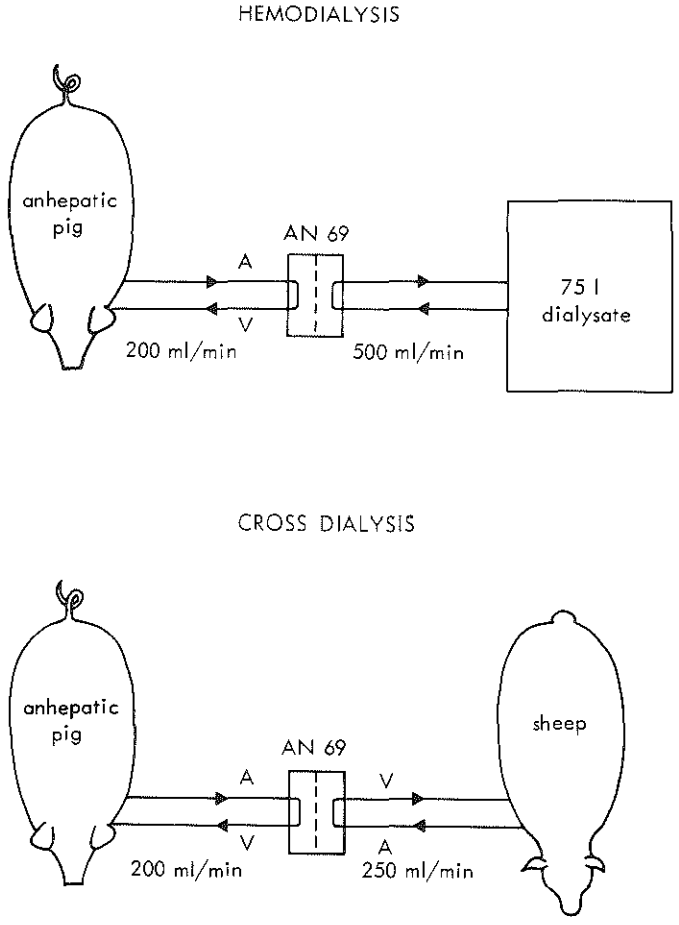

Fig.1. A schematic flow-diagram of the haemodialysis and cross-dialysis procedures.

Cross-dialysis (fig.1b)

Pigs undergoing cross-dialysis were connected to the disposable AN-69 haemodialyser as previously described. Normal animals were used as donors; they were connected to the dialysis compartment of the AN-69 haemodialyser in exactly the same fashion as the animals with ischaemic hepatic necrosis. For the donor, the blood flow was maintained at 250 ml/min, and the temperature at about 38°C. Anticoagulation of the donor's blood was induced by continuous infusion of heparin at a rate of 1500 IU/h, after a priming dose of 10,000 IU.

Measurements

Duration of survival was defined as the period between induction of ischaemic hepatic necrosis and time of death. All animals for the 6 h series and the series with continuous dialysis procedures were monitored in an identical way. The temperature, blood pressure, heart rate and response to various stimuli, such as pain and sound, were assessed every 6 h. Blood for biochemical analysis was collected every 6 h in ice-chilled plastic tubes containing disodium EDTA; within 20 min the plasma was separated by centrifugation at 4°C. After storage at -20°C for a maximum of 72h, deproteinization was performed by ultrafiltration (Amicon centriflow membranes CF25) at 4°C; the ultrafiltrate was frozen at -20°C until analysis. Ammonia was measured by an enzymatic method using glutamate dehydrogenase according to da Fonseca Wollheim (4). Glutamine and urea were also determined enzymatically by measuring the increase in the ammonia concentration after addition of glutaminase (5) or urease (6), respectively. Bile acids and total bilirubin in plasma were determined by a fluorimetric method (7) and the method of Jendrassik & Grőf (8), respectively.

Blood glucose electrolytes (Na^+, K^+, Cl^-), total protein , pH, pO_2 and pCO_2, platelets and clotting factors are the prothrombin complex (Normotest Nyegaart, Oslo, Norway) were measured by standard laboratory techniques. Blood samples were cultured in 60 ml of trypticase soy broth at 37°C and observed for 1 week.

Calculations

The measurements of ammonia, urea and glutamine were corrected for variations in plasma dilution induced by the haemodialysis procedures as follows:

$$\text{Measured concentration } (t_n) \times \frac{\text{total protein } (t_o)}{\text{total protein } (t_n)}$$

t_o = prior to the start of a dialysis procedure

t_n = n hours after the start of a dialysis procedure

The formulas for removal and clearance are: Removal=(A-V)xflow=umol/min; clearance=(A-V)flow/A=ml/min; where A=calculated arterial concentration of substance, and V=calculated venous concentration of the substance. Statistical methods included Student's t-test and Wilcoxon rank sum test for unpaired samples (9).

Results

Adequacy of the model

After induction of ischaemic hepatic necrosis all forty pigs (see Table I) showed neurological changes, including drowsiness and decreased responses to sound and pain, but no animal was completely comatose (no reaction to sound and pain). The temperature varied between 36.8 and 38.6°C for the majority of animals; preterminal hypothermia developed in six animals. All blood cultures were negative except one (E.coli); there were only minor variations in the levels of the electrolytes and blood gases. Blood glucose ranged between 5.2 and 15.4 mmol/l (mean ±SD 8.0±3.6); hypoglycaemia ($<$ 2.5 mmol/l) was never observed. Clotting factor values varied between 20% and 30% (mean ±SD: 29% ± 8; normal $>$ 75%) 18 h after ischaemic hepatic necrosis, and between 10% and 20% 24 h after ischaemic hepatic necrosis. Prior to the start of dialysis, plasma ammonia was markedly elevated in all animals (mean ±SD: 569±110 umol/l, normal $<$ 70). Post-mortem examination showed completely necrotic livers with the exception of a small area around the inferior vena cava. No statistical differences were observed for these features between the haemodialysis group, the cross-dialysis group and the control animals (Table 1).

Survival

For fourteen control pigs, survival after induction of ischaemic hepatic necrosis was 26.3±5.4 h. Pigs undergoing dialysis procedures (n=26) survived significantly longer (45.2±11.9 h, $p < 0.05$) than the control pigs. There was no significant difference in survival between haemodialysis and cross-dialysis, nor between a single 6 h dialysis procedure and continuous

Table 1. Clinical and biochemical data* for pigs 18 h after induction of ischaemic hepatic necrosis, immediately prior to dialysis.

	Haemodialysis (13)+	Cross-dialysis (13)	Controls (14)
Response to pain	+	+	+
Heart rate (beats/min)	102±25	98±20	100±28
Systolic blood pressure (mmHg)	95±10	108±15	100±12
Body temperature (°C)	37.8±0.7	38.2±0.4	37.0±1.4
Blood pH	7.51±0.02	7.50±0.03	7.46±0.08
Blood cultures, positive	0	0	0
Blood glucose (mmol/l)	8.0±4.1	8.0±3.7	8.0±3.2
Blood Normotest^{R} (%)§	27±9	29±6	29±9
Plasma ammonia (umol/l)	560±107	596±131	560±93
Plasma glutamine (umol/l)	752±230	640±186	1000±467
Plasma urea (mmol/l)	0.8±0.2	0.7±0.2	1.0±0.3
Serum bilirubin (umol/l)	12±8	8±4	13(8-15)++
Serum bile acids (umol/l)	1035±208	1134±288	1140(920-1250)++

* Mean ± SD.

\+ Number of experiments.

++ Data from three animals.

§ The Normotest measures the joint of the factors II, VII, X on the coagulation and is expressed as % of normal according to the manufacturer Nyegaard & Co (Oslo).

dialysis procedure. Pigs treated by continuous dialysis procedures had a prolongation of survival, but all animals ultimately died during extracorporal treatment and none had their life maintained by dialysis procedures (Fig.2).

Complications affecting survival were not observed in either the control group or the animals undergoing 6 h dialysis. In contrast, hypotension (< 80 mmHg) and intraperitoneal bleeding occurred in five out of fourteen animals during a continuous dialysis procedure (haemodialysis three, cross-dialysis two); in the majority of cases these complications occurred beyond 42 h of dialysis. All donor-animals survived the extracorporal procedures without untoward effects; some donors were used for 3 consecutive weeks.

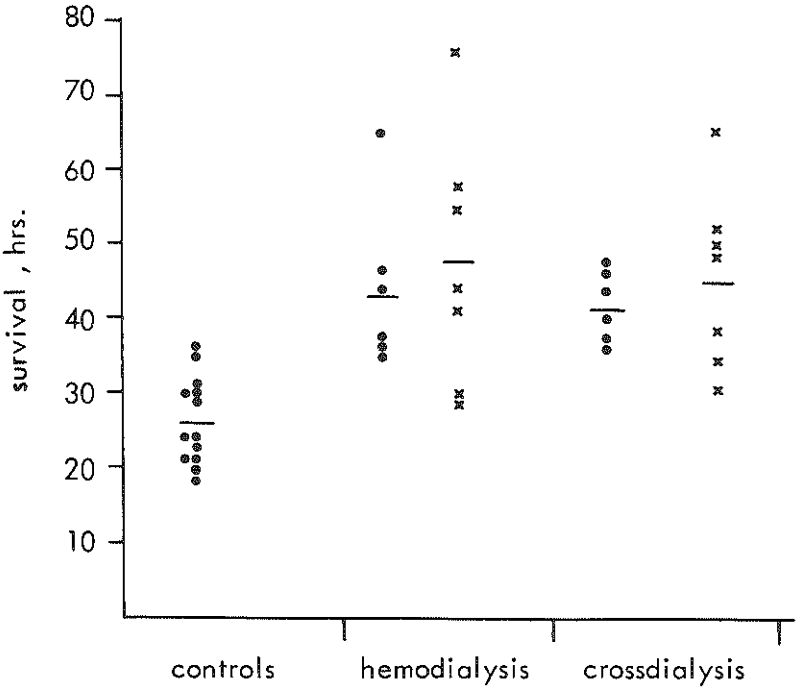

Fig.2. Survival after ischaemic hepatic necrosis for controls (N = 14), pigs undergoing haemodialysis for 6 h (●, N = 6) and continuously (x, N = 7) and pigs undergoing cross-dialysis for 6 h (●, N = 6) and continuously (x, N = 7).

Freely diffusible toxins

Eighteen hours after induction of ischaemic hepatic necrosis, blood ammonia was markedly elevated (569±110 umol/l). After the first 6 h of haemodialysis the blood ammonia had dropped to 210±51 umol/l whereas it increased to 699±199 umol/l in the control animals (Fig.3). The decrease in blood ammonia after 6 h was significantly ($p < 0.05$) less with cross-dialysis (398±81 umol) than with haemodialysis. In contrast, cross-dialysis removed significantly ($p < 0.05$) larger quantities of ammonia per minute than haemodialysis (Table 2). In spite of persistently efficient ammonia removal, dialysis procedures could not prevent a subsequent rise in blood ammonia; at 24 h the values corresponded to those of the control group (Fig.4).

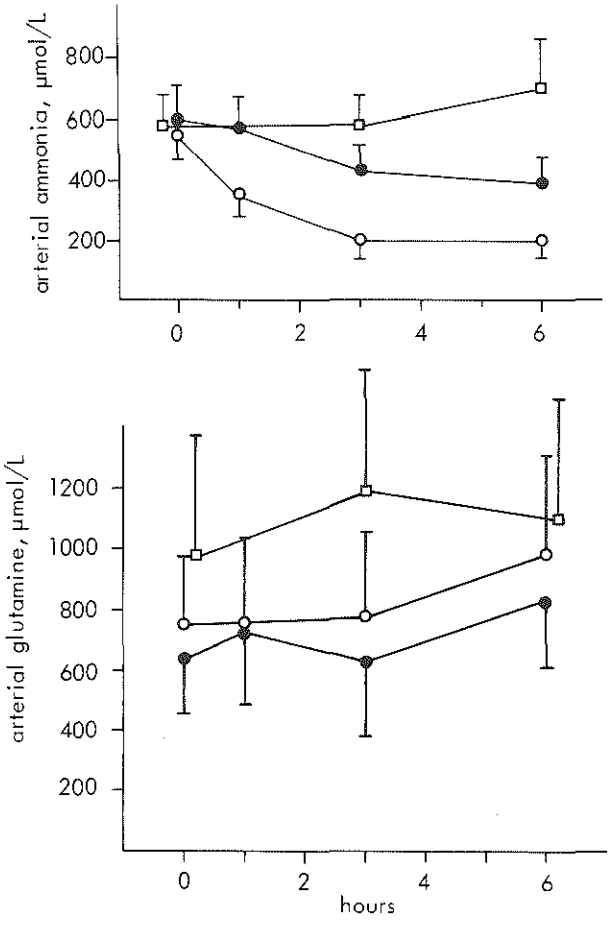

Fig.3. Blood ammonia (mean ± SD) and blood glutamine concentrations in pigs with ischaemic hepatic necrosis during haemodialysis (o, N = 6) and cross-dialysis (●, N = 6) and without dialysis (controls) (□, N = 14).

Table 2. Removal* of putative toxins (in umol/min) by continuous dialysis from pigs with ischemic hepatic necrosis.

	Haemodialysis+				Cross-dialysis+			
	1 h	6 h	12 h	24 h	1 h	6 h	12 h	24 h
Ammonia	20	15	20	15	13	30	33	30
Glutamine	49	71	103	90	12	53	51	57
Urea	30	30	34	34	0	-6	-++	-4
Bile acids	3.3	4.1	-++	3.6	2.0	4.0	0.9	5.4
Bilirubin	1.0	2.1	1.2	2.0	0	0	0	1.7

* (A-V)xflow.

\+ Mean value of seven experiments.

++ Deleted because of erratic measurements.

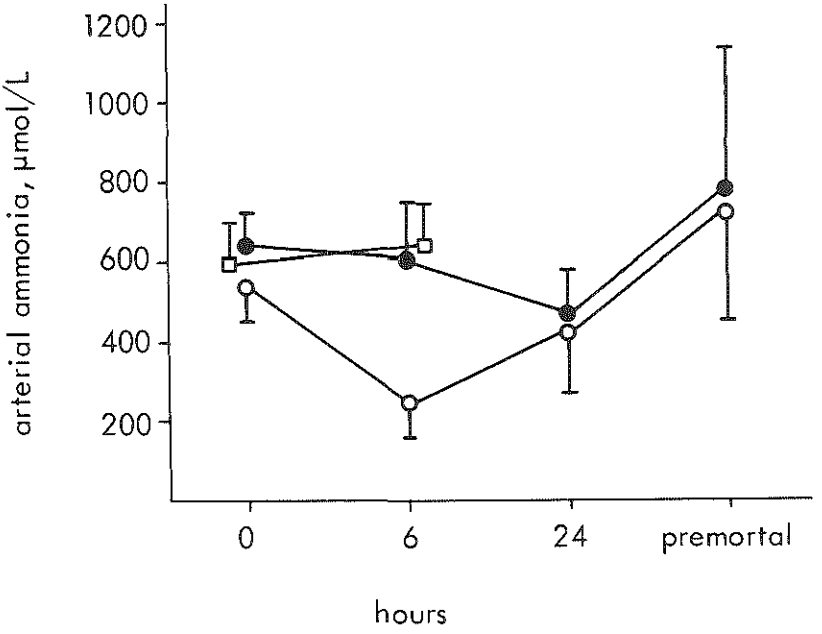

Fig.4 Blood ammonia concentrations (mean ± SD) in pigs with ischaemic hepatic necrosis during continuous haemodialysis (o, N = 7) and cross-dialysis (●, N = 7).

The blood glutamine concentrations were not significantly affected by dialysis. For both groups undergoing dialysis, the rise in the level of glutamine parallelled that of the control group (Fig.3), although appreciable quantities of glutamine were removed. Haemodialysis removed about twice as much glutamine as cross-dialysis (Table 2).

The levels of blood urea were low but within the normal ranges 18 h after induction of ischaemic hepatic necrosis. Haemodialysis produced a significant decrease in blood urea concentration from 785±258 umol/l to 334±108 umol/l at 6 h. In contrast, the blood urea levels rose during cross-dialysis to 3 times the initial values (2705±840 umol/l at 6 h). In the control animals the blood urea concentration did not change significantly (Fig.5). The quantity of urea removed by haemodialysis was significantly greater than that removed by cross-dialysis; in fact, in cross-dialysis a flux of urea from the donor animal to the animal with ischaemic hepatic necrosis was found (Table 2).

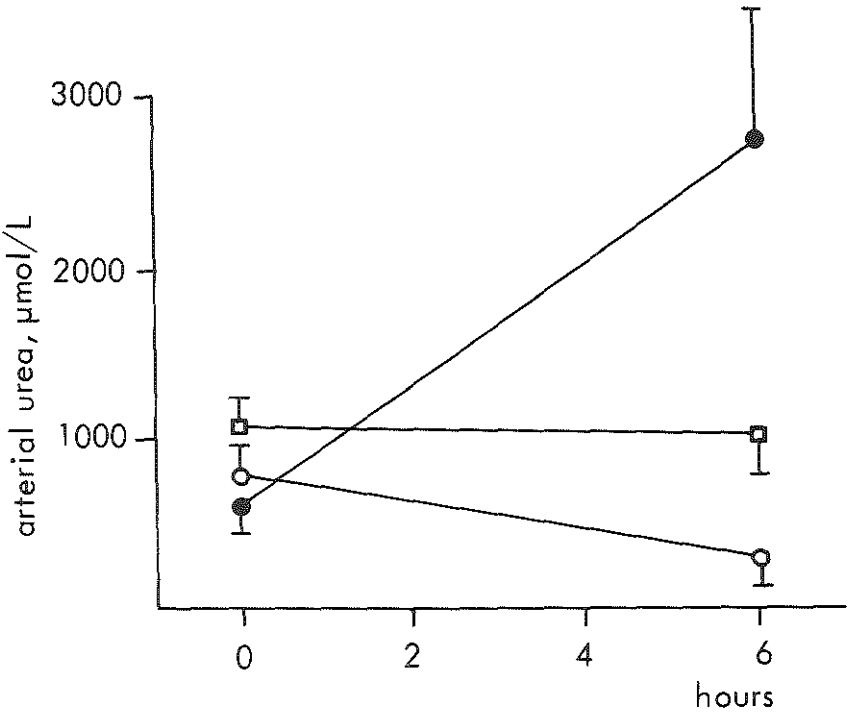

Fig.5. Blood urea concentrations in pigs with ischaemic hepatic necrosis during haemodialysis (o, N = 6) and cross-dialysis (●, N = 6) and without dialysis (controls), (□, N = 14).

Protein-bound substances

The concentration of bile acids in serum decreased slowly from 1084±248 to 680±248 umol/l at 24 h, while serum bilirubin rose from 10±6 to 100±20 umol/l; no differences were observed between haemodialysis and cross-dialysis as far as the blood levels of protein-bound molecules are concerned (Fig.6). The removal of bile acids and bilirubin was low compared to that of freely diffusible molecules (Table 2).

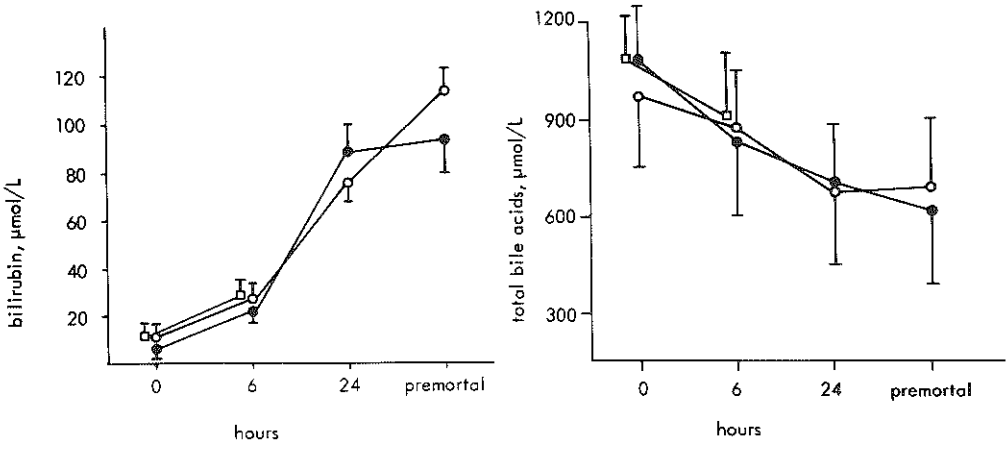

Fig.6. Blood bilirubin (mean ± SD) and bile acid concentrations (mean ± SD) in pigs with ischaemic hepatic necrosis during continuous haemodialysis (o,N = 7) and continuous cross-dialysis (●, N = 7) and without dialysis (□, N = 7).

Discussion

Both haemodialysis and cross-dialysis significantly prolonged the survival of pigs with ischaemic hepatic necrosis. In the past, the experiments by Opolon et al. (1) had only shown improvement in EEG recordings, but no effect on the duration of survival. A possible explanation for the discrepancy between effect on encephalopathy and survival was early mortality due to post-operative complications. We therefore chose a mode with a delay between the surgical trauma and the induction of hepatic failure. We have no reason to believe that the prolongation of survival in our experiments was due to incomplete devascularization of the liver. In fact, ammonia levels were markedly increased and the clotting factors were found to be below 30% in all pigs. Our results confirm the observations of Wallin and coworkers, who also demonstrated prolongation of survival with haemodialysis and cross-dialysis in hepatectomized dogs (10). However, in their series of experiments, as well as the present study, the control group was not selected by a randomization procedure; therefore we do not exclude the possibility that some of this effect on survival was due to selection. In our series, however, analysis of factors possibly affecting survival, such as temperature, positive blood cultures, elevation of blood ammonia and depression of clotting factors, did not show differences between the control group and the groups undergoing dialysis (Table 1).

The reason for the beneficial effect of dialysis remains unclear. Opolon (11) supports the middle molecule hypothesis since he did not find any improvement with cuprophan dialysis. We were surprised by the significant decrease in blood ammonia during haemodialysis and the lack of an appreciable drop during cross-dialysis. At first sight, this finding does not support the theory that ammonia plays a role in acute hepatic coma, but it should be noted that at 24 h hyperammonaemia was of equal magnitude for the two groups on dialysis and the premortal values did not differ from those of the controls.

Initially, ammonia removal apparently exceeded ammonia production since blood levels decreased. After 6–12 h, however, rising blood ammonia concentrations were observed despite constant ammonia removal, pointing to increasing ammonia production. Increased ammonia production in animals undergoing cross-dialysis is the only logical explanation for the lack of an

appreciable drop in blood ammonia, since removal of ammonia was excellent. One of the mechanisms by which ammonia production might be enhanced during cross-dialysis is transfer of an ammonia precursor to the animal with ischaemic hepatic necrosis. The levels of urea are higher in donor animals than in pigs 18 h after induction of ischaemic hepatic necrosis. This gradient induces a flow or urea from the donor to the pig with ischaemic hepatic necrosis, where urea apparently is converted to ammonia.

Therefore the removal of precursors of ammonia might be as important as the removal of ammonia itself in the treatment of hyperammonaemia. To express the removal of ammonia plus ammonia precursors we used the formula: removal of ammonia + 2x (removal of glutamine) + 2x (removal of urea)=total amount of ammonia equivalents removed. We assume that 2 moles of ammonia are taken up for the synthesis of 1 mole of either urea or glutamine (Table 3). In haemodialysis, the removal of ammonia equivalents amounted to 238 umol/min, while in cross-dialysis it was significantly less (113 umol/min, $p < 0.05$) due to the inferior removal of glutamine and urea. Thus the less pronounced decrease in the blood ammonia concentration during cross-dialysis can be explained.

Table 3. Removal* of ammonia equivalents§ (in umol/min) by continuous dialysis from pigs with ischaemic hepatic necrosis.

	Hours			
	1	6	12	24
Haemodialysis++	176	221	291	261
Cross-dialysis++	37	124	157	136

* (A-V)xflow.

§ Ammonia + (glutamine)x2+(urea)x2.

++ Mean value of seven experiments.

The concept of cross-dialysis is attractive because of the combination of toxins removal and supply of deficient factors. However, since no difference in prolongation of survival was observed between haemodialysis and cross-dialysis, we have not found any support for the hypothesis that deficient factors are responsible for hepatic encephalopathy. In contrast, we now have evidence that untoward metabolic effects can occur, such as the transfer of the ammonia precursor urea. These side-effects may out-weigh the potential benefits of supply of deficient factors so that in our opinion cross-dialysis procedures have no advantage over simple haemodialysis procedures with exclusively removal of toxins.

Protein-bound substances were removed rather ineffectively by both dialysis procedures. The presence of albumin on the dialysate side of the membrane in cross-dialysis did not increase the removal of molecules predominantly bound to albumin, as could be expected from a theoretical point of view. Still the concentrations of the protein-bound substances showed marked variations in relation to their production. Bile acids with their exclusive hepatic synthesis decreased appreciably during 24 h, while bilirubin with its non-hepatic origin rose.

We measured bile acids and bilirubin as markers for putative protein-bound toxins. Since concentrations of these markers in animals treated by a dialysis procedure were similar to those in controls, we think it is unlikely that removal of protein-bound toxins could be responsible for the observed prolongation of survival by dialysis.

Dialysis for 6 h was a procedure practically devoid of side-effects, in contrast to continuous dialysis; the latter was associated with hypotension and haemorrhagic diathesis. The increases incidence of complications can partly explain the absence of further prolongation of survival with continuous dialysis. From a theoretical point of view, Berk (11) suggested that an extracorporal procedure carried out twice a day for 4 h provides the maximum benefit in relation to efforts and risks; on the basis of our data it also seems worthwhile to evaluate treatment with alternating 4-6 h periods on and off dialysis.

In conclusion, haemodialysis is effective in prolonging survival in functionally anhepatic pigs, but life cannot be maintained by haemodialysis at present. The beneficial effect of haemodialysis could be due to removal of ammonia and ammonia-precursors, but other factors as well might be involved. The failure of continuous haemodialysis to sustain life of anhepatic animals

seems primarily related to the inability to correct biochemical abnormalities. Accelerated toxin production rather than decreased removal of toxins during haemodialysis in hepatic support can be expected if a more effective haemodialysis procedure can be combined with measures which prevent excessive toxin production.

References

1. Opolon P, Lavallard MC, Huguet CL, et al. Haemodialysis versus cross-dialysis in experimental hepatic coma. Surg Gynec Obstet 1976; 142:845-854.
2. Denis J. Opolon P, Nusinouici V, et al. Treatment of encephalopathy during fulminant hepatic failure by haemodialysis with high permeability membrane. Gut 1978;19:787-793.
3. Silk OBA, Trewby DN, Chase RA et al. Treatment of fulminant hepatic failure by polyacrylonitrile membrane dialysis. Lancet 1977;ii:1-3.
4. da Fonseca Wollheim, Direkte Plasma-Ammoniak Bestimmung ohne Enteiweissung. Z Klin Chem Biochem 1973;11:426-431.
5. Stahl A, Frick A, Imler M. Enzymatic micro-assay for blood glutamine. Clin Chem 1978;24:1730-1733.
6. Dutch Standardization Committee on Clinical Chemistry NEN 2410. UDC 612.124:547.435.2:543.432.
7. Mashinge F. A simple and sensitive assay of total serum bile acids. Clin Chem Acta 1976;70:79-86.
8. Jendrassik L, Grőf P. Photometrische Bilirubinebestimmung. Biochem Z 1938;297:81-90.
9. Swinscow TDV. Statistics at square one, 2nd ed.London: British Medical Association, 1977.
10. Wallin W, Turcotte JG, Sloan CH, et al. Conventional and cross-dialysis in hepatic failure. Curr Topic Surg Res 1970;2:75-86.
11. Berk PD. Possible importance of treatment schedule on effectiveness of artificial support systems in fulminant hepatic failure. (Abstract). Hepatology Rapid Literature Review 1977;2:358.

CHAPTER VI.

LARGE-PORE HEMODIALYTIC PROCEDURES IN PIGS WITH ISCHEMIC HEPATIC NECROSIS; A RANDOMIZED STUDY.

Gerrit H. de Groot M.D., Solko W. Schalm M.D., Ike Schicht M.D.,
Patricia Batavier, Minus de Jonge M.D., Jaap Lens M.D. and
Johannes L. Terpstra M.D.
Department of Internal Medicine II, University Hospital Dijkzigt,
Rotterdam.
Departments of Nefrology and Surgery, University Hospital, Leyden.

A grand-in-aid for this study was received from Diacare B.V., Holland.

Published in abstract form in Gastroenterology 1980;79:1022.
Hepato-Gastroenterology, 1984: no.4-5.

Abstract

In order to define further the therapeutic role of hemodialytic procedures in acute hepatic failure, 20 pigs with ischemic hepatic necrosis underwent via randomization hemodialysis against an electrolyte solution (n = 6, hemofiltration with re-infusion of an electrolyte solution (n = 5),control hemofiltration with re-infusion of autologous ultrafiltrate (n = 4) or no extracorporal procedure at all (n = 5).

Pigs on hemodialytic procedures survived significantly longer (51 ± 11 hrs) than controls (36 ± 8 hrs). There were no differences in the duration of survival between hemodialysis and hemofiltration, nor between controls undergoing and those not undergoing an extracorporal procedure. Electroencephalograms showed more rapid ($p < 0.05$) deterioration in control animals than in the treatment group. Putative toxins such as ammonia, glutamine, tyrosine, tryptophan,and methionine all decreased transiently in the treatment group; in the control group a continuous increase in the levels of the putative toxins was observed.

Comparison of all pigs surviving 35 hrs or less (n = 6) and animals surviving more than 45 hrs (n = 7) showed that long-term survival was significantly associated with lower plasma ammonia and methionine concentrations and fewer abnormalities on the electroencephalogram 10 hrs after the start of extracorporal procedures; moreover six of the 7 long-term survivors underwent hemodialysis or hemofiltration procedures.

We conclude that hemodialytic procedures prolong survival in pigs with ischemic hepatic necrosis by slowing the development of encephalopathy; this effect of hemodialytic procedures may be mediated by the lowering of plasma ammonia and methionine levels.

Keywords: acute hepatic insufficiency, hemodialysis, hemofiltration, ammonia, methionine, electroencephalogram.

Introduction

The acute hepatic failure syndrome is generally thought to be caused by accumulation of several toxins normally metabolized by the liver. Hemodialytic techniques in particular those using large-pore membranes have been proposed as a therapeutic device; however its effects in acute hepatic failure have not as yet been precisely defined (1-3). Reversal of electroencephalographic abnormalities and correction of brain neurotransmitter imbalance were reported by Opolon and co-workers (2,4) but no improvement in the duration of survival was observed. In contrast we found prolongation of survival in an irreversible model of acute hepatic insufficiency in pigs (5). Neither these studies nor the clinical studies reported to date (6,7) were of the randomized controlled type. In addition, only limited neurofysiological and biochemical measurements could be performed in our first study. It therefore remained unclear how the prolongation of survival was brought about by the hemodialytic procedures.

The aim of this investigation was to perform a randomized study comparing animals with ischemic hepatic necrosis undergoing large-pore hemodialysis with an adequate control group; the main goal was to study the effect on the duration of survival and on the neurologic state which was objectively monitored by means of an electroencephalogram. In addition, within the group undergoing large-pore hemodialysis we compared the effect of two variants of this technique: dialysis with a closed circuit dialysate, and hemofiltration which has a superior clearance for substances of 'middle molecular' size than hemodialysis (8). We also investigated by which mechanism the beneficial effects of these extracorporal procedures were obtained.

Material and methods

Animals Twenty 30-35 kg healthy White pigs underwent laparotomy for construction of a functional end-to-side portocaval shunt. All ligaments and peritoneal attachments to the liver were cut or coagulated. Subsequently, a loose silicon loop was placed around the isolated hepatic artery and common bile duct, and all other structures in the hepato-duodenal ligament were severed. A Scribner shunt was inserted, connecting the carotid artery to the

jugular vein. Two days after the construction of the portocaval shunt the bowels were cleaned with 30 g of magnesium sulfate and 150 ml of lactulose (50% w/v), to suppress excessive hepatic coma toxin production in the gut. One day later ischemic hepatic necrosis was induced acutely by tightening the loop around the hepatic artery without anaesthesia. Subsequently, in order to provide adequate nutritional support glucose (10 g/kg/24 hrs) and amino acids (1.3 g/kg/24 hrs, Aminosteril Hepa 8%, Fresenius, Germany, composition in g/l: Isoleu 10.4; Leu 13.9; Lys 6.88; Meth 1.1; Cyst 0.52; Phen 0.88; Thr 4.4; Tryp 0.7; Val 10.8; Arg 10.7; His 2.8; Gly 5.82; Ala 4.64; Ser 2.24; Acetic acid 7.25), mixed in one bottle, were infused continuously. Each bottle also contained penicillin G (9 mega U/24 hrs), kanamycin (3 g/24 hrs) as well as sodium, potassium and phosphate supplements (8 mmol/24 hrs, 37 mmol/24 hrs and 45 mmol/24 hrs, respectively). The environmental temperature was maintained at 25°C to ensure normothermia in the functionally anhepatic pigs.

AN-69 hemodialysis (Fig.1) Pigs undergoing AN-69 hemodialysis were connected to a disposable large-pore polyacrylonitrile (AN-69) membrane hemodialyzer (Rhone Poulenc, Paris, France) primed with 300 ml of 0.9% NaCl. Blood from the arteriovenous Scribner shunt was pumped through the hemodialyzer at a rate of approximately 200 ml/min, as measured by the air-bubble technique. Initially, only 4 of the 16 compartments of the hemodialyzer were used to prevent circulatory collapse; every 10 minutes additional compartments were opened until all compartments were in use.

A closed circuit 75 I Rhodial Apparatus (Rhone Poulenc, Paris, France) was used as dialysis machine. Dialysate flow was maintained at 500 ml/min and the temperature at 37°C. In this system the clearance of substances, which is dependent on molecular size, amounts to approximately 90 ml/min for ammonia (MW 17) and 60 ml/min for amino acids (MW 75-204) according to previous experiments (5).

The composition of the dialysate was Na^+ 135 mmol/l, K^+ 3 mmol/l, Mg^{++} 0.85 mmol/l, Ca^{++} 1.0 mmol/l, Cl^- 110 mmol/l, lactate$^-$ 5.0 mmol/l, bicarbonate$^-$ 28.0 mmol/l and glucose 4 mmol/l. Anticoagulation was achieved by regional heparinization. After a priming dose of 750 IU, heparin was infused continuously into the arterial blood line prior to its entry into the dialyzer at a rate of 1000 IU/hr (=10 mg/hr); similarly protamine chloride

was infused continuously into the blood leaving the dialyzer at a rate of 12 mg/hr.

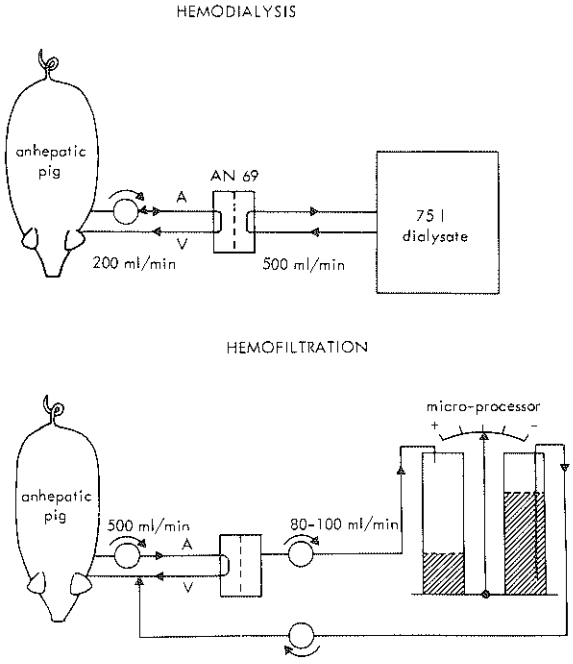

Fig 1. Schematic flow diagram of AN-69 hemodialysis and hemofiltration procedures.

Hemofiltration (Fig.1) Pigs undergoing hemofiltration were connected to a disposable large-pore cellulose tri-acetate hemofilter (Sartorius, Gottingen, Germany) primed with 75 ml of 0.9% NaCl. The cellulose tri-acetate membrane is permeable to molecules of up to 15,000-20,000 d; comparable to the AN-69 membrane, the sieving coefficient starts to fall at a molecular weight above 5000 d (Fig.2). Blood from the arteriovenous Scribner shunt was pumped through the hemofilter at a maximum rate of 500 ml/min. A hydrostatic pressure of about 200-300 mmHg was built up in the blood compartment on one side of the filter while a negative pressure was created by a second pump on the filtrate side of the membrane, leading to an transmembrane pressure of approximately 500 mmHg. Under such circumstances 60-80 ml of the ultrafiltrate per minute (15 l in about 4 hrs) are produced. The clearance of

all substances with a middle molecular size (up to 5000 d) is equal to the quantity of ultrafiltrate formed; i.e. 60-80 ml per minute; the clearance of molecules larger than 1500 d is thus superior with this technique than with hemodialysis (Fig.2). The ultrafiltrate flows into a barrel which is placed on a weight balance. A micro-processor ensures that exactly the same amount of substitution fluid (temp. 37°C) is pumped back into the venous line. These technical devices are enclosed in one apparatus (Hemoprocessor, type 40005, Sartorius, Gottingen, Germany). The composition of the substitution fluid was similar to that of the dialysate used in the hemodialysis experiments; it contained Na^+ 137 mmol/l, K^+ 4.3 mmol/l, Ca^{++} 1.0 mmol/l, Mg^{++} 0.87 mmol/l, lactate$^-$ 5.0 mmol/l, Cl^- 115 mmol/l, bicarbonate$^-$ 27 mmol/l, and glucose 4.0 mmol/l. The anticoagulation procedure was exactly the same as that followed for the animals undergoing AN-69 hemodialysis.

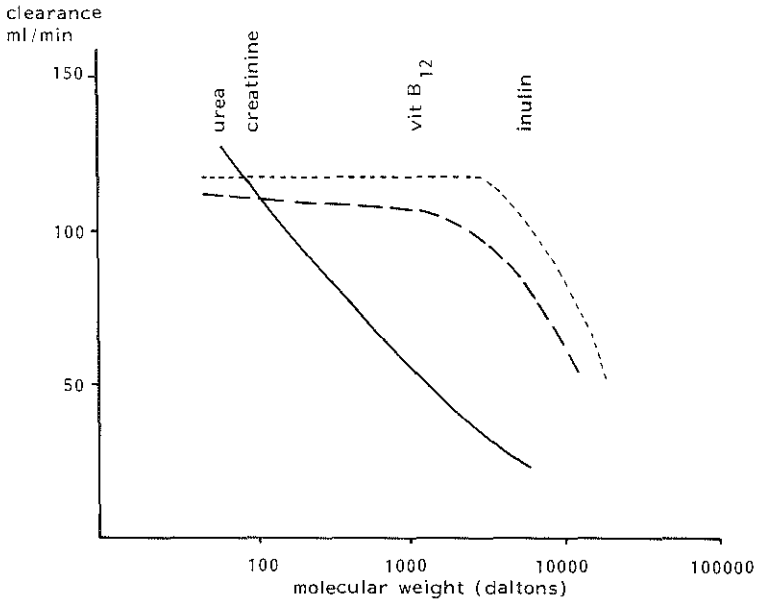

Fig 2. Clearances of substances in relation to their molecular weight for AN-69 hemodialysis (———), AN-69 hemofiltration (— — —), and cellulose tri-acetate hemofiltration (- - - -), the two procedures used in this study. For comparison, data are also given for AN-69 hemofiltration, used by Denis et al (8).

Control procedures. One group of animals underwent the ultrafiltration procedure described for hemofiltration, the only difference being that the animal's own ultrafiltrate was re-infused instead of a commercially prepared electrolyte solution.

The second group comprised animals who did not undergo any extracorporal procedure after induction of ischemic hepatic necrosis.

Experimental design Eighteen hours after ischemic hepatic necrosis was induced, the animals were assigned at random to the AN-69 hemodialysis, hemofiltration, control (no procedure), or hemofiltration control group. Extracorporal procedures lasted 4 hrs and were repeated twice daily, from 8-12 hrs and from 18-22 hrs, until the animal died or for a maximum of 60 hrs after induction of ischemic hepatic necrosis.

Measurements The duration of survival was defined as the period between the induction of ischemic hepatic necrosis and the time of death. Electroencephalograms were taken before induction of ischemic hepatic necrosis and before and after each extracorporal procedure. Six electrodes were placed on the shaved skin of the pig's head and five bipolar tracings were recorded on a Beckman apparatus (Accu tracer 110, Beckman, California, USA). To prevent low frequency artefacts, the pigs were strapped to a table with the eyes covered in a darkened room during electroencephalography. A thirthy-five Hertz filter was used to eliminate high frequency artefacts. The EEG recordings were analyzed independently by an electroneurologist who was not aware of the therapeutic procedures; the 5-grade classification as described by Opolon (2) was used.

Blood for biochemical analysis was collected before ischemic hepatic necrosis was induced and subsequently before and after each extracorporal procedure in ice-chilled polystyrene tubes containing disodium EDTA; centrifugation followed within 20 min at $4^{o}C$. The plasma was stored at $-20^{o}C$ for a maximum of 72 hrs, release of ammonia from plasma proteins is negligable for this period of time. Deproteinization was then performed by ultrafiltration (Amicon centriflow membranes CF 25) at $4^{o}C$; the ultrafiltrate was kept at $-20^{o}C$ until analysis.

Ammonia was measured by an enzymatic method using glutamate dehydrogenase, according to da Fonseca Wolheim (9). Glutamine and urea were determined emzymatically by measuring the increase in ammonia concentration after

addition of glutaminase (10) or urease, respectively. Tryptophan and tyrosine were measured by a fluorometric method, according to Dencla and Dewey (11) and Hochella (12), respectively. Methionine was determined by a modification of Collin's method (13), and bile acids by an fluorometric technique according to Mashige (14).

Blood glucose, electrolytes (Na, K, Cl), total protein, bilirubin and platelets were measured by standard laboratory techniques; clotting factors of the prothrombin complex were assayed by the Normotest (R) (Nyegaart, Oslo, Norway) method. Blood samples were cultured in 60 ml of Trypticase soy broth at 37°C and observed for 2-3 days.

Calculations and statistics To determine the efficacy of hemodialysis procedures the removal of substances was estimated as follows: removal (mmol/4 hrs) = dialysate concentration (mmol/l) x dialysate volume (L) or ultrafiltrate concentration (mmol/l) x ultrafiltrate volume (L/4 hrs).
For unpaired samples with a normal distribution of data points, the Student's t test was used, while the Wilcoxon rank sum test was used in the event of a skewed data profile. For comparison within a group, tests for paired samples were used. Changes in EEG grades with time were estimated by the linear regression method; the value representing the steepness of the curve (α) were used for comparison.

In additon to comparison of the groups undergoing various therapeutic procedures, we also compared animals with a short life span and those with prolonged survival. The groups were arbitrarily formed by selecting the animals in the upper and lower 33% of the survival range. Differences were assumed to be statistically significant when the p-value was less than 0.05.

Results

Adequacy of the model Eighteen hours after induction of ischemic hepatic necrosis, none of the twenty animals was clinically comatose; the EEG grade varied between grades 1 and 2. The temperature of the animals was between 37° and 39°C. With two exceptions, blood cultures were negative and endotoxinemia absent. Only minor variations in electrolyte levels were seen; blood glucose ranged between 5.5 and 14.0 mmol/l. Clotting factors as measured by 'Normotest' were 25% ± 8 (mean ± SD, normal > 75%) and plasma ammonia was markedly elevated with a median value of 380 umol/l (range 192-568)

Post-mortem examination showed completely necrotic livers with the exception of a small area around the inferior caval vein; one animal in the hemofiltration control group with a normal liver both macroscopically and microscopically was excluded from further analysis (this animal survived more than 60 hrs).

No statistically significant differences in the initial features (table I) were observed between the AN-69 hemodialysis group, the hemofiltration group, the hemofiltration control group, and the control (no extracorporal procedure) group.

Adequacy of AN-69 hemodialysis and hemofiltration procedures. Removal of substances by AN-69 hemodialysis and hemofiltration are given in table II. Both procedures were equally effective in removing the low-molecular substances. The efficacy of removal of putative toxins by AN-69 hemodialysis was equal to that found in previous experiments in which clearances were measured. Clearance in hemofiltration was equal to the quantity of ultrafiltrate formed per minute and amounted to 75 ml/min.

Survival (Fig.3) Pigs undergoing AN-69 hemodialysis or hemofiltration survived significantly ($p < 0.01$) longer (53 ± 11 hrs and 48 ± 11 hrs, respectively) than the control animals that did not undergo any procedure (33 ± 9 hrs). There was no significant difference in survival between AN-69 hemodialysis and hemofiltration, nor between untreated controls and controls on hemofiltration (Fig.3). Since no significant differences were observed for any variable (EEG, or biochemical measurements) between AN-69 hemodialysis and hemofiltration nor between untreated controls and controls on hemofiltration, we combined the data of the AN-69 hemodialysis and hemofiltration groups to form the treatment group, and the data of the two control groups to form the control group. The duration of survival for the treatment groups was 51 ± 11 hrs and for the control group 36 ± 8 hrs ($p < 0.04$).

Apart from cerebral oedema complications affecting survival were not observed in the control group, but intraperitoneal bleeding occurred in 6 out of 11 animals of the treatment group, particularly in those surviving more than 40 hrs.

Table I. Clinical and biochemical determinations for pigs before the start of extracorporal procedures, 18 hrs after ischemic hepatic necrosis.

	HEMODIALYSIS(6)		HEMOFILTRATION(5)*		HF CONTROLS(4)		CONTROLS(5)**	
	median	range	median	range	median	range	median	range
Temp,°Celsius	37.7	37-39	38.2	37-39	38.1	35-38	37.0	36-38
EEG,grades		1-2		1-2		1-2		1-2
Glucose, mmol/	7.9	6.2-12.8	7.0	5.5-8.0	7.1	5.6-8.1	8.6	6.1-14.0
Normotest,%	25	17-38	24	12-30	23	20-33	31	16-38
Fibronogen,g/1	1.7	0.7-1.8	1.7	1.6-2.0	1.1	0.6-1.7	1.7	1.2-1.9
Ammonia, umol/1++	347	298-444	341	211-568	438	291-481	396	192-416
Glutamine, umol/1+	1140	598-1418	1090	885-1238	1692	801-2020	1160	630-1409
Urea, mmol/1+	1.6	1.3-2.8	1.4	1.2-2.4	2.1	1.3-2.4	1.7	1.1-2.0
Tyrosine, umol/1+	205	129-385	222	148-257	224	176-285	256	230-524
Tryptophan (free),umol/1++	50	30-79	49	40-62	40	32-91	57	53-115
Methionine, umol/1+	204	169-309	218	186-244	192	144-280	239	179-417
Bilirubin, umol/1+	34	25-101	34	16-107	55	35-72	32	30-50
Bile Acids, umol/1+	363	227-999	430	206-556	479	202-968	248	235-952

* no significant differences between hemodialysis and hemofiltration.

** no significant differences between hemofiltration (HF) control group and the control group without an extracorporal procedure.

++ measured in plasma ultrafiltrate.

+ measured in plasma.

Table II. Removal of putative toxins in mmoles (mean ± SD) during 4 hrs AN-69 hemodialysis and hemofiltration.

	AN-69 hemodialysis (N = 20)	Hemofiltration (N = 18)
Ammonia	7,1 ± 3,8	4,4 ± 2,0
Glutamine	12,4 ± 2,5	18,5 ± 10,0
Urea	150 ± 20	135 ± 15
Tyrosine	2,5 ± 1,0	2,7 ± 0,9
Tryptophan	0,68 ± 0,20	0,67 ± 0,32
Methionine	1,9 ± 0,7	2,2 ± 0,6

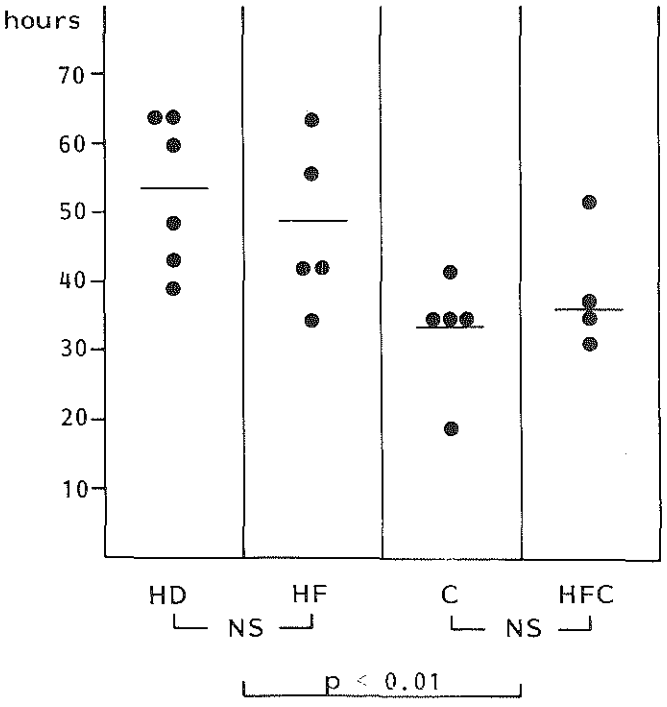

Fig 3. Survival after ischemic hepatic necrosis of pigs undergoing AN-69 hemodialysis (HD) and hemofiltration (HF) and controls undergoing no procedure (C) and hemofiltration with re-infusion of autologous ultrafiltrate (HFC). The bars denote median values.

Electroencephalogram The means of the EEG grades for the treatment and control groups are shown in Fig.4. The EEG grades gradually became more abnormal in the control group; similar but less severe changes were observed in the treatment group. After one extracorporal procedure, the grade of the EEG abnormalities for the treated animals was already significantly ($p < 0.03$) less than that for the controls.

A linear regression line was constructed using EEG grades at various points in time for each animal. The angle presenting the steepness of the line was significantly ($p < 0.05$) larger for the control group than for the treatment group, indicating a more rapid deterioration of the EEG in the control group.

A rapid improvement in EEG grading during or immedialtely after hemodialysis as reported by Opolon (2) was not observed; on the contrary, EEG grades after dialytic procedures were higher than EEG grades prior to dialytic procedures ($p < 0.001$, paired Wilcoxon test).

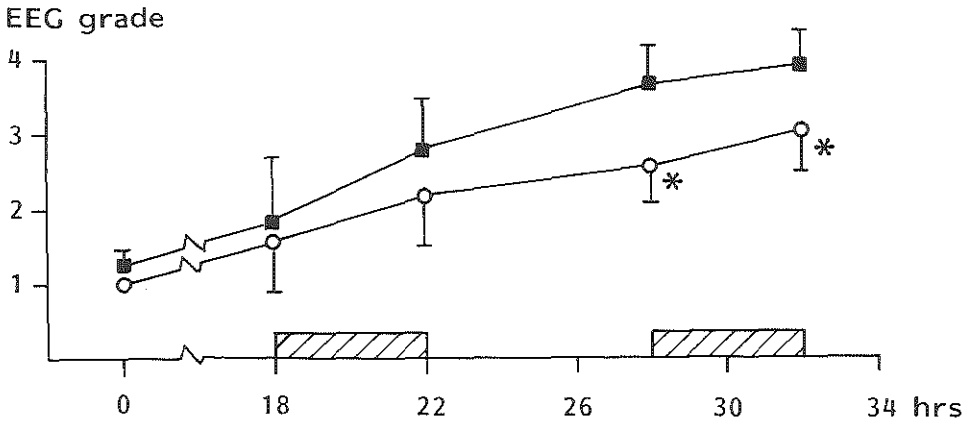

Fig 4. EEG grades (mean ± SD) for pigs with ischemic hepatic necrosis. Treatment group (HD+HF, n=11) o——o, and control group (C+HFC, n=9) ■——■
* $p < 0.05$, ** $p < 0.01$, comparison of treated and control animals.

▨ period of AN-69 hemodialysis, hemofiltration, or re-infusion of autologous ultrafiltrate.

Ammonia After 18 hrs of ischemic hepatic necrosis, the ammonia concentration in plasma was markedly elevated: 375 ± 122 umol/l (Fig.5). In the treated group the ammonia concentrations fell during an extracorporal procedure; normalization of ammonia concentrations (below 100 umol/l), however, could not be achieved. Between effective extracorporal procedures levels of ammonia rose again beyond initial levels. After two extracorporal procedures (32 hrs), the plasma ammonia concentration was 370 ± 160 umol/l. In the control group the plasma ammonia concentrations rose progressively to 615 ± 193 umol/l. After the first extracorporal procedure, the treatment group had significantly ($p < 0.04$) lower plasma ammonia concentrations than the control group (Fig.5).

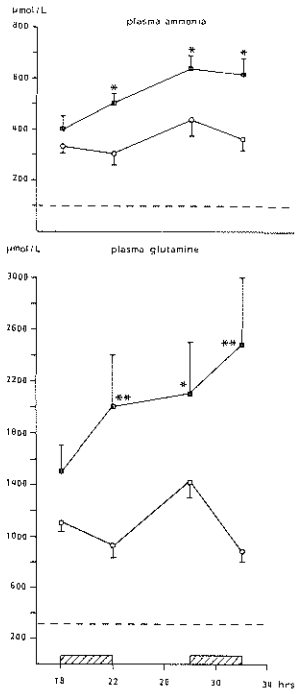

Fig 5. Plasma ammonia and glutamine (mean ± SEM) concentrations in pigs with ischemic hepatic necrosis.
Number of experiments and symbols as in Fig 4.

Amino acids (Fig. 5 + 6) The plasma levels of tyrosine, glutamine, free tryptophan and methionine showed an increase prior to treatment and a significant decrease after hemodialytic procedures. Between procedures the plasma amino acid concentrations rose to or beyond the initial levels. At 32 hrs (after two procedures) all amino acid levels were lower than the initial concentrations (18 hrs). In the control groups, there was a steady elevation of the plasma amino acid levels; as of the 22nd hour these levels were significantly ($p < 0.03$) higher than those found for the treatment group, except for free tryptophan.

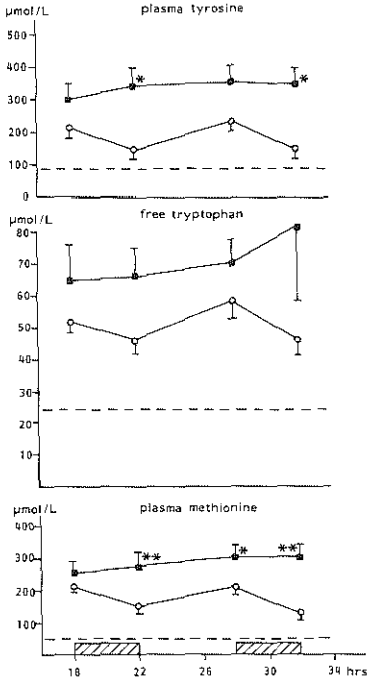

Fig 6. Plasma concentrations of tyrosine, free tryptophan and methionine (mean ± SEM) in pigs with ischemic hepatic necrosis.
Number of experiments and symbols as in Fig 4.

Short-term survivors and long-term survivors. Animals who survived 35 hrs or less were compared with animals surviving more than 45 hrs (table III). Eighteen hours after ischemic hepatic necrosis (thus before any procedure) the only difference between the two groups was the plasma ammonia concentration, which was significantly lower in the long-term survivors.

Table III. Biochemical determinations (mean ± SD) for short-term survivors (⩽35 hrs) and long-term survivors (>45 hrs), 18 and 28 hrs after ischemic hepatic necrosis was induced in pigs.

	18 hrs				28 hrs			
	Short-survivors (6)		Long-survivors (7)		Short-survivors (6)		Long-survivors (7)	
Ammonia, umol/l	446 ± 156		273 ± 73**		695 ± 143		358 ± 173**	
Glutamine, umol/l	1230 ± 530		1133 ± 437		1646 ± 620		1309 ± 467	
Tyrosine, umol/l	328 ± 149		241 ± 81		397 ± 155		294 ± 46	
Free tryptophan, umol/l	74 ± 31		56 ± 13		77 ± 20		59 ± 21	
Methionine, umol/l	328 ± 149		241 ± 81		325 ± 116		221 ± 37*	
Bilirubin, umol/l	44 ± 15		49 ± 29		71 ± 21		79 ± 31	
Bile Acids, umol/l	571 ± 271		416 ± 218		488 ± 238		413 ± 214	
Normotest,%	30 ± 5		25 ± 6		18 ± 7		17 ± 6	
Fibrinogen, g/l	1.5 ± 0.5		1.5 ± 0.3		1.0 ± 0.5		1.0 ± 0.2	
Glucose, mmol/l	8.9 ± 2.9		7.7 ± 2.6		10.6 ± 5.4		8.8 ± 4.3	
Urea, mmol/l	1.7 ± 1.1		1.4 ± 0.4		2.1 ± 1.4		1.3 ± 0.8	
EEG grade	2 ± 0		1.4 ± 0.5		3.5 ± 1.4		2.5 ± 0.5*	
Treatment hemodialytic procedures	-		-		1		6**	

* $p < 0.05$, (short-survivors vs. long-survivors).

** $p < 0.01$

Twentyeight hours after ischemic hepatic necrosis (6 hrs after the first treatment period), plasma ammonia was still significantly ($p < 0.03$) lower in long-term survivors and the difference between the ammonia levels found for the two groups had increased. Moreover plasma methionine was now significantly ($p < 0.04$) decreased in long-term survivors. The other putative toxins did not show significant differences. In addition, at 28 hrs the EEG grades were significantly ($p < 0.02$) lower for the long-term survivors, while no differences in EEG grades had been noted at 18 hrs.

Finally, six of the 7 long-term survivors underwent effective extracorporal procedures (4 animals on hemodialysis, 2 animals on hemofiltration), while none of the 6 short-term survivors received extracorporal treatment.

Discussion

In this strictly randomized study with adequate controls, both hemodialysis and hemofiltration significantly prolonged survival in pigs with irreversible ischemic hepatic necrosis. These findings confirm the observations of Wallin and co-workers (1), and our uncontrolled studies on hemodialysis and cross-dialysis (5). The prolongation of survival was associated with a less rapid development of EEG abnormalities. The beneficial effect of hemodialysis on the EEG observed in these experiments was different from that described by Opolon, who noted an improvement in the grade of EEG during or shortly after hemodialysis. We only observed a slower increase in EEG abnormalities in animals treated by hemodialytic procedures, without a detectable beneficial effect during the dialysis procedure itself.

The prolongation of survival and the effect on the EEG could be mediated by non-specific effects on body temperature or cerebral edema or by specific removal of the cerebral toxins involved in the pathogenesis of hepatic coma. Changes in temperature in the treatment group and the controls did not differ and a non-specific effect of hemodialysis on cerebral edema -so it be present- seems unlikely in view of the decrease in hematocrit and plasma albumin during the experiment.

The comparison of animals surviving less than 35 hrs with long-term survivors suggests that ammonia and methionine might be involved in the hepatic coma syndrome; we found no evidence that aromatic amino acids, tyrosine, and free tryptophan play a significant role in this respect. Other

putative toxins such as mercaptans, short chain fatty acids, and GABA were not measured and alterations in the concentrations of these substances could also explain the prolongation of survival and the delayed deterioration of the EEG brought about by treatment.

The findings on ammonia and methionine are similar to those of the very early studies of hepatic encephalopathy (15,16). Ammonia and methionine have long been suspected as direct or indirect causes of chronic hepatic encephalopathy; our experimental study suggests that these same compounds might also be involved in the acute hepatic coma syndrome. No supportive evidence was found for the role of 'middle molecules' in the pathogenesis of encephalopathy, since duration of survival was not different between hemodialysis and hemofiltration, which has a three-fold clearance for molecules between 1500-5000 d.

The difference in survival time between the control animals (26 ± 5 hrs) in our previous experiments (5) and the present controls (35 ± 8 hrs) is of interest; it could be explained by the ammonia lowering effect of lactulose given in the present study. In fact, the plasma ammonia levels found for control animals were significantly lower in the present study (411 ± 132 umol/l) than in the previous study (560 ± 93 umol/l) 18 hrs after induction of ischemic hepatic necrosis (5).

Although dialysis appeared effective in lowering plasma ammonia and methionine, it should be stressed that the procedure was unable to achieve normalization of plasma ammonia and methionine; in fact even a secondary rise in plasma ammonia to initial levels could not be prevented. That the life of functionally anhepatic animals cannot be sustained by hemodialysis appears to be due to insufficient toxin removal in relation to toxin production as well as the bleeding diathesis enhanced by extracorporal procedures.

Still, the definite prolongation of survival demonstrated in this study with an irreversible model of acute hepatic insufficiency justifies further evaluation of hemodialysis in potentially reversible model of acute hepatic failure. We have recently developed such a model in the pig by applying liver ischemia transiently (G.H. de Groot, unpublished observations). Additional complications may then arise from the release of products of damaged liver cells into the circulation.If bleeding diathesis can be controlled and excessive production of toxins prevented, life might be sustained thereby providing time for the liver to regenerate.

References

1. Wallin W, Turcotte JG, Sloan CH, et al. Conventional and cross-dialysis in hepatic failure. Curr Topic Surg Res 1970;2:75-86.
2. Opolon P, Lavallard MC, Huguet CT, et al. Hemodialysis versus cross-dialysis in experimental hepatic coma. Surg Gynec Obstet 1976;142: 845-854.
3. Santiago-Delpin EA, Callender C, Kjellstrand CM, et al. Crossdialysis in the treatment of experimental fulminant hepatic coma. J Surg Res 1975; 19:175-182.
4. Bloch P, Delorme ML, Rapin JR, et al. Reversible modifications of neurotransmitters of the brain in experimental acute hepatic coma. Surg Gynec Obstet 1978;146:551-558.
5. Groot GH de, Schalm SW, Schicht I, et al. Comparison of large-pore membrane hemodialysis and cross- dialysis in acute hepatic insufficiency in pigs. Eur J Clin Invest 1983;13:65-71.
6. Denis J. Opolon P, Nusinovici V, et al. Treatment of encephalopathy during fulminant hepatic failure by hemodialysis with high permeability membrane. Gut 1978;19:787-793.
7. Silk OBA, Trewby DN, Chase RA, et al. Treatment of fulminant hepatic failure by poly-acrylonitrile membrane dialysis. Lancet 1977;2:1-3.
8. Denis J, Opolon P, Delorme ML, et al. Long-term extra-corporal assistance by continuous hemofiltration during fulminant hepatic failure. Gastroenterol Clin Biol 1979;3:337-348.
9. da Fonseca Wollheim. Direkte Plasma-Ammoniak Bestimmung ohne Enteiweissung.
   Z Klin Chem Biochem 1973;11:426-431.
10. Stahl A, Frick A, Imler M. Enzymatic micro-assay for blood glutamine. Clin Chem 1978;24:1730-1733.
11. Dencla WD, Dewey HK. The determination of tryptophan in plasma, liver and urine. J Lab Clin Med 1967;69:160-169.
12. Hochella NJ. Automatid fluormetric determination of tyrosine in blood. Anal Biochem 1967;21:227-234.
13. Collins DJ, Robinson R, Knell AJ. Serum methionine: its rapid determination by continuous flow colorimetry. Clin Chem Acta 1978;88:277-281.

14. Mashige F, Osaga T, Imai K. A simple and sensitive assay of total serum bile acids. Clin Chem Acta 1976;70:79-86.
15. Phear EA, Ruebner B, Sherlock S,et al. Methionine toxicity in liver disease and its prevention by chlortetracycline. Clin Sci 1956;15:93-117.
16. Phillips CB, Schwartz R, Gabuzda GJ,et al. Syndrome of impending hepatic coma in patients with cirrhosis of the liver given certain nitrogenous substances. N Eng J Med 1952;247:239-246.

CHAPTER VII

STRATEGIES FOR HEPATIC SUPPORT IN ACUTE LIVER FAILURE.
Role of extracorporal devices vs auxiliary transplantation.

Gerrit H.de Groot[1], Solko W. Schalm[1], Thomas Fick[1], Cees B. Reuvers[2], Anton L. Boks[1], Onno T. Terpstra[2], Hans Jeekel[2] and Jacques Bruinvels[3].

1. Department of Internal Medicine II, University Hospital Dijkzigt, Rotterdam.
2. Department of Surgery, University Hospital Dijkzigt, Rotterdam.
3. Department of Pharmacology, Erasmus University, Rotterdam.

Supported by the Foundation for Medical Research FUNGO, and the Sophia Foundation for Medical Research, the Netherlands.

Submitted for publication.

Introduction

Acute hepatic failure (AHF) is one of the most dramatic situations in clinical hepatology, since this syndrome with its high mortality (80-90%) usually occurs in relatively young individuals who were previously completely healthy (1,2). The poor prognosis is due to the ensuing 1.cerebral edema, 2. additional complications and ultimately 3.a lack of liver regeneration.

Cerebral edema is now acknowledged to be a major cause of death in fulminant hepatic failure. Several autopsy studies have found that cerebral edema is present in 50%-100% of patients, with up to 20% showing brain stem herniation (3-6). The mechanism leading to cerebral edema in AHF is not yet fully understood. Alterations in cerebral blood flow due to dysregulation of cerebral vascular responses (7,8), hypercapnia and hypoxia (9), lactic acidosis (10) and fever are thought to play an additional role in the etiology of cerebral edema. There is evidence that cerebral edema is characterized by intracellular accumulation of water which can be seen microscopically as swelling of the astrocytes (11,12). This cytotoxic type of cerebral edema, that can also be seen after hypoxia and in the event of intoxication, is associated with inhibition of Na^+/K^+ dependent ATP-ase (13).Some authors suggest that changes in the blood-brain barrier cause the cerebral edema (14,15).

Other lethal complications also occur in patients with AHF. Major gastrointestinal hemorrhage contributes to death in 15-20% of the cases (6,16,17,18), and bacterial infection has been implicated as a main cause of death in 10% (6,19,20). Renal failure, electrolyte abnormalities (21-23), acid-base disturbances, respiratory and cardiovascular insufficiency (6,8,24,25,26) and hypoglycemia may lead to a fatal outcome. A reduction in the number of complications can probably be achieved with continuous and extensive monitoring, and intensive care has been claimed to improve the prognosis (27,28).

In the absence of the fatal complications mentioned above, recovery from AHF depends on regeneration of the liver or more precisely, on more liver regeneration than liver cell destruction. Milandri's study (29) suggested that regeneration of liver cells, assessed by the mitotic index and the hepatocyte DNA content, did occur in patients with AHF. However, the rate of

regeneration in relation to the rate of liver cell destruction has not been quantitatively assessed.

In view of these reflections, the goals of therapy in AHF should be the prevention of cerebral edema and other lethal complications, and the stimulation of liver cell regeneration with arrest of hepatic necrosis. The rationale for the use of the currently available artificial hepatic support systems is to replace the detoxifying function of the liver, i.e. to remove the putative toxins. Such devices should help prevent cerebral edema and also provide a favorable 'internal milieu' for hepatic regeneration. In the past twenty years many reports on succesfull hepatic support systems have appeared, but the efficacy of these treatment have never been proved in controlled studies nor confirmed by independent groups of investigators (75). Moreover, a centre reporting each time about succesfull treatment with plasma and heparin, charcoal hemoperfusion and large-pore hemodialysis, ultimately receded to early intervention hemoperfusion (76). Results with the latter techniques have been impressive in particular for patients with paracetamol intoxication or viral hepatitis; the system however was not efficacious for acute hepatic failure caused by rifampicin/isoniazid overdose, mushroom poisoning or halothane anesthesia. Charcoal hemoperfusion and large-pore hemofiltration each have its enthousiastic proponents, but in view of the lack or proven benefits also its sceptics. Therefore, in this article we will discuss the prospects of artificial hepatic support in acute hepatic failure, with emphasis on the efficacy of these artificial systems.

What should be removed?

Coma in AHF is thought to be the result of an accumulation of toxic metabolites. There are four major hypotheses on the pathogenesis of hepatic encephalopathy (30). The first hypothesis states that encephalopathy results from the synergistic effects of an excess of ammonia, short-chain fatty acids and mercaptanes (31,32), whereas hypoglycemia is thought to potentiate the neural inhibition of ammonia and free fatty acids (33).

The second hypothesis is based on the presence of a plasma-amino acid imbalance, increased brain uptake of free tryptophan, phenylalanine and tyrosine. An excess production of brain serotonin and octopamine as well as depressed synthesis of dopamine and norepinephrine are responsible for the disbalance between inhibitory and excitatory reflexes of the brain (34). An

inter-relationship between glutamine and neutral amino acids with regard to their passage through the blood-brain barrier and their intracerebral metabolism is suspected (34,35).

The third hypothesis relates to the inhibitory neurotransmitter GABA. It has been proposed that increased brain GABA is bound to an increased number of GABA receptors in post mortem brain tissue of rats and rabbits, resulting in sufficient neural inhibition to produce encephalopathy and coma (36-38). The mechanism of increased brain GABA is thought to be either an increased uptake or increased synthesis in the brain (39,40).

The fourth hypothesis originated from observations of the difference in the effects of cuprophane membrane and polyacrilonitrile membrane dialysis in AHF (41-43). It states that molecules that only pass through the polyacrylonitrile membrane and are characterized by a molecular weight of 1000-5000 daltons -the so called 'middle molecules'- play a role in the pathogenesis of hepatic failure. Recently 'middle molecules' have been detected in the sera (15,44,45) and brain (44) of comatose animals and men.

The uncertainty about the identity of toxins responsible for hepatic encephalopathy calls for removal of several classes of molecules, i.e. small water-soluble molecules (ammonia, glutamine, mercaptanes, neutral amino acids), middle molecules and protein-bound substances.

What can be removed?

The accumulation of a wide range of potentially toxic substances of different molecular sizes and physical properties suggests that a single narrow approach to artificial hepatic support for the treatment of AHF is hazardous. At present two techniques have evoked the most interest: hemoperfusion through adsorbents and hemodialytic procedures using high permeability membranes. Adsorbents allow the removal of free and protein-bound substances, whereas the dialytic procedures are only effective for the removal of free diffusable molecules. Freely diffusable substances can be divided into small and middle molecular substances (table I). Markers for small molecular substances are urea, ammonia, amino acids, free fatty acids and mercaptanes. As markers for the unidentified middle molecules vitamin B12 and inulin can be used. Bilirubin and bile acids have been proposed as markers for protein-bound substances. Bilirubin represents a

TABLE I. Removal of putative toxins by hepatic support systems.

		Hemodialytic procedures (large pore membrane)	Charcoal hemoperfusion	References
Free diffusable				
small molecular	ammonia	+	-	52,61,62
	amino acids	+	+	53,62.63
	phenols	+	+	64,65
	free fatty acids	+*	+	65,66
	mercaptanes	+	+	67
middle molecular	vit.B12, insulin	+	±	45,53,68,69
Protein-bound				
	bilirubin	-	±	70,71
	bile acids	-	+	47,72

\+ effective removal
± some removal of toxins or fast saturation of columm.
\- no removal
* personal observations

substance produced outside the liver and metabolized by the liver, while bile acids represent molecules produced and metabolized by the liver.

The total removal of toxins is dependent on multiple factors, including the amount of blood flowing through the support system per day and the clearance capacity of various systems. Table II presents data on blood flow through artificial systems and through the normal human liver. The quantity of blood passing through hemoperfusion or hemodialytic devices per minute is about 10-20% of the normal flow through the liver. Assuming that these systems are only in operation for the usual 4 hours per day then 1.6-3.3% of the blood flowing through the liver per day passes through the currently available clearing devices.

Extending dialysis-time to 24 hrs per day is feasible, but is associated with a markedly increased incidence of side effects (46-50). With regard to blood flow, it seems relevant to note that also in regular cross-circulation with a systemic-systemic connection, only 3% of the normal daily liver blood flow passes through the donor liver during 24 hrs. For, indeed, only 20% of the flow through the shunt will pass the donor liver, since liver blood flow is one-fifth of the total cardiac output (77). Thus the present available artificial organs usually do not have access to more than 3% of the daily liver blood flow.

The clearance capacities of various hemoperfusion (46,51,52) and hemodialytic procedures (42,44,46,47), as determined in previous investigations as well as from our own studies, are presented in table III. Charcoal powder hemoperfusion is the most effective as far as the removal of bile acids and some freely diffusable substances, such as amino acids, are concerned. Other substances are poorly cleared by hemoperfusion.
Hemodialysis and hemofiltration exhibit similar clearances for ammonia and its main precursors, urea and glutamine; these procedures are superior to charcoal hemoperfusion but are less efficient than a normal liver, as deduced from the cross-circulation experiments. Clearance of neutral amino acids did not differ markedly among the various systems. The clearance of protein-bound substances, such as bile acids, by hemodialysis was much lower than by charcoal hemoperfusion (table III).

Thus the currently available artifical hepatic support procedures have in general a clearance capacity for putative toxins which ranges from vastly inferior to about equal to that of the normal liver. When this is combined with the fact that only 3% of the daily hepatic blood flow passes through the

TABLE II. Blood flow through hepatic support systems in relation to the normal hepatic blood flow.

	flow 1/min	flow 1/24 hrs	% of normal hepatic liver flow/24 hrs	% of normal hepatic liver flow/4 hrs[2)]
Hemodialysis Hemoperfusion	0.2	288	13	2.2
Hemofiltration	0.4	576	27	4.5
Cross-circulation				
systemic-systemic	0.2	58[1)]	2.7[1)]	-
systemic-portal	0.4	576	27	-
In vivo liver flow	1.5	2160	100	-

1) flow through the donor liver is about 20% of the shunt flow.
2) generally regarded as optimal perfusion period as far as both effectiveness and side-effects are concerned.

TABLE III. Clearance by various procedures of substances (ml/min) in pigs with hepatic insufficiency.

	N	ammonia	urea	glutamine	bile acids	tryptophane	tyrosine
AN69 hemodialysis	20	84(60-104)	90(67-115)	58(46-70)	9(1-16)	40(28-55)	50(27-63)
Hemofiltration	18	50(45-70)	75(60-95)	54(48-60)	2(1-4)	26(15-37)	55(38-72)
Charcoal hemoperfusion	12	4(0-38)	10(0-50)	8(0-32)	40(10-123)	n.t.	91(31-147)
Selective ammonia[1] dialysis	7	85(6-127)	16(0-60)	27(0-67)	6(0-24)	n.t	n.t
Cross-circulation (systemic-portal)	2	304(286-325)	—	199(151-267)	195(157-257)	n.t	82(77-89)

1) In this procedure ammonia is removed directly from a small bathwater circuit by converting ionized ammonia to the volatile ammonia which leaves the system via gas-permeable membranes (73,74).
n.t.: not tested.

support system, then it seems probable that only 0.03-3.00% of the clearance capacity of the normal liver can be replaced by today's hepatic support systems.

How much should be removed?

The quantity of toxins that should be removed to correct the 'internal milieu' is dependent on the toxin production rate. In patients with terminal renal failure 4 hours of hemodialysis 3 times a week can correct the 'internal milieu'. This time schedule is based on the production rate of urea which is about 100 mmol/day on a low protein diet. In the event of hepatic insufficiency the situation appears quite different. Although the exact production rate of putative toxins is unknown, it can be estimated in various ways.

Hemodialysis induces a drop in the plasma ammonia and amino acid levels in the first hours after initiation of the procedure; subsequently however the plasma levels of ammonia rise again despite a constant rate of removal. Apparently initial removal exceeds production, but with time production increases in experimental acute hepatic failure (47). Similarly, cross-circulation is not capable of sustaining life (54,55), unless a high flow system is used (56,57). Here again the production of toxins must lie between the removal rates of the normal and the high-flow systems. We have used a third method to estimate the production rate of putative toxins. From our systemic-portal cross-circulation experiments (arterial blood from animal with liver failure flows (400 ml/min) into the portal vein of the donor) we calculated the daily capacity of the liver to metabolize putative toxins, using the formula: removal/min x 1440 min/day x 3. This latter factor is introduced into the formula because the value for removal/min is derived from cross-circulation experiments in which only 1/3 of the normal hepatic liver flow is processed. We assume that the removal capacity increases linearly with flow, since clearance was independent of the plasma concentration of putative toxins in our experiments (fig.1)(78). Since we know that life can be sustained as long as about 20% of the total liver remains functional, the removal capacity required for survival, which will approximate the daily production of putative toxins, has been estimated to be 20% of the daily removal capacity. In Fig.2 the capacity of currently available artificial

organs, expressed as a percentage of the removal capacity required for survival (or the daily production of toxins), is shown.
Assuming that the production rates for putative toxins in pigs with acute hepatic failure are not markedly higher than those for humans, we may conclude that the capacity of the currently available artificial organs is probably insufficient to restore the 'internal milieu'.

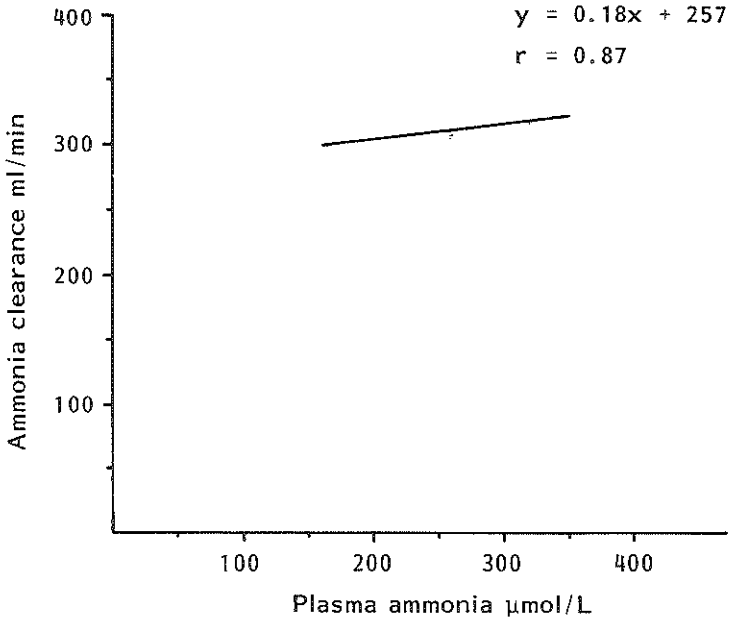

Fig.1. Relation between plasma ammonia concentration and ammonia clearance by the normal liver during systemic-portal cross-circulation (flow: 400 ml/min).

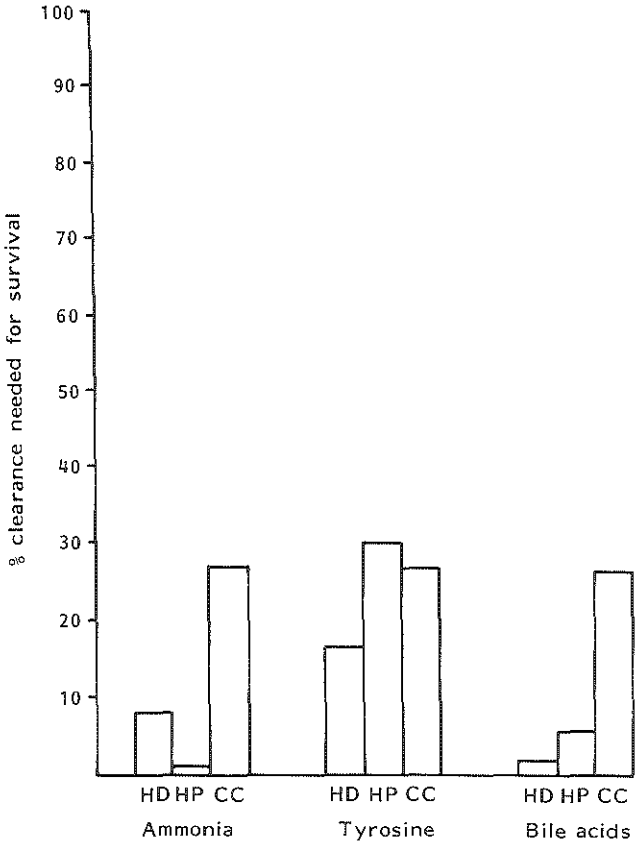

Fig.2. Efficacy of extracorporal support (calculated for procedures operating 4 hour per day) expressed as % of clearance needed for survival. The calculation of these data was based on the results of our cross-circulation, hemodialysis and hemoperfusion experiments (table III). The minimum removal rate for putative toxins required for survival, as calculated from the formula: removal/min x 1440 x 3 x 20% (see text), was 69 mmol for ammonia, 17 mmol for tyrosine, and 14 mmol for the protein-bound marker toxin bile acids. The removal of ammonia, tyrosine and bile acids by a 4-hour hemodialytic procedure was 6.9, 2.4, and 0.9 mmol, respectively. For charcoal hemoperfusion these values were 0.1, 8.4, and 3.4 mmol, respectively.
In the figure these data are presented as clearances. Sixteen hours of cross-circulation will achieve the 100% clearance needed for survival, whereas twentyfour hours of hemodialysis or hemoperfusion seem to be insufficient for clearance of the marker toxins ammonia and bile acids.

Perspectives of hepatic support.

From the data presented it can be concluded that none of the artificial systems used at present are sufficiently effective to act as a substitute for the detoxification capacity of 20% of the normal liver (table III + fig.2). Hemodialytic procedures and hemoperfusion would have to be maintained at least 24 hrs/day to stabilize the amino acid levels while ammonia and protein-bound putative toxins cannot be corrected during 24-hour treatment with the currently available artificial systems. Only systemic-portal cross-circulation can take over the detoxification function of 20% of the normal liver. The procedure must remain in operation for a minimum of 16 hrs per day. However, systemic-portal cross-circulation is not feasible for ethical reasons because fatal complications have occurred in donors (55). Other means should be found to remove toxins with an effectiveness similar to that of systemic-portal cross-circulation. In view of the above transplantation of part of a normal liver should lead to restoration of the 'internal milieu'. Blood flow through the transplanted liver will probably be larger than in cross-circulation, and the clearance capacity for all putative toxins should be similar to that of systemic-portal cross-circulation if the transplant is functioning well. The success of this procedure is illustrated in figure 3. Seven pigs underwent auxiliary heterotopic transplantation of 60% of a liver (ATPL). Four days after ATPL and ligation of the portal vein to the host liver, acute ischemic necrosis of the recipient liver was induced without surgery by external tightening an occluder around the hepatic artery for 6 hours (the vessel occluder was positioned at initial surgery). Auxiliary transplantation of 60% of the liver sustained life in pigs with acute hepatic failure for one month, whereas control animals undergoing ischemia of the liver for 6 hours without previous ATPL died within 72 hours. Marked changes in the electroencephalogram were not observed in ATPL-animals, while controls deteriorated to grade 4 encephalopathy within 48 hours. Moreover the synthetic function of the failing liver was adequately taken over by the transplanted liver, since clotting factors remained above 50% of control values and restored to normal. The excellent metabolic support of the partially transplanted liver resulted ultimately in long-term survival in 5 out of 7 animals (58).

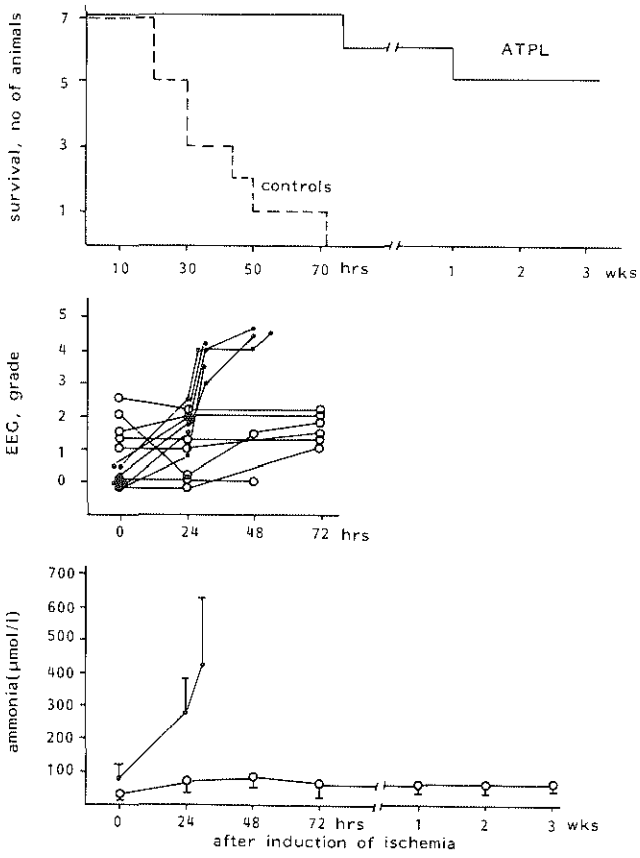

Fig.3. Data on survival, encephalopathy and plasma ammonia levels for pigs with acute hepatic failure without (controls, ●——●)) and with an auxiliary partial liver transplant (ATPL,o——o). Control animals died within 72 hours, after development of grade 4 encephalopathy and hyperammonemia.

Five animals with ATPL survived without showing marked metabolic abnormalities.

Acute hepatic failure was induced non-surgically by transient ischemia (59), and auxiliary transplantation of 60% of the liver was carried out by the technique of de Jonge (60) and Reuvers, et al(58).

Conclusion.

We believe that correction of the 'internal milieu' in acute hepatic failure is essential for the prevention of fatal complications and regeneration of the diseased liver. Currently available artificial support systems are limited by the low flow volume, the duration of treatment and clearance capacity. Even if used for 24 hours, the detoxification capacity of these systems will still be insufficient, as estimated from our systemic-portal cross-circulation experiments. Improvement in the survival of patients with acute hepatic failure persisting for several days might be enhanced by auxiliary transplantation of part of a liver. Efforts should be made to introduce this procedure into clinical practice.

Literature.

1. Berk PD and Popper H. Fulminant hepatic failure. Amer J Gastroent 1978;69:349-400.
2. Sherlock S. Diseases of the liver and biliary system. 5th Edition, Blackwell, Oxford 1975; 107-108.
3. Ware AI, D'Agostino A, Combes B. Cerebral oedema: a major complication of massive necrosis. Gastroenterology 1971;61:877-884.
4. Silk DBA, Hanid HA, Trewby PN et al. Treatment of fulminant hepatic failure by polyacrilonitrile membrane haemodialysis. Lancet 1977;ii:1-3.
5. Tholen H. Hirnödem: Eine Todesursache beim endogen Leberkoma. Klin Wschr 1972;50:296-301.
6. Gazzard BG, Portmann B, Murray-Lyon IM. Causes of death in fulminant hepatic failure and relationship to quantitative histological assessment of parenchymal damage. Quart J Medic 1975;44:615-626.
7. Trewby PN, Hanid MA, Mackensie RL, et al. The effects of cerebral oedema and arterial hypotension on cerebral blood flow in an animal model of hepatic failure. Gut 1978;19:999.
8. Trewby PN, Williams R. The pathophysiology of hypotension in patients with fulminant hepatic failure. Gut 1977;18:1021-1026.
9. Stanley NN, Kelsen SG, Cherniack NS. Effect of liver failure on the ventilatory response to hypoxia in man and the goat. Clin Sci Molec Medic 1976;50:25-35.
10. Record CO, Iles RA, Cohen RO et al. Acid-base and metabolic disturbances in fulminant hepatic failure. Gut 1975;16:144-149.
11. Hanid MA, Mackensie RL, Jenner RE, et al. Intracranial pressure in pigs with surgically induced liver failure. Gastroenterology 1979;76:123-131.
12. Klatzo I. In: Steroids and Brain Oedema edited by Reub HJ, Schuman K: Springer-Verlag 1972: pp. 1.
13. Hughes RO, Sewell RB, Poston L. Fractionation of fulminant hepatic failure serum to further characterise the leucocyte sodium transport inhibitor. Clin Sci 1981;60:23.
14. Klatzo I. J Neuropathology and Exp Neurology 1967;26:1.

15. Livingstone AG, Potrin M, Goresky CA. Changes in the blood brain barrier in hepatic coma after hepatectomy in the rat. Gastroenterology 1977; 73:697-704.
16. Clark R, Rake MO, Flute PT, et al. Coagulation abnormalities in acute liver failure; pathogenetic and therapeutic implications. Scan J Gastroenterol 1973;8:supp 19;57.
17. Opolon P, Caroli J, et al. The treatment of acute liver failure. Med et Hyg 1974;32:159.
18. Saunders SJ, Hickman R, McDonald R. Progress in Liverdisease IV, Ed. Popper H and Shaffner F. New York 1972;4:333-334.
19. Nusinovici V, Crubille C, Opolon P, et al. Hépatites fulminantes avec coma. Revue de 137 cas. Gastroentérologie Clinique et Biologique 1977;1:861-873.
20. Wijke RJ, Rajkovic IA, Eddleston ALWF, et al. Defective opsonisation and complement deficiency in serum from patients with FHF. Gut 1980;21:643.
21. Wilkinson SP, Moodie H, Arroyo VA, et al. Frequency of renal impairment in paracetamol overdose compared with other causes of acute liver damage. J Clin Pathol 1977;30:141-145.
22. Wilkinson SP, Arroyo VA, Moodie H, et al. Abnormalities of sodium excretion and other disorders of renal function in fulminant hepatic failure. Gut 1976;17:501-505.
23. Ring-Larsen H. Renal hemodynamics in the hepato-renal syndrome. Experimental aspect. Preceedings of the international symposium on the Hepato-renal syndrome. Sassari 1978;pp.155-171.
24. Roberts ICE, Poppell JW, Randell HI. Respiratory alkalosis. Ann of NY Acad Sci 1957;66:955-965.
25. Fischer JE, Baldessarini RJ. False neurotransmitters and hepatic failure. Lancet 1971;ii:75-80.
26. de Groot GH, Schalm SW, Dirksen R, et al. Effect of naloxone on shock in a patient with fulminant hepatic failure. Neth J Med 1983;26:77-79.
27. Auslander MO, Gitnick GL. Vigorous medical management of acute fulminant hepatitis. Arch Int Medic 1977;137:599-601.
28. Tygstrup N, Ranak L. Fulminant hepatic failure. Clin in Gastroenterol 1981;10:191-208.
29. Milandri M, Gaub J, Ranek L. Evidence for liver cell proliferation during fatal acute liver failure. Gut 1980;21:423.

30. Zieve L. The mechanism of hepatic coma. Hepatology 1981;1:360-365.
31. Gabuzda GJ. Hepatic coma: clinical consideration, pathogenesis and management. In: Dock W, Snapper I, eds. Advances in internal medicine. Vol 11, Chicago, Chicago Year Book, 1962:11.
32. Zieve L, Doizaki WM. Brain and blood methanethiol and ammonia concentration in experimental hepatic coma and coma due to inject of various combinations of these substances. Gastroenterology 1980;79:1070.
33. Zieve L, Lyftogt C, Draves K. Toxicity of a fatty acid and ammonia: interactions with hypoglycemia and Krebs cycle inhibition. J Lab Clin Med 1983;101:930-939.
34. James JH, Ziparo V, Jeppson B, et al. Hyperammonemia, plasma amino acid imbalance and blood brain amino acid transport: a unified theory of portal systemic encephalopathy. Lancet 1979;2:772-775.
35. Denis J, Delorme ML, Boschat M, et al. Respective roles of ammonia, amino acids and medium sized molecules in the pathogenesis of experimentally induced acute hepatic encephalopathy. J Neurochem 1983;49:10-19.
36. Schafer DF, Thakur AK, Jones EA. Increased gamma-aminobutyric acids receptors associated with acute hepatic encephalopathy in rabbit. Clin Res 1980; 28:485.
37. Schafer DF, Thakur AK, Jones EA. Acute hepatic coma and inhibitory neurotransmission increase of gamma-aminobutyric acid to neural membranes. Gastroenterology 1980;79:1123.
38. Baraldi M, Zeneroli ML. Experimental hepatic encephalopathy: changes in the binding of gamma-aminobutyric acid. Science 1982;216:427-429.
39. Schafer DF, Thakur AK, Jones EA. Acute hepatic coma and inhibitory neurotransmission: increase of gamma-aminobutyric acid levels in plasma and receptors in brain. Gastroenterology 1980;79:1123.
40. Ferenci P, Schafer DF, Kleinberger G, et al. Serum levels of gamma-aminobutyric-acid-like activity in acute and chronic hepatocellular disease. Lancet 1983;ii:811-814.
41. Denis J, Opolon P, Nusinovici et al. Treatment of encephalopathy during fulminant hepatic failure by hemodialysis with high permeability membrane. GUT 1978;19:787-793.
42. Opolon P, Lavallard MC, Hughet CL. Hemodialysis versus cross-dialysis in experimental hepatic coma. Surg Gynec Obstet 1976;142:845-854.

43. Silk DBA, Trewby PN, Chase RA, et al. Treatment of fulminant hepatic failure by polyacrylonitrile membrane dialysis. Lancet 1977;ii:1-3.
44. Faguer P, Delorme MC, Cueille G, et al. Demonstration of middle molecules in plasma and brain during experimental acute hepatic encephalopathy. ASAIO J 1980;3:153.
45. Leber HW, Klausmann J, Goubeaud G, et al. Middle molecules in the serum of patients and rats with liver failure: influence of sorbent hemoperfusion. In: Artificial liver support. Brunner G, Schmidt FW (eds), Springer-Verlag Berlin Heidelberg, New York,1981;96-102.
46. Opolon P. Artificial liver: issues and controversies. Artif Organs 1981; 5:21-28.
47. de Groot GH, Schalm SW, Schicht I, et al. Comparison of large pore membrane haemodialysis and cross-dialysis in acute hepatic insufficiency in pigs. Eur J Clin Invest 1983;13:65-71.
48. Denis J, Opolon P, Delorme ML, et al. Long-term extracorporal assistance by continuous hemofiltration during fulminant hepatic failure. Gastroenterol Clin Biol 1979;3:337.
49. Silk DBA, Trewby PN, Hanid A, et al. Use of the Rhône-Ponlenc polyacrilonitrile membrane in fulminant hepatic failure and its effects on amino acids levels. Proc Eur Soc Artif Org 1976;Vol III, 212-216.
50. Bartels O, Neidhardt M, Schellberger H. Hormone losses by charcoal hemoperfusion. In: Artificial liver support. Brunner G, Schmidt FW, (eds). Springer-Verlag, Berlin Heidelberg, New York, 1981, p.121.
51. Bartels O. Hemoperfusion through activated carbon adsorbents in liver failure and hepatic coma. Acta Hepato-Gastroenterol 1978;25:324-329.
52. Denis J, Opolon P, N'Guyen, et al. Nutritional implications of artificial liver support systems in fulminant hepatic failure. 2nd European Congres on Parenteral and Enteral Nutrition. Acta Clin Scand 1980;7:69.
53. Chase RA, Davies M, Trewby PN, et al. PLasma amino-acids profiles in patients with fulminant hepatic failure treated by repeated polyacrylonitrile membrane hemodialysis. Gastroenterology 1978;75:1033.
54. Sicot C, Fréjaville JP, Roche J. Six cas d'hépatite grave traitée par circulation croiseé interhumaine. Annales de Medicine Interne 1971;122:381-387.
55. Burnell JM, Runge C, Saunders FC. Acute hepatic failure treated by crosscirculation. Arch Intern Med 1973;132:493-498.

56. Stewart JV, Williams JS, Kluge DN, et al. Effect of crosscirculation on metabolism following hepatectomy. Ann Surg 1963;158:812-819.
57. Roche-Sicot J, Sicot C, Peignoux M, et al. Acute hepatic encephalopathy in the rat: the effect of crosscirculation. Clin Sci Mol Med 1974;47: 609-615.
58. Reuvers CB, Terpstra OT, de Groot GH, et al. Auxiliary transplantation of a partially hepatectomized liver in pigs with fulminant hepatic failure. Trans Proc in press.
59. de Groot GH, Reuvers CB, Schalm SW, et al. A reproducible model of transient ischemic liver necrosis in the pig. Submitted for publication.
60. de Jonge MCV. Thesis: Biliodigestieve anastomose auxiliare levertransplantatie. Krips, Repro, Meppel, 1983.
61. de Groot GH, Schalm SW, Schicht I, et al. Large pore haemodialytic procedures in pigs with ischemic hepatic necrosis; a randomized study. Hepato-Gastroenterology 1984; no.4-5:in press.
62. Gazzard BG, Weston MJ, Murray-Lyon IM, et al. Charcoal hemoperfusion in the treatment of fulminant hepatic failure. Lancet 1974;1:3101-1307.
63. Chang TMS. Microcapsule artificial kidney and medium weight molecules. Proc Eur Dial Transplant Assoc 1972;9:568.
64. Kaziuka FN, Chang TMS. In vitro assessment of the removal of phenols by ACAC hemoperfusion. Int J Artif Organs 1979;2:215.
65. Amano I, Kano H, Santo A, et al.Proc Eur Dial Transplant Assoc 1976; 13:262-269.
66. Chamuleau RAFM, Schoenmaker LP, Smit EN. In vitro adsorption of possible aetiological factors of hepatic encephalopathy. Int J Artif Organs 1979; 2:284.
67. Chang TMS, Lister C. Analysis of possible toxins in hepatic coma including the removal of mercaptanes by albumin-collodion charcoal. Int J Artif Organs 1980;3:108.
68. Asaba H, Bergström J, Furst P, et al. Removal of endogenous middle molecules by hemoperfusion. Artif Organs 1979;3:132.
69. Chang TMS. Hemoperfusion, exchange transfusion, crosscirculation, liverperfusion, hormones and immobilized enzymes. In: Artificial Liversupport, Brunner G, Schmidt FW (eds). Springer-Verlag. Berlin Heidelberg, New York, 1981, p.126.
70. Abouna GMT, Gilchrist JE, Pottit ND, et al. Hemoperfusion with activated charcoal in treatment of experimental acute hepatic failure. In: R.

Williams, IM Murray-Lyon (eds): Artificial Liver support, London-Torbridge, 1975.

71. Bartels O, Neidhardt M, Neidhardt H, et al. Untersuchungen und Erfahrungen mit der Kohle-Hämoperfusion bei Leberkoma. In: L.Demling, O.Bartels (eds): Entgiftung mit Hämoperfusion. Bindernagel-Verlag, Friedberg 1977, 119-131.
72. Van Berlo A, Verkooyen A, Tangerman A. The development of a new-hemoperfusion colomn: the filmadsorber. Int J Artif Organs 1982; Vol. 5, no.3:181.
73. Piskin E, Chang FMS. Effects of pH and urea concentration on urea removed from dialysate by enzymatic conversion and gasabsorption using a capillary hemodialyzer. Int J Artif Organs 1980;3:36-40.
74. Piskin E, Chang TMS. Effects of temperature on the removal of urea as ammonia by enzymatic conversion and gasabsorption using an oxygenerator. Int J Artif Organs 1981;4:45-48.
75. Benhamou JP, Rueff B, Soest C. Severe hepatic failure: an article study of current therapy. In:Liver and Drugs, Orlandi F and Jezequel AM (eds), Academic Press, New York;pp.213-228.
76. Gimson AES, Brande S, Mellon PJ et al. Earlier charcoal hemoperfusion in fulminant hepatic failure. Lancet 1982;ii:681.
77. Tijgstrup N, Fundung J, Juul-Nielsen J et al. The function of the isolated perfused and the in vivo pig liver. Scand J Gastroenterol 1971;6:131-138.
78. Williams RL. Drug therapy: drug administration in hepatic disease. New Eng J Med 1983;309:1616-1622.

CHAPTER VIII.

SUMMARY AND CONCLUSIONS

After working for 5 years on the development of methods for the treatment of acute hepatic failure (AHF), the following conclusions can be drawn.

The first purpose of this thesis was to develop an animal model that could be considered comparable to the human acute hepatic failure syndrome and, secondly, to develop a reliable and objective method for measuring the cerebral disturbances which are closely associated with acute hepatic failure. Both are necessary for the evaluation of therapeutic modalities for acute hepatic failure.

In chapter II an animal (pig) model for acute hepatic failure that is induced by transient ischaemia of the liver is described. Four hours of ischaemia were tolerated by 4 out of 8 animals while 6 hours resulted in death within 50 hours in 6 of the 7 animals. Severe encephalopathy, which was followed by death, developed within 30 hours in all animals undergoing 6 hours of hepatic ischaemia. With respect to the potential reversibility of this model we conclude that 1) hepatic circulation was restored in all animals; 2) histological data for all animals undergoing 6 hours of ischaemia revealed necrosis of less than 75% of the liver cells. Therefore more than 25% of liver tissue remained available for recovery and possible regeneration. This large animal model for acute hepatic failure fulfills the accepted criteria of death by hepatic failure, reproducibility and potential reversibility and allows sufficient time to start liver support procedures. The disturbed 'internal milieu', as previously described in acute hepatic failure, could be confirmed. New interesting data included the plasma amino acid pattern during the development of hepatic encephalopathy. The ratio of tyrosine to the sum of all neutral amino acids increased markedly and correlated with the degree of hepatic necrosis; the same was found for the ratio of phenylalanine. Correlations between the degree of liver cell

necrosis and other neutral amino acids ratio's were not found. Assuming that disturbances in the transport of amino acids across the blood brain barrier are best expressed by plasma ratio's, tyrosine and phenylalanine appear to be the major abnormality of neutral amino acids in hepatic encephalopathy in the pig. Tryptophane ratio did not change in pigs in contrary to the data found in men and rats. Plasma arginine disappeared after induction of ischaemia and re-appeared 24 hours later in animals that survived.

In chapter III methods for objective measurement of hepatic encephalopathy are described. The so-called power spectrum - a computer-assisted transformation of the electroencephalogram - was evaluated since conventional EEG readings remain subjective and clinical assessment of pigs is not feasible. The power spectrum can be expressed by the mean dominant frequency and the relative power spectra of the various frequency bands. The mean dominant frequency and the relative power of the delta frequency band were sensitive parameters of hepatic encephalopathy up to grade 3. Visual and brainstem auditory-evoked responses were neither sensitive nor specific indicators of the course of encephalopathy.

In chapter IV the incidence of endotoxaemia and its influence on survival were determined in pigs with permanent ischaemic hepatic necrosis. The incidence of endotoxaemia increased markedly before death; endotoxaemia could be prevented by the oral administration of magnesium sulfate and lactulose. A significant relation between endotoxaemia and survival was not found, irrespective of pretreatment with lactulose. Haemodialysis procedures did not increase the risk of endotoxaemia even when dialysates were contaminated with endotoxins.

In chapters V and VI we studied the effect of haemodialysis, cross-dialysis and haemofiltration (intermittent and continuous) on survival and the neurological state of pigs with ischaemic hepatic necrosis. In addition we determined the removal and clearance capacities of several liver support systems for putative toxins. Large-pore haemodialysis, cross-dialysis and haemofiltration (intermittent or continuous) prolong survival slightly, and reduce the rate of deterioration of the EEG. Pronounced improvement of the neurological state was not found. Although haemofiltration should remove middle molecules more efficiently, this procedure was not superior to conventional large-pore haemodialysis as far as survival, neurological improvement and removal of small molecular substances were concerned. During the dialysis procedures the plasma levels of small molecular toxins, such as

ammonia, glutamine, tyrosine and methionine, decreased but normalisation of blood levels could not be achieved. In advanced hepatic coma ammonia and glutamine increased in spite of efficient removal.

In chapter VII the data acquired in our studies are discussed in relation to the prospectives of artificial liver support. It is concluded that only high-flow systemic portal cross-circulation (400 ml/min) for 16 hours a day is sufficient to correct the internal milieu. All other systems are inadequate because of their failure to remove 'all' toxins efficiently. Since cross-circulation is considered unethical in man, another procedure must with an efficacy comparable to that of systemic portal cross-circulation be found. Partial auxiliary transplantation of a liver (ATPL) yields a toxin removal that is at least similar to that of systemic portal cross-circulation. The preliminary results of ATPL in pigs with ischaemic hepatic necrosis are encouraging. Efforts should be made to introduce this procedure into clinical practice.

SAMENVATTING EN CONCLUSIES.

Vijf jaren onderzoek naar de ontwikkeling van methoden voor de behandeling van acute leverinsufficiëntie, hebben geresulteerd in de hiernavolgende conclusies.

Een belangrijk doel van het onderzoek beschreven in dit proefschrift was het ontwikkelen van een diermodel, dat kan worden vergeleken met het humane acute leverinsufficiëntie syndroom, en het ontwikkelen van een betrouwbare en objectieve methode voor het meten van hepatische encephalopathie. Beide onderdelen zijn noodzakelijk voor de evaluatie van therapeutische toepassingen bij acute leverinsufficiëntie.

In hoofdstuk 2 wordt het diermodel van varkens met acute leverinsufficiëntie beschreven. Acute leverinsufficiëntie wordt veroorzaakt door tijdelijke ischaemie van de lever. Ischaemie van de lever gedurende 4 uur werd door 4 van de 8 dieren verdragen, terwijl ischaemie gedurende 6 uren binnen 50 uur tot de dood leidde bij 6 van de 7 dieren. Ernstige encephalopathie (graad 3 à 4), ontwikkelde zich binnen 30 uur in alle dieren met 6 uur ischaemie van de lever. De leverbeschadiging in dit model heeft theoretisch de mogelijkheid van reversibiliteit: 1. vanwege het herstel van de leverdoorstroming na de ischaemische periode; 2. daar de hoeveelheid necrose van de lever na 6 uur ischaemie niet meer dan 75% bedraagt, blijft meer dan 25% van de levercellen over voor regeneratie. Met dit diermodel is voldaan aan de volgende criteria:
Reproduceerbaarheid, potentiële reversibiliteit en dood door leverinsufficiëntie met daarbij een voldoende lang tijdsinterval om een lever ondersteunende behandeling te kunnen starten. Abnormale aminozuur spectra, zoals uitvoerig bij een acute leverinsufficiëntie beschreven, kon ook in dit model worden aangetoond. De metingen van de neutrale aminozuren werden niet uitgedrukt als concentraties, maar als plasmaratio's (verhouding tussen elk afzonderlijk neutraal aminozuur en de som van alle neutrale aminozuren), omdat de individuele aminozuurratio het beste de influx van neutrale aminozuren naar de hersenen weergeeft. De ratio van tyrosine nam sterk toe na inductie van levernecrose en bleek te correleren met de mate van levercelnecrose. Ook de ratio van phenylalanine en methionine nam toe, doch

een correlatie met uitgebreidheid van levernecrose kon niet worden gevonden. De ratio's van tyrosine en phenylalanine normaliseerden zich 30 uur na inductie van de levernecrose bij dieren die overleefden. De tryptofaan ratio veranderde niet in varkens, in tegenstelling tot gegevens over mensen en ratten. Aannemende dat verstoringen in het transport van aminozuren via de bloed-hersen barrière het beste worden weergegeven door de plasmaratio's van de neutrale aminozuren, lijken de ratio's van tyrosine en phenylalanine het meest afwijkend te zijn bij hepatische encephalopathie in varkens. Het plasma arginine verdwijnt nagenoeg volledig na inductie van levernecrose; na 24 uur stijgt de plasma arginine concentratie bij dieren die overleven.

In hoofdstuk 3 worden methoden beschreven voor het objectief meten van hepatische encephalopathie. Het zgn. 'powerspectrum' een door een computer verrichte transformatie van het EEG - werd geëvalueerd, daar conventionele beoordeling van het EEG subjectief is en klinisch-neurologisch onderzoek bij varkens niet betrouwbaar is. Het EEG kan op deze wijze worden vertaald in de gemiddelde frequentie en de 'powerspectra' (de som van golfamplitudes als functie van iedere frequentie) van verschillende frequentiebanden. De gemiddelde frequentie en de relatieve 'power' van de deltafrequentieband waren gevoelige parameters voor hepatische encephalopathie tot graad 3. 'Visuele evoked potentials' en 'brainstem auditory evoked potentials' waren sensitieve noch specifieke indicatoren om encephalopathie te meten.

In hoofdstuk 4 wordt de incidentie van endotoxinaemie en de invloed hiervan op de overleving bepaald bij varkens met permanente ischaemie van de lever. Voor de dood nam de incidentie van endotoxinaemie aanzienlijk toe: endotoxinaemie kon worden voorkomen door toediening per os van magnesiumsulfaat en lactulose. Significante relaties tussen het optreden van endotoxinaemie en de overleving werden niet gevonden, onafhankelijk van behandeling met lactulose. Haemodialyse procedures vergrootten het risico voor endotoxinaemie niet, zelfs niet wanneer het badwater gecontamineerd was met endotoxines.

In de hoofdstukken 5 en 6 wordt het effect van haemodialyse, kruisdialyse en haemofiltratie op de overleving en neurologische status van varkens met ischaemische levernecrose bestudeerd. Tevens werden de verwijderings-en klaringscapaciteit voor verschillende toxinen door de verschillende dialyse

systemen berekend. Haemodialyse met een grote-gatenmembraan, kruisdialyse en haemofiltratie verlengden de overleving enigszins en lieten minder snel verslechtering zien van het EEG. Duidelijke neurologische verbetering werd niet gevonden. Haemofiltratie bleek niet superieur aan haemodialyse met een grote-gatenmembraan, ondanks het feit dat haemofiltratie efficiënter middelgrote moleculen verwijderde. Plasmaconcentraties van kleine moleculaire toxinen zoals ammoniak, glutamine, tyrosine en methionine daalden tijdens de dialyse procedures, doch normale plasmaspiegels van de toxinen konden niet worden bereikt. Bij vergevorderde hepatische encephalopathie namen de plasmaconcentraties van ammoniak en glutamine zelfs toe, ondanks efficiënte klaring.

In hoofdstuk 7 worden de gegevens, verkregen uit vorige onderzoekingen besproken. Daarbij wordt uitgebreid ingegaan op de vooruitzichten van een zgn. kunstlever bij acute leverinsufficiëntie. De uiteindelijke conclusie is dat alle huidige systemen een onvoldoende capaciteit hebben om welke toxinen dan ook effectief te kunnen verwijderen. Alleen systemische-portale kruiscirculatie met een hoge bloedflow kan het gestoord 'milieu interne' volledig normaliseren, mits deze procedure 16 uur per dag aangewend wordt. Door fatale complicaties bij donoren van kruiscirculatie kan deze procedure bij mensen niet gebruikt worden, zodat een andere procedure met dezelfde capaciteit als kruiscirculatie moet worden gevonden. Partiële auxiliaire transplantatie van de lever kan in theoretisch opzicht efficiënter en effectiever dan kruiscirculatie de detoxificatie functie van de lever overnemen. De voorlopige resultaten van partiële auxiliaire transplantaties van de lever bij varkens met acute leverinsufficiëntie lijken bemoedigend. Aanbevolen wordt deze procedure toepasbaar te maken voor patiënten met acute leverinsufficiëntie.

VERANTWOORDING

Dit proefschrift werd bewerkt op verschillende experimentele chirurgische laboratoria. Het slagen van de experimenten was afhankelijk van de enorme inzet van velen. Het werken op dergelijke laboratoria was voor mij als internist een bijzondere ervaring. De coördinatie was voor mij leerzaam, boeiend en veelzijdig; selectie der dieren, huisvesting der dieren, prae-operatieve voorbereiding, operatieve procedures, post-operatieve zorg, uitdenken en uitvoeren van dialysetechnieken, verrichten van metingen en bloedbepalingen, obducties, histologische bewerkingen en rubriceren van data konden alleen verwezenlijkt worden door medewerking van velen. De hemodialyse experimenten werden uitgevoerd in het experimenteel chirurgisch laboratorium van het Academisch Ziekenhuis Leiden. Dank aan alle medewerkers van het laboratorium onder leiding van Drs.H. Stol, tevens aan Hans Terpstra, Jaap Lens en Minus de Jonge voor hun voortreffelijke operatieve assistentie.
Patricia Batavier, Ieke Schicht en alle co-assistenten worden bedankt voor hun hulp t.a.v. de dialyse experimenten. De ontwikkeling van een representatief diermodel voor acute leverinsufficiëntie en het uitvoeren van hemoperfusie en kruiscirculatie experimenten werden gerealiseerd in het experimenteel chirurgisch laboratorium van de Erasmus Universiteit onder leiding van Prof.Westbroek en Rob ten Berge.
De inzet van Cas, Roy, Enno, Rob, Erik, Pim, Wiebeke en Jannie maakte het uitvoeren van vele experimenten mogelijk.
Bijzondere dank ben ik verschuldigd aan Kees Reuvers, die, ondanks zijn klinische werkzaamheden, wekelijks tijd vrij kon maken voor de chirurgische ingrepen.
Ook de hulp van Onno Terpstra en Ton Fick was onontbeerlijk.
Grote bewondering heb ik voor het uithoudingsvermogen van Jan Boot, Wim v.d. Berg en alle anderen van het laboratorium Interne II, die gedurende 5 jaar met grote nauwkeurigheid de bepaling van toxinen hebben verricht en daarnaast hebben meegedacht in de opzet van de experimenten.
Dank aan Ton Boks die vaak met grote technische vindingrijkheid de haemoperfusie experimenten kon laten slagen.

De hulp van de afdelingen Pharmacologie (Prof.Bruinsveld), Electrofysiologie (Prof.de Vlieger en medewerkers), Pathologie (Dr.F.W.J. ten Kate, Dhr.Vroonhoven, Mej. Kiers), Bacteriologie (Prof.Michel, Mw.Maas), Centrale Research Werkplaats en Bureau Medische Technicologie van de T.H. Eindhoven (Ad van Berlo) en Diacare Nederland (Jan Smetsers) is zeer gewaardeerd. De apothekers van het Academisch Ziekenhuis Dijzigt, Academisch Ziekenhuis Leiden en Holy Ziekenhuis worden bedankt voor hun hulp bij de hemofiltratie experimenten.

Dank aan Ivonne Pijpers, die nauwgezet alle tikwerk voor dit proefschrift heeft verricht.

Prof.Frenkel, van wie ik mijn internistische opleiding mocht genieten, heeft mij altijd gestimuleerd en de gelegenheid gegeven om dit wetenschappelijk werk te voltooien. Het is voor mij een grote eer hem als promotor te mogen voordragen.

Last, but not least, wilde ik Solko Schalm bedanken voor zijn enorme inzet om dit werk te kunnen voltooien. Solko, meer dan wie ook weten wij wat het is om dergelijke experimenten, waarbij zo veel mensen betrokken waren, tot ieders tevredenheid af te ronden. Mede door jouw optimisme, doorzettingsvermogen, medewerkzaamheid en enthousiasme is dit gelukt.

Tenslotte dank aan allen, die direct of indirect door middel van vriendschap en steun mij in de gelegenheid gesteld hebben om dit werk te kunnen afronden.

CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren op 21 maart 1951 te Vroomshoop.

In 1970 werd het eindexamen H.B.S.-B aan het Christelijk Lyceum te Almelo behaald.
Aansluitend werd de medicijnen-studie aangevangen aan de Erasmus Universiteit te Rotterdam, alwaar in 1975 het Doctoraal-examen en in 1977 het Artsexamen werd afgelegd.

Op 21 Februari 1977 werd de opleiding tot internist aangevangen in het Academisch Ziekenhuis Rotterdam, Dijkzigt, onder leiding van Prof.Dr.M. Frenkel.
In Februari 1982 werd de schrijver in het Specialisten Register ingeschreven.

Vanaf October 1981 tot en met Februari 1983 bekwaamde hij zich op de afdeling Interne Geneeskunde II van het Academisch Ziekenhuis Rotterdam in de hepatologie en de gastro-enterologie onder leiding van Dr.S.W. Schalm.

Hij is thans werkzaam als internist in het Sint Jozef Ziekenhuis te Heemskerk.