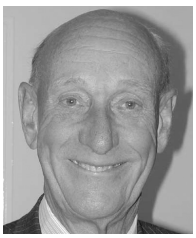
The elusive goal of liver support – quest for the Holy Grail

Roger Williams



This article is based on the Fitzpatrick Lecture given at the Royal College of Physicians on 10 April 2006 by **Roger Williams** CBE MD FRCP FRCS FRCPE FRACP FMedSci FRCPI(Hon) FACP(Hon), Director, UCL Institute of Hepatology, Division of Medicine, University College London

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ABSTRACT – The history of liver support devices is traced from early attempts with dialysis based on the known dialysability of ammonia – the major identified toxin in liver failure – and exchange transfusion with removal of protein-bound toxins, to the later techniques based on whole organ perfusion in extracorporeal circuits. Perfusion through charcoal as an adsorbent represented a major advance and remains a component of more recently introduced devices based on bioreactors of cultured hepatocytes and in the albumin dialysis techniques of molecular adsorbent recirculating system and the Prometheus device. The latter are the most highly efficient to date in toxin removal but whether survival is improved and the need for liver transplantation remain to be proven.

KEY WORDS: albumin dialysis, bioartificial devices, charcoal haemoperfusion, liver failure, water-soluble/protein-bound toxins

Thomas Fitzpatrick

Dr Thomas Fitzpatrick was a distinguished graduate of Dublin University, obtained his MD degree in 1862, was admitted MD Cambridge in 1867 and became a Member of the Royal College of Physicians of London (RCP) a year later. After a period travelling abroad, he settled in private practice in London. According to a most moving obituary in the *Lancet* dated 16 June 1900, he was a much loved physician, generous and kind to all those who required his skills. Blessed with a facile and graceful pen, he was a classical scholar and linguist of the highest order, fluent in Italian, German, French and Greek, with a remarkable memory for reciting prose and poetry. I certainly could not match these skills but there were two aspects of his life where I did feel some affinity. Firstly, he was a man of the sea. He worked for a time as an assistant surgeon in the service of the Honourable East India Company and he wrote two books on his travels. One, entitled *A transatlantic holiday*, recounts a voyage to the New England territories. The other, *An autumn cruise in the Aegean* or *Notes of a voyage in a sailing yacht* describes the 126-ton schooner, *Linda* (derived from the Spanish '*lindo*' meaning elegant and well proportioned), which was built in 1869 and had been made available to Fitzpatrick and his party shortly thereafter. The yacht was commanded by Captain R Diaper of the famous professional yachting family (the subject of an exhibition in Southampton Maritime Museum later in 2006). Fitzpatrick and friends boarded *Linda* in Piraeus as at that time the Italian and southern French ports were experiencing outbreaks of cholera. In the book, he writes of being caught in one of the sudden squalls for which the Aegean Sea has been notorious since the days of Homer. In his own words, 'I lent a hand at the ropes until we had secured and covered the mainsail'.

Secondly, Fitzpatrick had a considerable interest in the liver. In 1865, his work on *Chronic diseases of the liver*, was awarded the silver medal of the Pathological Society of Dublin. Although I had hoped to learn of his thoughts on liver failure, I have sadly not been able to trace this book. By the 1860s, liver atrophy, as it was then called, was well recognised and in Frerichs textbook and that of George Budd of King's College London there is much speculation at to how such a widespread and rapid loss of liver cells is initiated. Not surprisingly, hepatologists have long desired to provide patients suffering from liver failure with something comparable to the dialysis machine that revolutionised the care of patients with renal disease. But the liver has metabolic functions in addition to its excretory actions and replacing both functions with a machine is much more challenging.

Background

By way of background, some information on the various types of liver failure encountered in current day clinical practice is in order. Acute liver failure, often referred to as fulminant hepatic failure (FHF) because of the rapidity of the clinical course, is a rare syndrome with a high mortality occurring without previous liver disease. Surprisingly with the severity of the liver damage, if recovery occurs normal liver structure is restored. 'Acute-on-chronic liver failure' is the term given to an acute decompensation of a

previously well compensated cirrhosis precipitated by sepsis, variceal bleed or an alcoholic binge. This is the commonest type of liver failure seen in our hospital wards and intensive care units. It overlaps with the third variety, namely chronic decompensation from end-stage disease, where the influence of a precipitating factor is less evident. Manifestations of all three categories of liver failure are similar, with encephalopathy, jaundice, impaired coagulation, marked susceptibility to infection and finally multi-organ failure leading to death.

An effective liver support device would facilitate the processes of regeneration in acute-on-chronic liver failure to mitigate the harmful effects of the additional insults to the liver and, in chronic liver decompensation or failure, to serve as a bridge to transplantation. The liver is known to have an extraordinary regenerative capacity, but knowledge of how this is affected by the severe toxaemia of liver failure is limited. Many years ago, Robin Hughes in my unit at King's College Hospital showed that serum from patients with FHF inhibits Na^+, K^+, ATPase activity of cultured cells *in vitro,* proportional to the grade of coma present.[1] As to the nature of the toxaemia, severe hyperammonaemia remains centre stage along with a number of endogenous putative toxins many of which are protein-bound including aromatic amino acids, bile acids, unconjugated bilirubin, digoxin-like substances, benzodiazepines, indols, mercaptans, middle- and short-chain fatty acids, phenols, prostacyclins, tryptophan. Some of them may act synergistically but the fact that so many potential toxins continue to be listed is an indication that no one simple substance can be targeted in liver support.[2]

The known dialysability of ammonia was the basis for the first real attempts at liver support. In 1958, Kiley, Gundermann and Lie[3] reported marked improvements in encephalopathy in four out of five patients with chronic liver disease but with no apparent improvement in long-term survival, a result which sadly applies to many of the studies on liver support. Other types of dialysis membrane were subsequently investigated including much later the large-pore polyacrylonitrile membrane of Opolon and colleagues[4] who reported reversal of coma in 54% of patients treated.

Recognition of the need for a more aggressive approach to the removal of protein bound molecules led to the use of exchange transfusion, first described in 1958 in a single case report from Australia[5] and the subject of a landmark paper by Saunders and colleagues from Cape Town in 1966 (Table 1).[6] In the latter, all seven treated patients recovered completely after failing to respond to other forms of treatment and five survived to leave hospital. The technique was developed further using plasmapheresis machine and in recent years the Copenhagen Study Group for Liver Diseases introduced high volume plasmaphoresis with 16% of body weight exchanged with fresh frozen plasma daily. Successful bridging to transplantation was reported in a number of patients with FHF along with measurable improvements in cerebral blood flow and oxygen delivery;[7] the results of an ongoing controlled clinical trial are awaited with interest.

Table 1. Early clinical applications of temporary liver support for liver failure illustrating different approaches at the various times.

1958	Haemodialysis
1958	Exchange transfusion
1965	Porcine liver perfusion
1966	Cadaveric liver perfusion
1967	Human–human cross-circulation
1968	Plasma exchange (plasmapheresis)
1972	Coated-charcoal haemoperfusion
1976	Polyacrylonitril membrane haemodialysis

It was Eiseman, an American surgeon, who wrote so aptly in 1965:

> *The metabolic complexity of the liver makes it unlikely that attempts to remove a simple toxic product of liver failure will meet with much lasting success. In all likelihood only another normal liver adequately substitutes for one that is failed.*

He was the first to demonstrate improvement in encephalopathy with perfusion of porcine livers in an extracorporeal circuit, a technique which was used on and off for years with numerous reports attesting to improvements in the clinical state of the patients. A recent meta-analysis of 198 patients in the world literature showed an overall survival of 28%.[8] There was some evidence of a survival benefit when baboon or human livers were used but the complexity of the technique clearly limited its applicability. Abouna and his colleagues from Newcastle hold the record for the number of perfusions employed, reporting in 1970 a case of hepatorenal failure secondary to chronic hepatitis who had a total of 16 extracorporeal perfusions – 10 pigs, 3 baboons, 1 calf, 1 monkey, 1 human, and the patient survived![8a]

A more straightforward approach to whole liver support was reported by Burnell and colleagues in 1967,[9] namely cross-circulation with a human. The three patients treated had acute hepatic necrosis induced by the anaesthetic agent halothane, and one made a complete recovery. I vividly remember the only patient I ever treated by cross-circulation. The young woman had taken a large overdose of paracetamol after her husband left her. She was deteriorating fast but, overcome by remorse, the husband returned and without further ado he agreed to be linked up to his wife. As her bilirubin level and prothrombin time came down so his values went up and he looked increasingly unwell. Fortunately both survived.

Charcoal haemoperfusion

The introduction of charcoal haemoperfusion was a major breakthrough in liver support. The patient's blood is passed in an extracorporeal circuit through a column of activated charcoal which is a powerful absorbent for a number of the putative toxic substances in liver failure (Table 2). Chang and colleagues from Canada reported the first clinical use in a patient with hepatic coma in 1972[10] following a series of experimental animal studies. Together with the Research and Development Department of Smith and Nephew Ltd, we were able to develop

a column for clinical use in which the charcoal was coated with a membrane to prevent release of small charcoal particles, known as fines, into the patient's bloodstream, thereby improving biocompatibility.[11] We soon learned, however, that, as in any extracorporeal circuit in a patient with liver decompensation, the already primed coagulation system leads to further platelet activation and intravascular coagulation. We showed that this was partially correlated by an infusion of prostacyclin,[12] work incidentally which was part of John Vane's studies on prostacyclin at the Wellcome Laboratories, gaining him the Nobel Prize.

The first patient I treated at King's had been flown in with FHF from Jersey and made what seemed to be a magical recovery. In a series of grade IV encephalopathy cases published in 1974, survival was considerably improved: 45% compared with 10% in historical controls.[13] A later series showed additional benefit accruing when daily perfusions were started earlier at the stage of grade III encephalopathy.[14] But in a randomised controlled clinical trial, we were unable to show survival benefit for 10- versus 5-hour perfusion in grade III encephalopathy, and in grade IV cases no perfusion gave the same outcome as the 10-hour ones.[15] Clearly, in retrospect there were flaws in the design of the trial and as pointed out by Paul Berk, then Editor of *Hepatology*, the probability of a type II error was large. Nevertheless, the results halted further development of the charcoal haemoperfusion device, although further controlled trials in experimental animal models of liver failure combined to show statistically significant improvement in survival duration.[16,17] Its use, though, has not been lost completely as columns of absorbent charcoal have become an important component of a number of liver support devices. In other studies at that time, we were able to show, using perfusion of plasma through an ion exchange resin, satisfactory removal of bile acids – 200 to 400 μmol in the first hour with corresponding reduction in high serum levels.[18]

Table 2. Comparison of removal efficacy by coated charcoal, neutral resins and high flux dialysis for potential toxins in liver failure.

	Coated charcoal	Neutral resins	High flux dialysis
Ammonia	–	–	++
Aromatic amino acids	+	–	++
Fatty acids	+	–	+
Mercaptans	+	–	–
Bile acids	–	++	++
Bilirubin	–	+	+
Middle molecules	+	+	–
Cytokines	+	+	–
Endotoxin	–	–	–

The next phase in the history of liver support was marked by a return to the perceived requirement for a biological component to provide a full spectrum of liver functions. Based on a bioreactor containing isolated functioning hepatocytes, an absorbent column was often incorporated in the circuit for the removal of toxins, potentially damaging to the hepatocytes, hence the name bioartificial device. One of the earliest of such devices to undergo evaluation was known as extracorporeal liver assist device (ELAD) in which the C3A clone of hepatoblastoma cells was grown to confluence around a hollow fibre cartridge (Fig 1) through which blood was perfused. In a controlled trial of 24 patients with acute liver failure (ALF), we were unable to show a survival advantage although there were decreases in the number

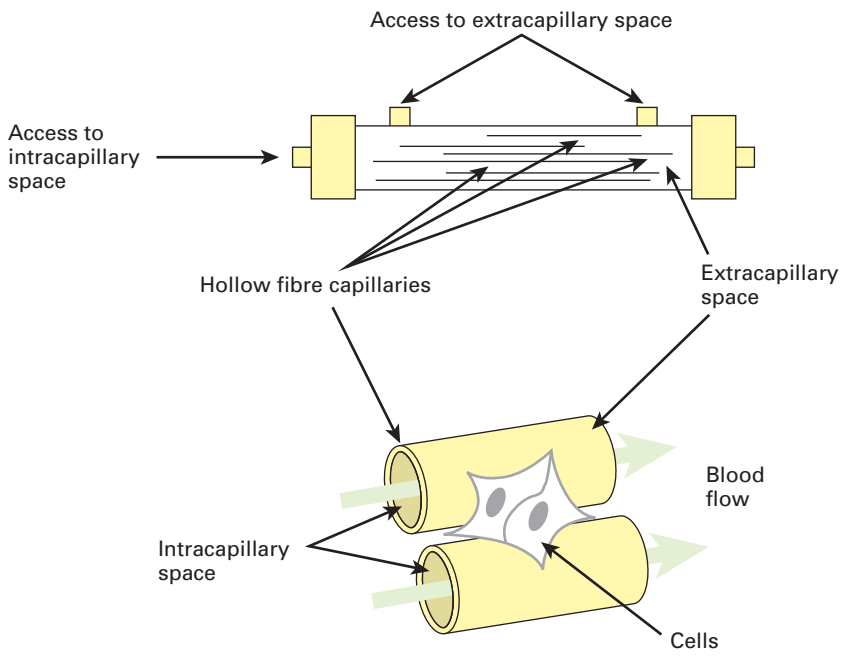

Fig 1. Diagram of a hepatocyte bioreactor in the extracorporeal liver assist device (ELAD) with hepatoma cells cultivated on the exterior surface of the semipermeable hollow fibres contained with the cartridge. In the bioartificial liver device of Demetriou, microcarrier-attached porcine hepatocytes are seeded into the extra fibre space. Reproduced with permission from Cambridge University Press.[19]

of patients showing deterioration in encephalopathy grade and in cardiovascular state.[19] The first person at King's to benefit from the device was a 14-year-old boy with ALF of undetermined cause. He was maintained on the device continuously for 60 hours during which time there was some improvement in prothrombin time and serum bilirubin, until an auxiliary graft transplant could be performed. When his own liver showed signs of recovery, after three months, immunosuppression was withdrawn allowing the graft to atrophy. He remains well today. Improved versions of the ELAD device using plasma for perfusion instead of blood and with an oxygenator in the circuit have shown greater metabolic activity with apparent clinical benefit.[20]

Various sources for the hepatocyte component have been tried with the aim of obtaining long-term differentiated function in culture. Porcine liver cells are easy to obtain and have been shown to retain some functions, but the potential for transmission of porcine endogenous retroviral infection to the patients causes anxiety although no evidence of this has ever been demonstrated. Human primary hepatocytes are difficult to maintain in a functioning state for more than a few weeks. Hepatocytes immortalised in culture through the introduction of simian tumour antigen gene, which prior to use can be excised using a transfected herpes simplex virus gene, have shown good function in experimental studies.[21] But it is not only the source of the cells which is important but how they are grown. Hugo Jauregui of Brown University, USA, an enthusiast in this field commented, 'When culturing liver cells – they've got to look like bricks; if they look like fried eggs they don't work properly.' Many different approaches have been tried including culture on extracellular matrix, co-culture with non-parenchymal cells, and three-dimensional culture by attachment to micro carrier beds, semipermeable fibers or a polyester fabric. The physical design of the bioreactor is also important and perfusion should preferably mimic the sinusoidal low diffusion gradient of the normal liver. There is also the question of what constitutes the critical cell mass for a bioreactor; 6 x 10^{10} cells is equivalent to 30% of a normal liver size which we know from surgical hepatectomies is sufficient to sustain life. Interestingly, in a rat model of ALF, the equivalent of 2% of liver mass was sufficient to enhance regeneration through removal of inhibitory substances including TGFβ.

Of the latest designs of bioartificial livers, the modular extracorporeal liver support (MELS) device developed in Berlin uses human hepatocytes laden into a cartridge containing three intertwining capillary systems which allows the hepatocytes to develop their own bio-matrix and sinusoidal structure. Of the eight patients treated at the time of the first clinical report, six were successfully bridged to transplantation. The bioartificial liver at the academic Medical Centre at the University of Amsterdam, uses porcine hepatocytes grown on a polyester-fibre membrane in a radial flow system and has given encouraging results in a large animal model of liver and in early clinical trials.

The largest controlled clinical trial carried out to date of a bioartificial device, much modified and developed by Demetriou and his colleagues from Cedars-Sinai Medical Center, Los Angeles, was finally published in 2004.[22] One hundred and seventy-one patients with ALF were randomised to BAL with an average of 2.9 perfusions per case or standard medical care. Thirty-day survival between treated and non-treated groups was not significantly different. Transplantation, performed in 54% of cases, had a profound impact on survival and when the data were analysed to take account of this, survival in fulminant/subfulminant groups was statistically just significant in those treated with the BAL device.

The huge cost and impracticability of such bioartificial devices makes the whole approach unattractive, certainly for me. Indeed, during the last few years there has been a resurgence of interest

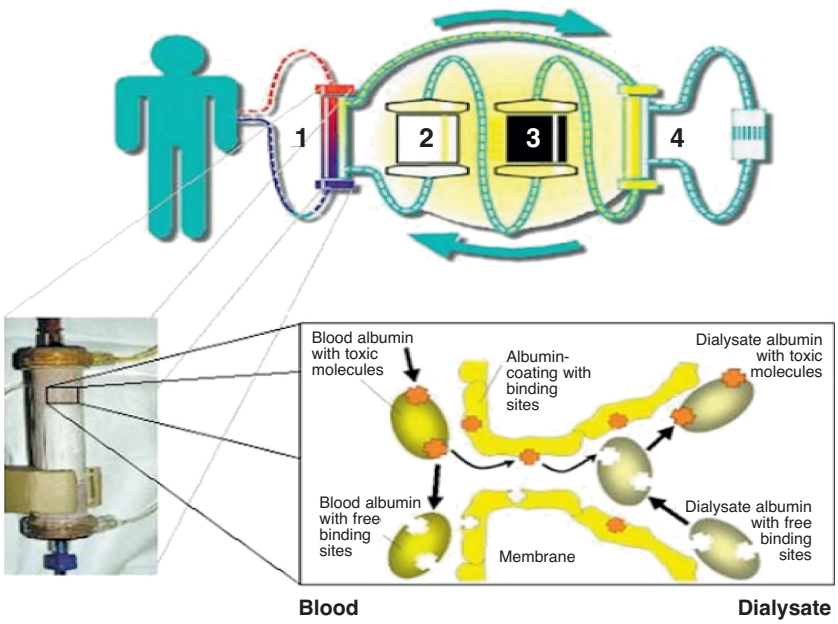

Fig 2. Diagram of the molecular adsorbent recirculating system (MARS) circuit illustrating primary circuit of blood past albumin impregnated membrane with passage of protein bound toxins across to the second albumin recleansing circuit through charcoal and ion exchange resin. Copyright © 2002 American Association for the Study of Liver Diseases. Reprinted with permission of Wiley-Liss, Inc, a subsidiary of John Wiley & Sons, Inc.[24]

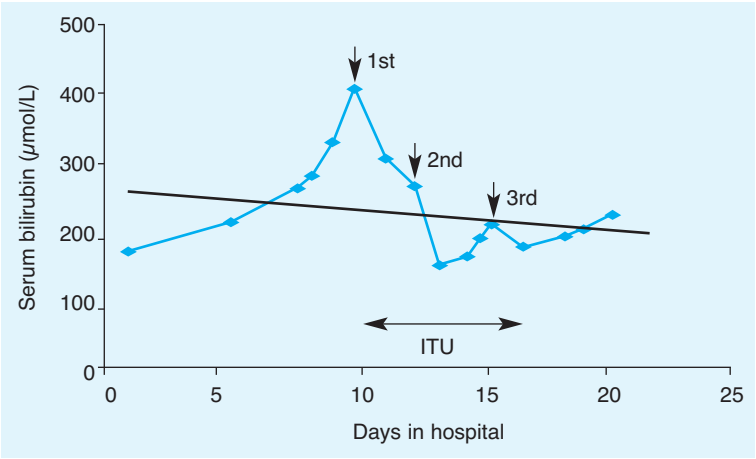

Fig 3. Effects of three treatments with molecular adsorbent recirculating system (MARS) on serum bilirubin level in a patient admitted with acute-on-chronic liver failure from an acute alcoholic hepatitis.

in artificial systems. In the molecular adsorbent recirculating system (MARS), developed in the early nineties by Stange and Mitzner of the University of Rostock, the patient's blood is dialysed across a high flux membrane impregnated with albumin (Fig 2).[23] The pore size of 50K daltons prevents loss of valuable hormones, growth factors and, of course, albumin. Toxins are thought to be stripped off the circulating plasma albumin because of the higher affinity of albumin when attached to polymers and reattach to molecules in the albumin solution on the other side of the membrane. This is then cleansed by recycling through absorbent charcoal and resin columns. The circuit includes a standard haemodialyser for the removal of water soluble toxins. The first patient we treated had an acute alcoholic hepatitis and was deteriorating progressively after admission, as they so characteristically do, with the serum bilirubin level climbing to 400 μmol/l (Fig 3). Each of the three treatments with MARS produced a marked fall in level along with improvement in encephalopathy, the patient finally recovered sufficiently to leave hospital. Over 5,000 patients worldwide have now been treated with the device. In addition to its use in liver failure, it has been applied in the treatment of severe cholestasis with effective reduction in bilirubin and bile salt levels.

A major outcome study in acute-on-chronic liver failure was reported by Hassanein and colleagues in the past year.[25] This was a prospective controlled multicentre trial of patients receiving either standard medical treatment or daily albumin dialysis for six hours over five consecutive days. A two grade improvement in hepatic encephalopathy was reached significantly faster and more frequently in the MARS group. Significant decrease in ammonia, bilirubin, bile salts, creatinine and aromatic amino acids were demonstrated. The number of patients dying during the 180 days of follow-up and the number having liver transplants, however, were not significantly different in the two groups. Resolving coma within five days was shown to have a strong impact on the two-week transplant-free survival: 81% compared with 34% in those remaining in coma ($p<0.001$).[26] Among the many research studies on the effects of MARS on the disturbed pathophysiology of acute-on-chronic liver failure, those of Rajiv Jalan and his group in the UCL Institute of Hepatology, University College London, have shown reduction in whole body nitric oxide production and in oxidative stress as well as reversal of renal vasoconstriction in parallel with a reduction in norepinephrine and renin levels[27] consistent with well documented evidence of improvement in hepatorenal syndrome. I would also draw attention to other measured effects namely an increase in cerebral blood flow and lessening of the indices of brain swelling including ICP elevation and high jugular bulb oxygen saturation.[28,29]

Another artificial device based on albumin dialysis currently under clinical evaluation is named after the Greek God, Prometheus.[30] The dialysis membrane is of large pore size freely permeable to albumin which is then passed through absorbent columns to remove the bound toxins so a better term for it is fractionated plasma separation. In a comparison study with MARS, there was little difference in the plasma clearances of the various substances except for unconjugated bilirubin which was better removed by the Prometheus device.[31] Undoubtedly the stimulus of these new techniques will lead to new knowledge on the functionality of albumin in relation to the binding of toxic substances.

Conclusion

Finally, there is one more concept that I would like to discuss arising from three theoretical outcomes for a patient with liver failure depending on the severity of the initial injury. In the first, the patient, although deteriorating to below the coma limit, is able through spontaneous recovery to improve above that again. With the second outcome, the deterioration is further to beyond survival level but not to below regeneration limit and the patient could recover if treated by hepatic assist. The third outcome follows from when the liver injury takes patients to below regeneration limit and the only chance for survival is with a liver transplant. Such theoretical considerations help in focusing minds on what are the important prerequisites to effective liver support in the context of the underlying disease processes and allow me to pose the following questions. Are we paying enough attention to the processes set in motion in the liver that lead to progressive deterioration in function and structure? Will control of toxaemia and the addition of certain metabolic functions through liver devices ever lead to an improvement in at least medium-term survival without the need for liver transplant? Should we not be directing our efforts towards transplantation of living cells – whether stem cells or liver progenitor derived – into the diseased liver with the potential for repopulation and remodelling of the organ, which has already been demonstrated in animal models, or is this too pious a hope? In other words, are we asking the right questions? This naturally brings me back to the subtitle of this article, the 'Quest for the Holy Grail' of Arthurian legend. Despite all our desires for a liver machine and

the inventiveness of those who have worked on it, can this elusive goal ever be reached and will we ever identify the important question that Sir Perceval – better known as Wagner's Parsifal – had to ask before the Fisher King could be healed?

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