

## Leading article

# Artificial livers – what's keeping them?

The development of artificial organs must go down as one of the major areas of medical advance in this century. Temporary replacement of heart and lung function has permitted miracles of cardiac surgery; renal dialysis has revolutionised the prognosis in acute and chronic renal failure. The analogy with renal disease points to the areas in which artificial livers are required – temporary replacement of function in acute hepatic failure until regeneration has had time to restore normality, and buying time in chronic liver disease until transplantation can be performed. Why have they been so long in coming?

The answer is simple – none of the organs currently replaced artificially are particularly complex. Hearts are pumps, kidneys are filters, lungs are membranes; in that sort of language, what is a liver? A chemical factory, a detoxification plant, and a nutrient processor, with a sophisticated command and control function as a biochemical servo-mechanism. More scientifically, provision of an artificial liver should address its synthetic functions, its metabolic role in the control of carbohydrate and fat metabolism, and its role in the removal and detoxification of both endogenous and exogenous substances. In view of this complexity, it is perhaps not surprising that relatively simple mechanical approaches to replacing liver function have proved unsuccessful.

Various forms of haemodialysis have been tried,<sup>1–4</sup> but these can clearly substitute only for the filtering and detoxifying properties of the liver. Simple filtrative haemodialysis and haemofiltration have been further refined to allow an additional absorptive step, and particular attention has been paid to absorbing molecules thought to be relevant to the development of hepatic encephalopathy.<sup>5</sup> In the 1950s Schechter proposed the use of Dowdex resin to absorb ammonia.<sup>6</sup> A variety of synthetic resins were experimented with in the 1960s,<sup>7,8</sup> and charcoal was in extensive use through the 1970s and early 1980s in haemoperfusion systems.<sup>9</sup> The initial enthusiasm with which charcoal haemoperfusion was greeted reflected good survival rates in hepatic failure compared with historical controls in early studies. However, controlled trials, comparing different durations of charcoal haemoperfusion in patients with grade 3 encephalopathy, and comparing haemoperfusion with no perfusion in grade 4 coma, lead to the conclusion that the apparent good results of those early studies represented the effects of a general improvement in the care and monitoring of patients with fulminant hepatic failure.<sup>10</sup>

A number of physiological problems – relevant to any form of artificial liver – were highlighted by experience with haemoperfusion. These included incompatibility between blood and the extracorporeal circuit, particularly the absorptive surface, causing problems such as complement activation<sup>11</sup> and the initiation of platelet activation and leucopaenia,<sup>12</sup> and removal of desirable molecules such as coagulation factors<sup>13</sup> and hormones.<sup>14</sup> Prostacyclin infusion,<sup>15</sup> heparinisation,<sup>16</sup> extensive use of Silastic,<sup>17</sup> and thrombolytic and antithrombotic agents such as the polydeoxyribonucleotide defibrotide<sup>18</sup> have all been assessed to

help overcome these problems. Studies on new forms of absorbent columns continue, aimed at developing more specific ligands for removal of different molecules including Amberlite<sup>19</sup> which removes a number of cytokines, polymixin to remove endotoxin,<sup>20</sup> and hydrophilic liquid membranes<sup>21</sup> to remove ammonia, phenols, cresols, and fatty acids. Problems of biocompatibility of these absorbents with formed elements of the blood in most experimental systems are now being circumvented by a plasma separation step in the extracorporeal circuit.<sup>22</sup>

A theoretical objection to plasmapheresis, dialysis, and absorption columns, is that factors helpful to the repair of the damaged liver may be removed. Plasma from patients with fulminant hepatic failure does indeed contain stimulators to hepatic regeneration, such as hepatocyte growth factor<sup>23</sup> and the albumin-bilirubin complex,<sup>24</sup> although inhibitors of regeneration are also reported.<sup>25</sup> Such considerations support the alternative approach to liver support – some form of bioartificial liver. The temporary harnessing of functional liver tissue, although complex and difficult, allows the possibility of replacing the whole spectrum of deficient liver function.

The use of xenogeneic tissue – canine,<sup>26</sup> pig,<sup>27</sup> or baboon livers<sup>28</sup> – in extracorporeal systems has not proved successful in clinical practice. Most clinical reports of these approaches are now 20 years old; but this idea is re-emerging. Recent limited studies in the USA show that an auxiliary liver will function short term, and also reported the development of intravascular coagulation<sup>29</sup>; even if temporarily successful, however, the animal proteins generated are likely to induce immunological responses. Ex vivo perfusion through human liver,<sup>30</sup> though technically feasible, has not been extensively explored in recent years, probably for the pragmatic reason that the proven efficacy of transplantation dictates that usage of available organs.

The use of isolated hepatocytes is now attracting attention. The potential for cryopreserving hepatocytes, and the techniques now applicable to human liver for preparing single cell suspensions, indicate there is no insuperable bar to obtaining hepatocyte suspensions as required.<sup>31</sup> Theoretically, isolated hepatocytes might be used in an extracorporeal bioreactor or else implanted within the patient in sites such as the peritoneum, and both approaches are being explored experimentally. For use in an extracorporeal system,<sup>32</sup> the classic tissue culture technique of cell monolayers is clearly unsuitable, as the surface area to volume considerations are grossly unfavourable, but developments over the last few years offer the ability to culture a large number of cells in a substantially smaller volume; these include the use of microcarriers,<sup>33–36</sup> hollow fibre support systems,<sup>37,38</sup> multilayered monocultures separated by glass plates,<sup>39</sup> or cell aggregates trapped between glass beads.<sup>40</sup> Each of these has advantages in cell performance over simple suspension cultures of hepatocytes in which specialised hepatocyte functions are not well maintained; provision of some form of matrix for hepatocyte support seems to play

an important role in maintaining differentiated hepatocyte function.<sup>41</sup>

Microcarriers, typically spheres of 100–300 µm in diameter, of various materials, have been assessed for use in a bioartificial liver. Many microcarrier coatings optimised for use in tissue culture media are unsuitable for use in plasma, as they induce fibrin deposition, but materials such as Biosilon avoid this complication provided a minor level of anticoagulation is provided.<sup>42</sup> Linear rates of carbohydrate metabolism, protein synthesis, and drug removal are achieved and sustained in plasma perfused columns. Prolongation of survival and improvement of biochemical parameters of liver failure in rats with CCl<sub>4</sub> or galactosamine induced liver failure have been reported.<sup>34</sup> An unusual feature of this report, however, was a lower transaminase activity in the microcarrier supported animals, so these experimental conditions may have reduced the damage induced rather than treated the consequences of hepatic damage.

One potential advantage of the microcarrier based system is the direct contact established between the blood or plasma and the hepatocytes, allowing free interchange of metabolites and synthesised proteins; this advantage is, to some extent, counteracted by the potential for cell debris, including DNA, to be carried into the body. If liver support systems involve direct exposure of plasma to liver cells, design features must address both these problems, although in the context of the treatment of acute hepatic failure, the long term risks of receiving an infusion of a small amount of DNA needs to be kept in perspective.

Hollow fibre systems, in contrast, separate hepatocytes (in a chamber through which fibres pass) from blood which is pumped through the fibres.<sup>37 38</sup> The hepatocytes may be in suspension, attached to microcarrier, or attached to the fibres.

In experimental models such as the hyperbilirubinaemic Gunn rat,<sup>38 43</sup> these systems can be shown to conjugate bilirubin, and reversal of paracetamol induced hepatic failure in two dogs has been reported in a pilot study.<sup>37</sup> The interposition of the membrane of a hollow fibre is likely to retard significantly the diffusion of a number of larger molecules, although fibre characteristics are varied, and Jauregui *et al* have commented that such devices are most likely to be of help in the reversal of those aspects of hepatic failure, such as encephalopathy, which probably reflect accumulation of low molecular weight substances such as ammonia, mercaptans, fatty acids, phenols, and benzodiazepine-like molecules.<sup>38</sup> These considerations emphasise one difficulty of designing experiments to test artificial livers. Animal experiments (if ethical) probably involve anaesthetics, and improvement attributed to an artificial liver may correspond to removal of low molecular weight anaesthetic agents.

An alternative approach using isolated hepatocytes is that of direct implantation, within the peritoneal cavity<sup>44–46</sup> or into the splenic pulp or the liver via the portal circulation.<sup>47</sup> Isolated liver cells are capable of surviving and fulfilling specialised hepatic functions in these sites, and the potential for treating inborn errors of metabolism has been explored in rats.<sup>45</sup> Bilirubin conjugation defects<sup>36 46 48</sup> and analbuminaemia<sup>46</sup> have been ameliorated, at least for several weeks; however, allogenic hepatocytes are rejected unless the implanted cells are protected in some way, either by immunosuppression,<sup>45</sup> or by protection within a semipermeable membrane.<sup>48</sup> One attractive approach to protection is micro-encapsulation of hepatocytes within an alginate covering.<sup>48 49</sup> This is achieved by mixing hepatocytes with alginate and forming droplets by extrusion through a needle; encapsulated cells maintain biological function *in vitro*, particularly if they are in

spherical aggregates.<sup>50</sup> Within the capsule, more prolonged amelioration of metabolic defects can be achieved when alginate coated cells are transplanted,<sup>46</sup> and rapid rejection of allogenic hepatocytes can be avoided.<sup>48</sup> For experimental manipulation, encapsulated hepatocytes seem to be both more robust than single cells and to survive longer.<sup>51</sup> There is also a moderate body of literature indicating that implanted hepatocytes can ameliorate experimental hepatic failure.<sup>46 52</sup> Earlier enthusiastic research in this field<sup>53</sup> was slowed in the 1970s when it was shown that implanted hepatocytes, alive or dead, and even a cytosolic hepatocyte extract, could also improve survival, perhaps reflecting release of stimulators of regeneration.<sup>54</sup> More recently this approach has again been tried, with studies showing that intraperitoneal injection of micro-encapsulated hepatocytes improves survival after initiation of galactosamine hepatitis in rats when compared with empty alginate microcapsules.<sup>46</sup> In view of the previous demonstration of the apparent benefit from dead hepatocytes or hepatocyte extracts, it is unfortunate that studies still omit this vital (or non-vital!) control. Critical studies have shown that cultures of microencapsulated hepatocytes *ex vivo* can release hepatic stimulatory substances capable of improving survival in the rat galactosamine model.<sup>55</sup>

For any system using hepatocytes, availability of suitable cells presents a challenge. In 1987, Matsumura *et al* wrote a clinical report of the use of isolated rabbit cells in an extracorporeal system,<sup>56</sup> and recently pig cells, maintained on microcarrier cells in a hollow fibre system have been used to treat patients both in the presence of an acutely failing liver and during a brief anhepatic phase before transplantation.<sup>57 58</sup> Thus far the documentation of improvement is brief. For general usage, a source of human cells seems desirable, unless sophisticated genetic modification of animal cells to prevent generation of antigenic proteins can be achieved: still a remote goal. Available normal human hepatocytes are scarce, although cryopreservation should ease this problem, but for the moment some competition between transplant surgeons and the artificial liver seems inevitable. Cultured fetal hepatocytes or immortalised lines from normal adult cells are potential prospects, but any manipulation that maintains hepatocytes as dividing cells gives rise to some disquiet, as does the use of tumour derived cell lines even if they are well differentiated. One recent pilot study reports the use of a well differentiated human tumour derived cell line which maintains the property of contact inhibition, so that cartridges of these cells can be maintained in a perfused system 'at the ready'<sup>37</sup>; regrettably this cell line is the subject of a US patent.

In recent months, abstracts of reports of clinical usage of artificial liver support systems using hollow fibre and whole perfused human liver as well as animal cells have been published.<sup>59 60</sup> A clinical trial of the device using human tumour cells has begun; initial reports have not (yet) confirmed a benefit.<sup>61</sup> Review of previous clinical studies in this area emphasises (and it seems to need emphasis) that proving the efficacy of complex interventions in the unpredictable setting of severe acute hepatic failure is difficult. Let us hope that workers are self critical in assessing these interventions, and that institutional ethics committees recognise the importance of establishing the true value of these interventions. Despite these recent reports, artificial livers remain tantalising – theoretically impeccable, but still practically remote. The disappointing outcome of adsorption columns, and the failure to show significant benefit from plasmapheresis, seem to leave a bioartificial liver using extracorporeal or implanted hepatocytes as the most promising prospect. Problems of

biocompatibility of the hardware, availability of hepatocytes, and ensuring blood or plasma are exposed adequately to the cells to allow exchange to occur, seem likely to employ innovative researchers for some years yet.

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