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Discussion

DR. WILLIAM V. MCDERMOTT, JR. (Boston, Massachusetts): I had the opportunity to read the manuscript that formed the basis for this excellent report you have just heard and that provided a summation of this long period of excellent and quite prodigious labors over a period of time. I will not comment on the hepatocyte microcarrier system; I will leave that to those who are expert in this field or who have a direct interest in this problem, perhaps through similar problems encountered by those investigators working with islet cell transplants. The objective of this report was to develop an extracorporeal support system that worked, and of course this has been an objective of many of us over many years.

(Slide) Some of the historical background, which I won't go into in detail, shows that in many instances it goes back to Claude Bernard's canine perfusion system and then carries through from the 19th century over the subsequent decades. You can see here that Ben Eiseman, as in so many instances connected with liver problems, was one of the pioneers in this system. As he developed this and began to use it in humans, he was of immense support and help to us in our work, and we collaborated and exchanged information over a period of time. Ben not only pursued this with the porcine and canine liver but also with hepatocytes.

(Slide) I would like to talk briefly about the extracorporeal support system that we utilized for a period of time. This is a description of the pig liver and its attachment in man and the various technological mechanisms in the clinical project.

(Slide) These are the first five patients whom we selected to be supported by this device. They all had a similar clinical situation, of deep, unresponsive coma and anuria or extreme oliguria, a combination which at that time was invariably lethal. This is the type of intermittent perfusion that we were using at that time.

(Slide) Without going into more than superficial details of this system and its clinical utilization, you can see here the effect on the bilirubin level of serial perfusion over several weeks in a patient.

(Slide) Some of the interesting things, some of which are still puzzling, show the protein characterization in the patient's sera, which I think demonstrates the presence of both porcine analbumin and alpha-1 and -2 globulins. The ascites is really the lymphatic flow from the extracorporeal liver. The bile made during the perfusion by the porcine liver shows the presence of human albumin, and again the globulins with human serum components.

One of the intriguing things was the antibody determinations that in all patients were negative by the relatively primitive techniques available at that time.

(Slide) In a brief summation of this, the pig liver produced bile containing human proteins and lymph containing both human and pig proteins and resulted in circulating pig albumin and alpha-2 globulin in the patients.

(Slide) Immunologically, although perfused and preterminal, these patients did have 7S gamma globulin prior to the perfusions and, we thought, had potential antibody formation capacity, but they showed no demonstrable antibody response despite serial testing over many weeks. I have no real explanation for this except perhaps a massive antigen overload.

I will conclude by saying that we gave this up for an obvious reason. Even though it was dramatically successful repeatedly and intermittently in restoring both renal function and responsiveness, none of the patients survived, and, at post mortem, microscopic studies all showed complete absence of hepatocytes and no evidence of regeneration. So, clearly, utilizing these terminal patients, we have felt that this type of support had no long-term clinical application.

DR. BEN EISEMAN (Denver, Colorado): Only those of us who have worked in the still unsolved area of artificial liver support can appreciate the significance of the advance made by this work.

One of the major problems that we found in our many trials illustrated by these slides (Slide) was to keep the liver cells suspension bound within the chamber. Dr. Levenson and co-workers have clearly shown this can be done by attaching the cells to a carrier.

I believe the autologous cells from a patient with liver failure will be of little help: they are too badly damaged. This will mean that the cells will either have a very short active life or immunosuppressives will have to be used in the clinical setting involving transplants.

I am delighted that this problem, which so long occupied our efforts, is yielding to the imaginative efforts of Stan Levenson and his colleagues.

DR. DAVID E. R. SUTHERLAND (Minneapolis, Minnesota): Dr. Levenson was kind enough to send me the paper, and I would like to discuss it and ask some questions that were raised in the presentation.

At the Transplantation Laboratories at the University of Minnesota, we have had a longstanding interest in hepatocyte transplantation. Ten years ago, Art Matas, who was with our group then, did some experiments in Gunn rats in which he transplanted hepatocytes, and he was able to effect a lowering of bilirubin that was not sustained. That was before Dr. Carl Hanson at NIH had developed the inbred strains of RAJ rats that Dr. Demetriou used to avoid the rejection problem and effect a 21-day reduction in bilirubin.

Dr. Marco Cavallini in our laboratory did auxiliary liver transplants in RAJ rats. He also did pancreas, heart, and kidney transplants, and he was able to show that even a kidney transplant would lower the bilirubin because there is glucouronyl-transferase in the kidney. However, ultimately even in liver transplant recipients the bilirubin increased, and it was thought that with the lack of portal perfusion these livers atrophied. I wonder if in these hepatocyte transplants this might be a problem if you carried out your experiments for longer than 21 days.

You did demonstrate that the hepatocytes actually function. One problem with hepatocyte transplant experiments that have been done in the past has been the use of a liver failure model. In liver failure models survival of the animals can be increased just by giving liver cell culture supernatants or other manipulations, and it appears that it may not be the hepatocytes that sustain the animal but some factors that accelerate liver regeneration.

One thing that was not clear to me was what your results were without using the microcarrier. You presented excellent results using the microcarrier, but I really wonder if you proved that it does improve the results. What would happen if you just gave the hepatocytes transplanted intraperitoneally? I also was not certain to what degree you corrected the jaundice. The bilirubin levels fell to nearly 1 ml per deciliter, but you did not show what the level is for normal rats. Also the concentration of conjugated bilirubin in the bile increased after transplantation, but you did not give the figures for what the usual normal concentration would be, which might be an indication of how nearly normal you made your animals.

This was an excellent paper with very interesting results.

DR. ACHILLES A. DEMETRIOU (Closing discussion): I would like to thank the discussants for their kind remarks. Drs. McDermott, Eiseman, and Sutherland have carried out pioneering work in the surgical treatment of liver disease, development of extracorporeal liver support systems, and hepatocyte transplantation. We have developed an extracorporeal liver "superfusion" system that successfully carried out protein synthesis, bilirubin synthesis and bilirubin conjugation. We believe that the microcarrier collagen matrix is crucial in maintaining hepatocyte viability, function, and differentiation. We do not think that preventing cell washout from the column by the attachment to the microcarriers is the only factor responsible for the demonstration of hepatocyte function by this "superfusion" system. As a potential method for artificial liver support, our system offers several advantages over systems used previously by other investigators. It allows use of the whole metabolically intact liver cell, it is simple, and it allows use of cryopreserved microcarrier-attached hepatocytes.

In our hepatocyte transplantation experiments we had direct evidence of function of the transplanted microcarrier-attached normal hepatocytes by demonstrating presence of bilirubin conjugates in the bile of Gunn rats that lack the ability to conjugate bilirubin and by showing increased plasma albumin levels in analbuminemic rats that have only trace levels of plasma albumin because of a genetic defect in albumin synthesis. The decrease in serum bilirubin seen in the Gunn rats cannot be explained only by excretion of bilirubin conjugates in the bile because the amounts found were small. However, it is possible that there is increased excretion of conjugated bilirubin in the urine. We are now carrying out studies in an attempt to determine the levels of bilirubin in the urine and other body fluids. This method has not been successful in prolonging survival in rats with D(+)galactosamine-induced acute liver injury; however, we have been able to improve survival in rats with acute liver insufficiency due to 90% partial hepatectomy. We only transplant 1×10^7 cells intraperitoneally, which represents approximately 1% of the liver mass in the rat. We are now carrying out studies to determine whether the transplanted microcarrier-attached hepatocytes proliferate in the host following transplantation intraperitoneally.

I would like to thank the Association for the privilege of allowing us to present our work.