Liver Preservation by Single Passage Hypothermic "Squirt" Perfusion

R. Y. CALNE, D. C. DUNN, B. M. HERBERTSON, E. M. GORDON, H. BITTER-SUERMANN, A. J. ROBSON, A. S. MACDONALD, D. R. DAVIS, D. P. SMITH, F. H. REITTER, L. M. WEBSTER

British Medical Journal, 1972, 4, 142–144

Summary

A single passage, hypothermic, intermittent squirt perfusion of the pig's liver is described which has allowed preservation of porcine livers for up to 17 hours. After preservation, orthotopically allografted livers can maintain recipient animals in good health. If this technique can be applied to clinical liver transplantation it should be possible to move livers from one hospital to another in the same way that kidneys are transported. This should increase the availability of donor livers for transplantation and avoid the serious disadvantages of moving the sick recipient just before the operation to the hospital where the donor has died.

Introduction

The two methods of organ preservation that have been most widely adopted are cold storage at 0-4°C after preliminary flush-out with cold innocuous fluid (Collins *et al.*, 1969) and continuous high-flow perfusion at 10°C (Belzer *et al.*, 1967). Non-perfusion cold storage is certainly simpler but the results from using continuous perfusion have been better, particularly for long-term preservation. The apparatus is complicated, expensive, and requires expert supervision. It was felt that if the advantages of the lower temperature used in simple storage could be combined with those of continuous perfusion a useful system of organ preservation might be developed. We reported experiments in which a hypothermic continuous single passage trickle perfusion was used for storage of both the kidney and liver followed by subsequent transplantation (Calne *et al.*, 1972). Canine kidneys preserved for between 24 and 26 hours could maintain the life of dogs when autotransplanted, contralateral nephrectomy being performed at the same time. Porcine liver preservation was successful up to 11 hours as tested by orthotopic allografting. Dye studies and angiography of organs preserved by this method suggested that parenchymal perfusion could be inadequate owing to leakage of perfusate into pericapsular arterioles at these very low flow rates. It was decided, therefore, to modify the technique so that the perfusate was administered intermittently at higher pressures as a squirt of a bolus of perfusate. This produced superior results with liver preservation. Considerable further improvements followed the use of perfusates at a pH of 6·8 instead of 7·4. These experiments will be reported.

Departments of Surgery and Pathology, University of Cambridge
R. Y. CALNE, M.S., F.R.C.S., Professor of Surgery
D. C. DUNN, F.R.C.S., Assistant Director of Research, Department of Surgery
B. M. HERBERTSON, M.D., M.R.C.PATH., Lecturer, Department of Pathology
E. M. GORDON, M.B., F.R.C.S., Research Fellow in Organ Transplantation, Department of Surgery
H. BITTER-SUERMANN, M.D., Research Fellow in Organ Tranplantation, Department of Surgery
A. J. ROBSON, F.R.C.S., Assistant Director of Research, Department of Surgery
D. R. DAVIS, Chief Technician, Department of Surgery
D. P. SMITH, Technician, Department of Surgery
F. H. REITTER, Technician, Department of Surgery
L. M. WEBSTER, Theatre Sister, Department of Surgery

Dalhousie University, Halifax, Nova Scotia, Canada
A. S. MACDONALD, M.D., F.R.C.S., Assistant Professor of Surgery

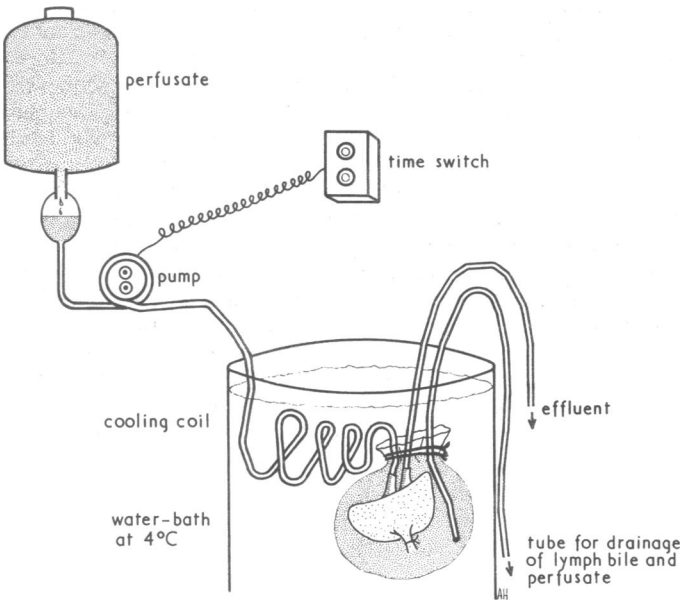
Materials and Methods

Cross-bred landrace/large white pigs of either sex between 20 and 30 kg were used as donor and recipient. The technique of liver removal and orthotopic transplantation has been described previously (Calne *et al.*, 1968). During the anhepatic state in the recipient operation a shunt was used from the portal vein to jugular, but not from the inferior vena cava, which was clamped. Vagotomy and drainage were not performed. While the donor was exsanguinated via aortic and inferior vena caval cannulae, the portal vein was perfused with between 500 and 1,500 ml of chilled electrolyte solution containing 1,000 units of heparin. In most experiments Hartmann's solution was used but in two experiments the initial cooling was with Collins C_3 solution. The hepatic artery was perfused with 100 ml of the chilled solution.

The liver was then weighed, inserted into a plastic bag with a drainage cannula to remove the effluent, and the bag was immersed in a second bag containing cold saline solution. The temperature was maintained between 3° and 5°C in a water-bath cooled by a Churchill refrigeration unit. The temperature of the water-bath was monitored.

The effluent was removed by gentle suction. The perfusate was pumped from litre bottles by a Watson-Marlowe pump connected to a timing system. The pressure close to the pump was recorded direct on a manometer, the tubing limb to which acted as a bubble trap. The perfusion line passed through a blood cooling coil before reaching the liver (see Fig.).

The interval between each bolus was five minutes. The bolus consisted of 20 ml which was introduced in 15 seconds. The pressure recorded was between 40 and 60 mm Hg. The perfusate consisted of plasma protein fraction kindly supplied by the Blood Transfusion Service. To each litre were added 15 mEq $K_2H\,PO_4$ and 250 mg dextrose in 15 ml, 5 ml 10% $Mg\,SO_4$, 250 mg hydrocortisone, 500 mg ampicillin, and N/10 HCl to lower the pH to 6·8 at 37°C.



Apparatus used in liver preservation. Timer controls pump to squirt a bolus of 20 ml through the portal vein in 15 sec with an interval between each bolus of 5 min. The water-bath is kept between 3° and 5°C.

Liver Preservation Times and Clinical and Laboratory Findings in Recipient Pigs

Pig No.	Preservation Time (Hours)	Survival	Cause of Death	Histological Findings	Days Post-operative	Bilirubin	SGOT
1	12	18 hours	Haemorrhage from split liver	Extensive centrilobular necrosis			
2	12½	9 days	Peritonitis	Normal lobular structure with well preserved liver cells. Slight oedema of portal tracts with slight mononuclear cell infiltration	+1	1·0	1,080
					+5	3·5	176
3	14	3½ hours	Hydrothorax	Substantial degeneration and necrosis of liver cells in lobular centre			
4	14	37 days	Intestinal obstruction	Healthy liver cells. Moderate fibrosis of portal tracts and interlobular septa with moderate bile duct proliferation and slight mononuclear cell infiltration	0	<0·5	588
					+1	<0·5	896
					+2	<0·5	378
					+3	<0·5	31
5	15	57 days	Peritonitis and intestinal obstruction	Normal lobular architecture and well preserved liver cells. Conspicuous portal tract fibrosis with slight mononuclear cell infiltration	+2 hr	<0·5	420
					+1	<0·5	676
					+2	<0·5	308
6	16½	Alive, 5½ months			+57	<0·5	49
					+162	<0·5	22
7	17½ (500 ml Collins + 100 mg isoprenalin solution)	51 days	Peritonitis and intestinal obstruction	Well preserved liver cell columns with moderate portal tract fibrosis, slight bile duct proliferation, and lymphocytic infiltration	+2	<0·5	784
					+8	12·8	188
					+15	12·8	140
8	17½ (500 ml Collins + 100 mg isoprenalin solution)	3 hours	Haemorrhage	Necrosis and disruption of liver cell columns in lobular centres			
9	17	12 hours	Atelectasis	Necrosis of central parts of all liver lobules. Liver cells at lobular periphery well preserved			
10	16	Alive, 2 months			+1	2·7	3,440
					+2	1·1	1,400
					+4	7·6	
					+5	10·1	672
					+43	4·0	164
11	15½	Alive, 7 weeks			+1	<0·5	700
					+2	<0·5	264
					+40	<0·5	80
12	16½	Alive, 4 weeks			0	<0·5	348
					+1	<0·5	420
					+2	<0·5	328
					+14	2·6	48
13	16½	6 hours	Mechanical vena caval obstruction				
14	16½	Alive, 3 weeks			+1	1·8	736
					+3	<0·5	80
					+12	<0·5	136
15	17	Alive, 2 weeks			+2	<0·5	756
					+9	7·6	209

The livers were preserved for between 12 and 17½ hours and were then weighed and prepared ready for grafting. The constituents of aliquots of the perfusate and effluent sampled at varying times were compared. Estimations were made of sodium, potassium, calcium, magnesium, phosphate, chloride, glucose, free fatty acid, amino acid, pH, pCO_2, and pO_2. No immunosuppressive agents were given. A small specimen for biopsy was taken from the liver after revascularization just before closure of the abdominal wound. Blood was taken at varying intervals after operation. Estimations were performed of the serum bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, glucose, and protein electrophoresis.

Results

The 15 experiments are summarized in the Table. There was no significant weight change during preservation in any of the experiments. Attempts to extend the period of preservation by this method beyond 18 hours were unsuccessful. After 18 to 24 hours of preservation, although the livers appeared to be in good condition after revascularization and the animals recovered consciousness, they died 6 to 8 hours after operation with convulsions, acidosis, and hypoglycaemia despite intensive efforts to combat these changes.

Microscopical examination of the initial biopsy specimens in all experiments showed some necrotic liver cells. In the experiments where liver preservation was successful the degree of necrosis was slight. When the liver was preserved between 18 and 24 hours the liver cell damage was more severe, and it was felt that the initial biopsy did correlate reasonably well with the success of the preservation. The liver cell necrosis was always more prominent towards the centre of the lobules. The histological appearance at necropsy of preserved allografted livers from pigs that survived 37, 51, and 57 days showed complete liver cell regeneration but moderate fibrosis of the portal tracts and interlobular septa with varying degrees of mononuclear cell infiltration.

The pO_2 of the perfusate at room temperature varied between 100 and 160 whereas the pO_2 of the effluent was below 50. The other biochemical estimations performed on the perfusate and effluent showed no significant differences. There appeared, therefore, to be no gross shifts of electrolyte between the perfusate and the liver or evidence of utilization of constituents in the perfusate with the exception of oxygen consumption.

The biochemical studies of liver function after transplantation showed early raised aminotransferase levels which subsequently fell towards normal. The bilirubin and alkaline phosphatase levels were initially normal but later rose in some experiments. The blood glucose tended to fall in the first few hours after transplantation and intravenous glucose was always given. Subsequently blood sugar levels became normal. The protein pattern on electrophoresis showed slight lowering of the serum albumin and variable slight rises in the globulin fractions. The aspartate aminotransferase (SGOT) and serum bilirubin estimations are shown in the Table. Of the last six consecutive experiments in which the liver was preserved from between 15½ and 17 hours, five animals were alive at the time of writing.

Discussion

Because of the extreme sensitivity of the liver to ischaemic damage and the shortage of suitable donors in the United Kingdom, we have performed many transplants outside our own institution by transferring medical and nursing personnel together with the sick patient to the hospital where the donor has died. This has caused various logistic difficulties and requires extreme goodwill on the part of the hospital receiving us. The procedure has deterred many other hospitals from informing us of suitable donors, and the journey just before a major surgical operation has added to the risks for the patient.

Perfusion of the liver via the portal vein with innocuous cold fluid and storage in ice without further perfusion can keep the organ in excellent condition for 3 to 4 hours both experimentally and clinically (Schalm, 1968; Calne and Williams, 1968). Complicated continuous perfusion apparatus with hyperbaric oxygen

and refrigeration have been required for prolonged preservation. Brettschneider *et al.* (1968) were able to preserve dog livers consistently for eight hours. Over the last four years much effort has been expended in our laboratory to obtain consistent and prolonged preservation of the liver. Until recently the best result obtained in pigs was seven hours of preservation by using an initial cold flush and then storage in ice (Hadjiyannakis *et al.*, 1971). This was tested by orthotopic allografting. The technique described here has permitted the storage time to be increased to 17 hours, and in the last six consecutive experiments five animals survived the procedure with good liver function. We feel that the failures have been due to technical errors either in the surgery or in the perfusion.

In one preliminary experiment where the perfusion failed owing to a mechanical fault, although the liver was kept at 4°C throughout the preservation, its consistency was grossly abnormal at the time of transplantation. It was very soft, and disintegrated when handled gently. This observation together with previous experience with non-perfused livers and the consistent finding of oxygen utilization by the livers during preservation indicate that the perfusion plays an essential part in the increased preservation times. The pig's liver is very susceptible to temperature changes during preservation, and if the temperature is inadvertently allowed to rise above 5° damage occurs and the organ fails to support life.

Single passage perfusion of kidneys was reported by Hermann and Turcotte (1969). The advantages of this system are that no filters are necessary, constituents in the perfusate cannot be exhausted, waste products are removed, and the apparatus is simple. Turner and Alican (1970) found that canine liver preservation was better when the pH of the perfusate was 7 as opposed to 7·4. The perfusate that we used consisted of a basis of human plasma, the protein fraction being mainly albumin. The added potassium, phosphate, and magnesium alter the electrolyte content towards that of intracellular fluid. A pH of 6·8 is probably close to the pH within the cell. We feel that the intermittent squirting of the perfusate not only has advantages of parenchymal perfusion due to the pressure achieved with each bolus, but also in the interval between perfusion there is an opportunity for osmotic equilibrium to occur. It has been notable that significant weight change of the liver during preservation has not been observed. The disadvantages of this technique are that it is extravagant in the use of perfusate, some 4 litres being required for 17 hours' preservation, and essential cellular constituents could be washed out of the liver.

It is possible to modify the perfusion by analysis of the difference of the constituents in the perfusate compared with those in the venous effluent. Estimations so far of electrolytes, amino-acids, fatty acids, and dextrose show no significant change. There is, however, a consistent alteration of pO_2 indicating oxygen consumption by the preserved organ. No attempts were made to oxygenate the perfusate, which had a pO_2 of between 100 and 160 mm Hg while the pO_2 of the effluent was under 50 mm Hg. We are modifying the apparatus so that it can be transportable, and hope that application to man will allow us to transport livers from other institutions to our own hospital where clinical liver transplantation can be performed without having to move the sick patient.

We wish to thank the biochemistry department of the John Bonnett Clinical Laboratories for performing many of the estimations, Dr. David Bowyer for assays of free fatty acid, and Mrs. M. Sheldon and the department of medical illustration for preparing the Figure.

References

Belzer, F. O., Ashby, B. S., and Dunphy, J. E. (1967). *Lancet*, **2**, 536.
Brettschneider, L., *et al.* (1968). *Surgery, Gynecology and Obstetrics*, **126**, 265.
Calne, R. Y., and Williams, R. (1968). *British Medical Journal*, **4**, 535.
Calne, R. Y., Yoffa, D. E., White, H. J. O., and Maginn, R. R. (1968). *British Journal of Surgery*, **55**, 203.
Calne, R. Y., Dunn, D. C., Gajo-Reyero, R., Hadjiyannakis, E. J., and Robson, A. J. (1972). *Nature*, **235**, 171.
Collins, G. M., Bravo-Shugarman, M., and Terasaki, P. I. (1969). *Lancet*, **2**, 1219.
Hadjiyannakis, E. J., Calne, R. Y., Marshall, V. C., and Davis, D. R. (1971). *British Journal of Surgery*, **58**, 835.
Hermann, T. J., and Turcotte, J. G. (1969). *Archives of Surgery*, **98**, 121.
Schalm, S. W. (1968). Ph.D. Thesis, University of Leiden, Leiden, Holland.
Turner, M. D., and Alican, F. (1970). *Cryobiology*, **6**, 293.

Fingerprints in Patients with Coeliac Disease and their Relatives

M. MYLOTTE, B. EGAN-MITCHELL, P. F. FOTTRELL, B. McNICHOLL, C. F. McCARTHY

British Medical Journal, 1972, 4, 144–146

Summary

The incidence of fingerprint pattern abnormality in 53 patients with coeliac disease and 82 of their relatives was not greater than that found in 58 control subjects. This finding contradicts an earlier report that most patients with coeliac disease had abnormal fingerprints.

Regional Hospital, Galway, Eire
M. MYLOTTE, M.B., B.A.O., Research Fellow
B. EGAN-MITCHELL, M.B., D.C.H., Research Fellow
B. McNICHOLL, M.D., F.R.C.P., Paediatrician, and Professor of Paediatrics, University College, Galway
C. F. McCARTHY, M.D., F.R.C.P.I., Physician, and Associate Professor of Medicine, University College, Galway

University College, Galway, Eire
P. F. FOTTRELL, M.SC., PH.D., Associate Professor of Biochemistry

Introduction

The description by David and colleagues (1970) of abnormal fingerprint patterns in most patients with coeliac disease aroused considerable interest not only in medical circles but also in police departments (*Sunday Express*, 1971). Since then a report from Scotland (McCrae *et al.*, 1971) disagreed with the findings and showed that fingerprints are normal in coeliacs. Verbov *et al.* (1971) found no evidence of epidermal ridge atrophy in 37 patients with dermatitis herpetiformis. In an attempt to resolve this discrepancy, fingerprint examinations were carried out on coeliac patients and on their first-degree relatives who were being studied for other reasons (Mylotte *et al.*, 1972). Fingerprints taken from patients undergoing gastrointestinal investigation and hospital staff were used as controls.

Subjects and Methods

Altogether 53 patients with coeliac disease, 18 males and 35 females, were studied. All had subtotal villous atrophy of the