Conservation of the Liver

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DURING the past two years, a system of isolated ex-vivo liver preservation has been developed which combines the use of hypothermia, hyperbaric oxygenation, and low flow perfusion with fresh diluted blood.[1] The effectiveness was measured by the ability of the organs to sustain life both acutely and chronically when transplanted as orthotopic homografts in immunosuppressed canine recipients. Uniform success was obtained following storage for 8 to 9½ hours, and in 60% of the experiments for as long as 25 hours. The same technique has been successfuly employed in the preservation of 11 human livers for up to 7½ hours prior to orthotopic transplantation, but the need for fresh homologous blood in this system is inconvenient in the clinical setting. Consequently a study was undertaken to evaluate various acellular perfusates which could be made readily available, including an oncotically balanced salt solution, a hemoglobin solution, unaltered plasma, and the desilted plasma described by Belzer.[2]

METHODS

The basic experimental model was the same as previously described.[1] In summary, mongrel donor dogs were cooled to 32° to 34° C and sacrificed at the time of hepatectomy. The donor organ was cleared of blood, and further cooled by an intravascular infusion with a chilled (4° C) balanced salt solution. The organ was then placed in a refrigerated (4° C) hyperbaric oxygen chamber which was pressurized to 40 PSIG over 20 minutes. The portal vein and hepatic artery were continuously perfused with the appropriate solution at a flow rate of 4.8 and 1.2 milliliter/gram liver/hour, respectively, and stored for 8 to 25 hours. The chamber was then decompressed over 3 to 4 hours. Orthotopic transplantation was performed to nonrelated mongrel dogs who received an immunosuppression regimen consisting of heterologous antilymphocyte globulin (ALG), azathioprine, and prednisone. Only the perfusate was varied in the different experimental groups as indicated below.

Group 1—Fresh homologous blood was added to an equal volume of a buffered (pH 7.4-7.5) balanced salt solution containing 5.0 gm% low molecular weight dextran, 150 mgm% glucose, 2 milliequivalents per liter magnesium sulfate, 50 mgm per liter procaine and 100 mgm per liter heparin.

Group 2—The balanced salt solution containing low molecular weight dextran solution described in Group 1 was employed without the addition of blood.

Group 3—Fresh hemoglobin solution was prepared by Folkman's modification (3) of Pennell's technique (4). One part of the balanced and oncotically controlled buffered salt solution described above was added to 3 parts hemoglobin solution, resulting in a final hemoglobin concentration of 5 to 7 gm%. The pH and electrolyte composition was adjusted to that of normal whole blood.

Group 4—Pooled fresh heparinized homologous plasma was added to an equal volume of the diluting solution described in Group 1.

Group 5—Pooled fresh heparinized plasma was frozen for 12 hours, quickly thawed and passed through a series of millipore filters to remove the lipoprotein flocculate as described by Belzer.[2] This perfusate was not diluted.

RESULTS

Group 1—As previously reported,[1] 5 livers perfused for 8-9½ hours with diluted fresh homologous blood had an average weight loss of −4.2%, (range +4.6 to −12.5%), and inevitably functioned well immediately following transplantation.[1] All 5 recipients of these organs survived the operation and

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Table 1.—Experimental Groups

GROUP	PERFUSATE	NUMBER OF EXPERIMENTS	AVASCULAR PERIOD (Hours)	AVERAGE POST-PERFUSION WEIGHT GAIN (%)	OPERATIVE SURVIVAL (24 hours)	SURVIVAL GREATER THAN 5 DAYS
Controls	----------	12	½ - 1½	----------	12/12	11/12
1 A	Diluted Blood	5	8 - 9½	—4.2	5/ 5	5/ 5
1 B	Diluted Blood	5	24¾ - 25⅙	4.3	3/ 5	3/ 5
2	Asanguinous	5	21¼ - 24½	—1.9	1/ 5	1/ 5
3	Diluted hemoglobin solution	5	8 - 10½	—0.7	1/ 5	0/ 5
4	Diluted plasma	5	9½ - 10¾	—0.9	3/ 5	2/ 5
5	Desilted plasma	5	9½ - 11	—9.4	0/ 5	0/ 5

lived for more than 5 days (Table 1, Group 1A). Chronic survival did not significantly differ from a series of control experiments in which transplantation was carried out after core cooling without a period of interim preservation (Table 1, controls).

When the preservation period was extended to 24–25 hours, the 5 organs had an average weight gain of 4.3% (range +1.9 to 9.6%). Two of the 5 recipients died within 24 hours from an uncontrollable hemorrhagic diathesis. The remaining 3 animals lived for 8, 46 and 128 days (Table 1, Group 1B).

The pH of the perfusate remained within normal limits during the 8–9½ hour experiments and minimal addition of buffering agents was required in the 24 hour experiments (Table 2).

Group 2—Five homografts perfused with an asanguinous solution for between 21 to 24 hours had an average weight loss of −1.9% (range −9.1 to +9.4%). Moderately severe acidosis developed during storage with occasional intraperfusion reductions in the pH to as low as 7.10 in spite of active attempts at buffering (Table 2). Four of the 5 animals receiving these organs died of hepatic insufficiency and hemorrhage within 24 hours. The fifth lived for 9 days with persistently poor liver function.

Group 3—The 5 livers perfused with diluted hemoglobin solution for 8 to 10½ hours had an average weight loss of −0.7% (range +5.4 to −7.9%). During preservation, the perfusate invariably became alkalotic (Table 2). Of the 5 dogs receiving these organs, 4 died of hemorrhage and hepatic insufficiency on the day of operation. The remaining recipient demonstrated poor hepatic function and died of intussception on the 5th postoperative day (Table 1).

Group 4—Five livers perfused with diluted unaltered plasma had an average weight loss of −0.9% (range +12.2 to −6.6%) following preservation for 9½ to 10¾ hours. Acid-base relationships remained normal during perfusion (Table 2). Two recipients died within 24 hours of orthotopic transplantation, one from hemorrhage and the other with extensive ascites and atelectasis. Three animals survived operation, one dying after two days from hemorrhage and hepatic insufficiency. The remaining two recipients survived for 19 days with death due to homograft rejection in one case, and a perforated duodenal ulcer in the presence of good liver function in the other.

Group 5—Desilted plasma was used to perfuse 5 livers for 9¾ to 11 hours with an average post-preservation weight loss of −9.4% (range from −5.4 to −16.3%). Prior to preservation, the osmolarity of their perfusate was low but increased during perfusion at a rate much greater than seen in the other experiments (Table 2). In addition, the specially prepared plasma tended to be acidotic prior to perfusion with further

Table 2.—Analysis of Pre-Preservation and Post-Preservation Perfusates

	NA (meq/L)		K (meq/L)		Cl (meq/L)		Osmolarity (mOsm)		pH		Plasma (mgm Hemoglobin %)	
GROUPS	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
1 A	142±1.8	139± 2.2	4.8±1.2	11.2±3.1	133± 6.3	135± 3.1	321± 5.8	326± 2.5	7.46± .09	7.35± .01	1.8±2.1	2.3±0.92
1 B	146±1.8	146± 1.8	4.8±.42	9.4±5.7	130± 6.8	133± 3.6	319± 5.6	330±13.6	7.48± .03	*7.38± .03	1.8±1.0	10.0±9.8
2	145±1.9	150± 4.9	5.3±0.8	10.8±1.3	135± 1.3	138± 3.0	318± 8.5	339±11.7	7.41±0.4	*7.29±1.3		
3	134±6.9	139±14.8	4.5± .3	11.9±3.1	104±20.2	107±15.3	272±20.7	288±16.7	7.45±0.1	7.64±0.7		
4	149±2.4	147± 1.4	3.4±0.5	5.3±2.9	111± 4.1	122± 8.5	311± 6.7	335± 9.2	7.43±0.05	7.40±0.07		
5	138±1.6	138± 2.9	4.4±9.4	10.1±.65	69± 1.9	78± 2.9	263± 1.6	336± 5.3	7.31± .35	7.21±0.01		

* Average intrapreservation buffering per experiment: Group 1B: Tromethamine 144 mgm, $NaHCO_3$ 1.78 meq
Group 2 : Tromethamine 648 mgm, $NaCHO_3$ 7.12 meq

Mean values and standard deviation

reductions in pH developing during storage. All 5 recipients died within 24 hours following transplantation, of hemorrhage, ascites and/or acute hepatic insufficiency.

Discussion

Throughout the developmental period of the preservation technique used in these experiments, a systematic evaluation has shown that elimination of hyperbaria, rapid decompression of the hyperbaric chamber, omission of perfusion or reduction in the rate of blood flow yielded inferior results when the preserved organs were tested as orthotopic homografts.[1] In addition, the beneficial effect of hypothermia in reducing the ischemic injury to a hepatic graft prior to revascularization is well documented.[5,6]

When fresh homologous blood was used as the perfusing solution, well preserved livers were uniformly obtained following storage for 8 to 9½ hours and frequently for as long as 25 hours. However, the use of fresh blood has its disadvantages. The potential hazard of hemolysis and resulting tissue damage is always present and the necessity for compatible blood in the clinical application of this technique may preclude its use if a suitable blood donor is not readily available. Consequently, various acellular solutions were tested as the only variable in the storage system.

Perfusion with oncotically controlled buffered balanced salt solution, hemoglobin solution, unaltered plasma, and desilted plasma produced livers of poor quality which were generally unable to sustain life following transplantation (Table 1). The explanation for the inferior results obtained with acellular perfusates is not clear. Upon revascularization, homogeneous perfusion of the graft was invariably obtained and the development of outflow block was almost never encountered. Nevertheless, most of the recipients developed a hemorrhagic diathesis within 30 minutes following reestablishment of blood flow and/or exhibited severe hepatic insufficiency manifest by the development of massive ascites, "third-space" sequestration, and failure to recover from anesthesia.

A frequently proposed explanation for the graft injury caused by prolonged organ perfusion has been the development of progressively rising intravascular resistance and edema with consequent tissue damage.[2,7,8] This explanation does not appear to be applicable to our system, as we have shown that organ edema, at least as measured by weight gain, can be virtually eliminated with hyperbaric oxygenation and slow terminal decompression. In the experiments herein described, significant weight gain was rarely seen regardless of the perfusate used (Table 1).

The importance of maintaining a normal acid-base relationship during storage of isolated organs has been frequently emphasized.[7,9] Buckberg et al,[10] Eiseman et al[11] and others[12,13,14] have demonstrated the accumulation of acid metabolites in the effluent of livers subjected to minimal anoxia and/or isolated perfusion. The development of progressive acidosis during preservation with the asanguinous perfusate reflects the inefficient buffering capacity of non-blood containing solutions in a recirculating system and may explain the poor results obtained in Group 2.

Hemoglobin was added to the asanguinous perfusate (Group 3) in an effort to provide improved buffering capacity and better oxygenation during the initial cooling phase in the chamber. A high perfusate pO_2 was invariably obtained. Instead of developing acidosis as with the other acellular solutions, the perfusate now tended to become progressively alkalotic. The explanation for this finding is not clear, nor is it evident why there was uniform graft failure in this group of experiments.

The results obtained with unaltered homologous plasma (Group 4) were better than with the other acellular perfusates but

mediocre when compared to the blood perfusions. Therefore, a final group of experiments (Group 5) were performed using lipoprotein depleted plasma, shown by Belzer to improve the results in his plasma perfusion system for kidney preservation. This solution differed from the other perfusates in two important ways, the pH and osmolarity being significantly lower (Table 2).

During perfusion with Belzer's solution the pH tended to decrease further, resulting in a degree of acidosis significantly greater than that seen with the other perfusates. Although the final pH was lower, the degree of change during perfusion was the same, suggesting that the buffering capacity of the desilted plasma was not inferior to the other perfusate containing unaltered plasma. The osmolarity markedly increased during the interval of storage, a fact which is not surprising in view of the prior removal of lipoproteins which would predictably lower the oncotic pressure of the solution. It has been suggested that oncotic pressure is not necessarily important in renal preservation.[7] However, the large electrolyte shifts occuring with the desilted plasma may have a deleterious effect during liver storage, accounting in part for the almost invariable graft failure seen in this group.

In view of the poor results obtained with the acellular solutions tested in our preservation system, it would seem logical to continue using fresh homologous blood as the perfusate. Although occasional recipient survival was obtained with unaltered plasma, there was almost always evidence of moderate to marked hepatic insufficiency manifest by a bleeding diathesis following orthotopic transplantation. However, in the application of preservation to human liver transplantation, only a system providing repeated survival can be justified. As fresh homologous blood is the only perfusate providing a high degree of reliability in our system, its continued use would appear to be advisable.

Summary

A system of whole organ liver preservation has been developed and tested by means of transplanting the organs as orthotopic homografts. The separate components of hypothermia, hyperbaric oxygenation and continuous low flow blood perfusion have been shown to contribute to the efficiency of the method in which uniform success was obtained following storage for 8 to 9½ hours and often for as long as 25 hours. Various acellular perfusates were tested as the only variable in the storage technique. Perfusion with oncotically controlled buffered balanced salt solution, hemoglobin solution, unaltered plasma or lipiprotein depleted plasma yielded inferior results when compared with the original diluted blood perfusate.

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