Factors Determining Successful Liver Preservation for Transplantation

LUIS H. TOLEDO-PEREYRA, M.D., RICHARD L. SIMMONS, M.D., JOHN S. NAJARIAN, M.D.

Auxiliary liver allotransplants will survive for relatively long periods of time after 24 hour hypothermic (10–12C), pulsatile perfusion. The best perfusate was a silica gel fraction of dog plasma with added potassium chloride gel made hyperosmolar with glucose. Further improvement could be achieved with added allopurinol and methylprednisolone. Nonpulsatile flow or lower temperatures were less effective preservation techniques.

ALTHOUGH the *in vitro* storage of kidneys for transplantation is now routine, preservation of the liver by similar techniques has been limited to a few hours.[2,26] Belzer[2] has noted endothelial damage and sinusoidal disruption after 10 hours of hypothermic perfusion. Impairment of mitochondrial functions, specifically the oxidative phosphorylation, the respiratory control and the capacity to oxidize substrates are also commonly observed after short periods of either normothermic or hypothermic perfusion.[7,24] Attempts to maintain the viability of the organ by means of vascular perfusion and oxygenation have frequently failed.[2,4,26] The use of continuous pulsatile perfusion[27,36] plus hyperbaric oxygen have only partially improved the preservation of liver homografts.[5,32]

In previous experiments utilizing hypothermic plasma perfusion of the kidney and small intestine we have developed several techniques[37,41] which permit preservation for very long periods. These include modifications[37,39] and additives[38,42] to the plasma perfusate as well as modifications in pressure and flow.[38,39,41,42] The present study was designed to study these and other modifications in attempting to prolong the survival of dog livers *in vitro* prior to auxiliary allotransplantation.

Submitted for publication September 3, 1974.

Supported by Grant AM 13083 from the United States Public Health Service and grants from the Minnesota Medical Foundation and the University of Minnesota Graduate School.

Reprint requests: Dr. Toledo-Pereyra, Box 223, University of Minnesota Hospitals, Minneapolis, Minnesota 55455.

From the Department of Surgery, University of Minnesota Hospitals, Minneapolis, Minnesota.

Materials and Methods

Adult unrelated mongrel dogs of either sex underwent auxiliary liver transplantation according to a modified technique of Bengochea-Gonzalez and associates.[3] Recipient animals weighed 19-29 kg and donor dogs weighed 8–14 kg. All animals were anesthetized with sodium methohexital for induction and Fluothane for maintenance. Oxygen was used during ventilation. Ringer's lactate was given intravenously during surgery. Isoproterenol HCL 1:5000 (0.2 mg) and heparin (500 U/kg) were administered intravenously 20 minutes before the graft was removed from the donor.

The liver graft was removed from the donor dog with attached long segments of supra and infra-hepatic vena cava, portal vein and celiac axis. The donor's liver was flushed through the celiac axis and portal vein with cold (4C) Ringer's lactate solution containing heparin (10,000 U/L) and procaine (1 gm/L), until the venous effluent was clear. Usually 2 L of flushing solution was utilized for a complete washout.

Immediately after flushing, the liver was preserved by hypothermic perfusion for 24 hours (Figs. 1 and 2) except the livers of dogs from Group I which were transplanted immediately. The livers of dogs in Group II were perfused with cryoprecipitated plasma (CPP) prepared according to standard techniques.[1] The livers of dogs in group III were perfused with a silica gel fraction (SGF) of plasma recently described by our group.[37] SGF is basically a plasma fraction treated with silica gel resulting in removal of all cholesterol and fibrinogen and most of the lipoproteins.[37] Both CPP and SGF have a normal concentration of extracellular electrolytes (sodium 140 mEq/L, potassium 4.0 mEq/L), and osmolarity (290 mOsm/L) with total proteins of 4.0 gm% and

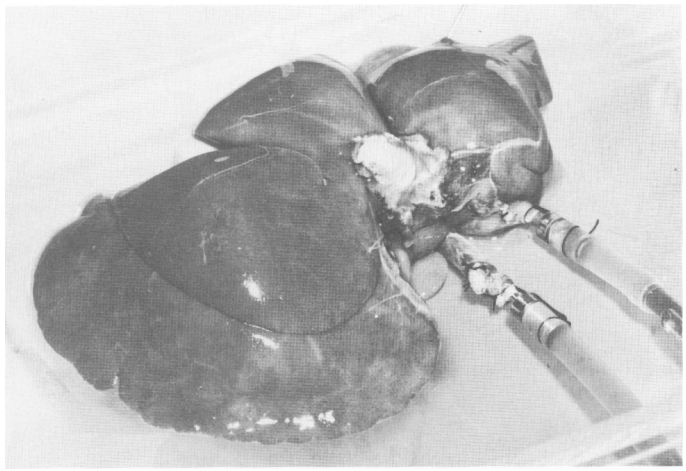

FIG. 1. Canine liver being perfused through the celiac axis and portal vein. The common bile duct is cannulated and the bile collected outside the perfusion cassette. Perfusate comes out the superior and inferior vena cava and recirculates again into the system.

albumin 2.9 gm%. The livers of dogs from Group IV to VII were perfused with SGF to which KCL, dextrose and salt poor human albumin (Hyland, Travenol Laboratories, Costa Mesa, Calif.) had been added (MSGF). The final concentration of these additives in the perfusate was K +100 mEq/L, glucose 1050 mg/L, total proteins 5.0 gm%, albumin 3.7 gm%, and osmolarity 560 mOsm/L. Additives to all perfusates included phenolsulfonphthalein (PSP) (2 ml/L), insulin (80 U/L), methylprednisolone (800 mg/L), and ampicillin (1 gm/L).

The perfusion system consisted of a Mox-300 machine (Waters Instruments, Inc., Rochester, Minn.) that was primed with 1800 ml of the perfusate maintained at 10–12C, pH 7.35 (10C), pO_2 800 mmHg. The livers were perfused through the celiac axis or hepatic artery with a systolic perfusion pressure of 60 mmHg, pulse rate 60/min, and through the portal vein by means of gravity flow (12 cm H_2O) (Figs. 1 and 2). The bile was continuously obtained from a catheter placed in the common bile duct. Perfusate flow and pressure were periodically determined until the end of preservation. During perfusion, samples were taken from the perfusate every two hours for the first six hours, then every six hours until the end of perfusion. Electrolytes, osmolarity, pH, pO_2, pCO_2, glucose, alkaline phosphatase, lactic acid dehydrogenase (LDH), glutamic oxaloacetic transaminase (GOT), β-glucuronidase, lactic acid, bilirubin and ammonium were determined in all samples (Bilirubin, LDH, and SGOT were determined according to the techniques of Bio-dynamics Inc., Indianapolis, Ind. Lactic acid was obtained from Sigma Chemical Co., St. Louis, Mo., and ammonia from Hyland, Travenol Laboratories, Costa Mesa, Calif.

Following 24 hours of perfusion, the liver was reflushed

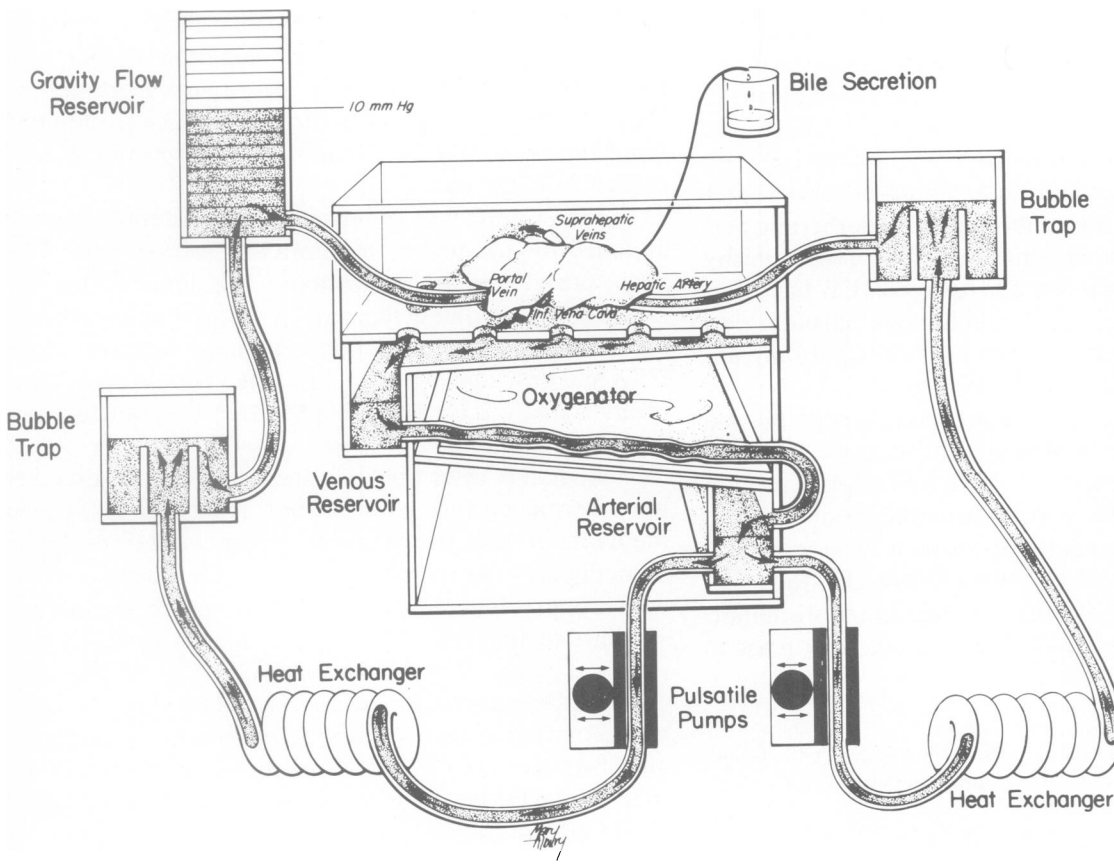

FIG. 2. Diagram of the liver perfusion system. The liver is perfused through the hepatic artery or celiac axis with pulsatile flow (60 mmHg) and through the portal vein with gravity flow (12 cm H_2O). The perfusate comes out both vena cava and circulates through the oxygenator (pO_2 800 mmHg), arterial reservoir, pulsatile pumps and heat exchanger. The flow that goes to the portal vein is derived in a gravity fashion and the arterial flow in a pulsatile modality. Bile is collected from the common bile duct.

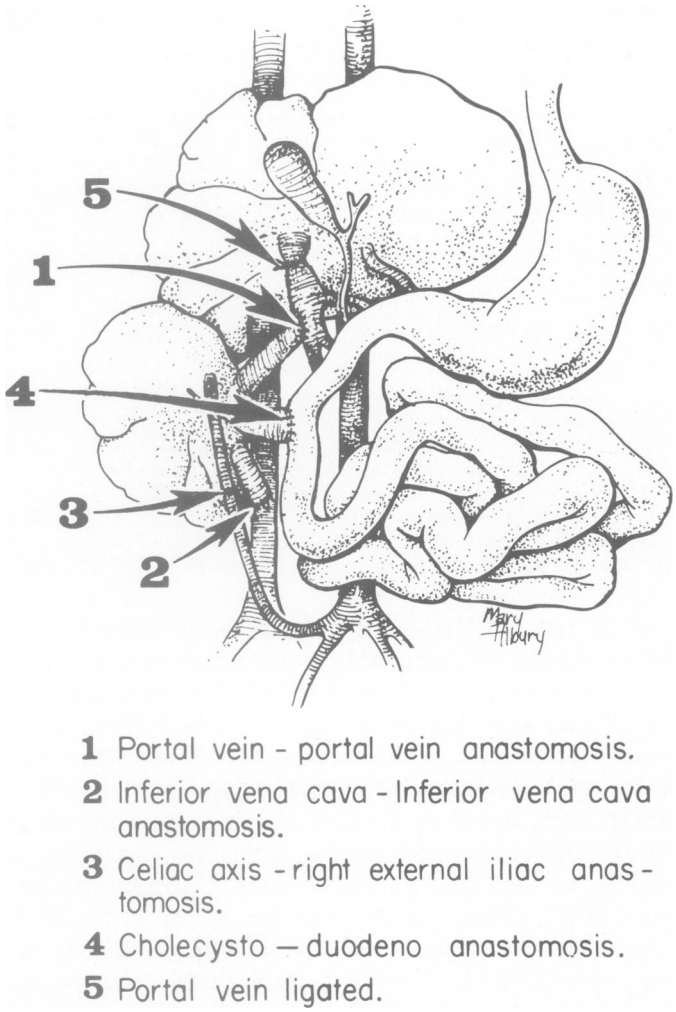

FIG. 3. Technique of auxiliary liver transplantation. The portal vein was ligated in the host's liver (5), and the splanchnic flow was directed to the transplanted liver through an end-to-end portal to portal anastomosis (1). The celiac axis and inferior vena cava anastomosis were performed end-to-end to the right external iliac artery and end-to-side to inferior vena cava respectively (2,3). A cholecysto-duodeno anastomosis was performed (4).

through the portal vein and the hepatic artery with a total of 1 L of cold (4C) Ringer's lactate to wash out the excessive K + before auxiliary transplantation into an unrelated recipient animal. The donor liver was continuously flushed with this solution while being transplanted. It was placed in the right side of the abdomen with its hilum towards the midline. Two end-to-side venous anastomoses were performed: portal vein to either superior mesenteric vein or portal vein of the recipient and inferior vena cava to infrarenal vena cava. One end-to-end arterial anastomosis was done, celiac axis of the donor to right common iliac of the recipient. The recipient's portal vein was then ligated to direct host splanchnic blood flow into the auxiliary liver. A side-to-side, one layer cholecystoduodeno anastomosis completed the operation (Fig. 3).

After transplantation the dogs were treated with intravenous Ringer's lactate to which dextrose 50% (50 ml/L), salt poor albumin 25% (50 ml/L), multivitamins (2 ml/L) and potassium chloride (20 mEq/L) were added. Chloramphenicol 500 mg intravenously twice a day and kanamycin 500 mg intramuscularly every 12 hours were given for three days after surgery. Oral feeding was started progressively at the 5th postoperative day. All dogs were treated with azathioprine 5 mg/kg/day for the first three days and then 2.5 mg/kg/day until death. Such an immunosuppressive regimen will not normally prolong the survival of nephrectomized kidney allograft recipients in dogs.[40] Blood samples were obtained from recipients daily for two weeks, and twice a week thereafter for: hemoglobin, hematocrit, leukocyte count, electrolytes, lactic acid, bilirubin, alkaline phosphatase, LDH, SGOT, and ammonium.

Weekly ^{99m}Tc-pertechnatate and ^{131}I rose bengal gamma scanning were obtained. Postmortem examination was performed in all animals. Biopsies from both livers were taken weekly and final microscopic study was performed at

TABLE 1. *Perfusion Characteristics During Liver Preservation for 24 Hours (Mean Values* ±SE)

Group	Perfusate	Flow Rate (ml/min/gm) I	Flow Rate (ml/min/gm) F	Weight Gain (%/24 hrs)	Fluid Loss (L/24 hrs)	Bile Production (ml/gm tissue/24 hr)
II	CPP	0.6±0.1	0.5±0.1[a]	21.8±4.5[b,c]	0.5±0.1[b,c]	0.2±0.06[c,e]
III	SGF	0.7±0.1	0.8±0.2	17.3±5.1	0.3±0.1	0.3±0.08[e]
IV	MSGF	0.8±0.1	0.9±0.2	10.1±3.5[c]	0.2±0.1[c,d]	0.4±0.1[e]
V	MSGF with Gravity flow	0.8±0.2	0.6±0.1[a]	20 ±8.5[b]	0.4±0.2[b]	0.04±0.01[b,e]
VI	MSGF with temperature at 7 C	0.7±0.2	0.6±0.1[a]	17 ±6.2	0.5±0.1[d]	0.2±0.05[d,e]
VII	MSGF with methylprednisolone and allopurinol	0.8±0.1	1.1±0.2[a]	8.6±3.5[b]	0.1±0.05[b,d]	0.5±0.1[b,d,e]

I = Initial
F = Final

[a]$p<0.05$ when final values of Groups II, V, VI were compared to final values of Group VII.
[b]$p<0.05$ when values of Groups II, V were compared to values of Group VII.
[c]$p<0.05$ when values of Group II were compared to values of Group IV.
[d]$p<0.05$ when values of Group VI were compared to values of Groups IV, VII.
[e]$p<0.05$ when values of Group V were compared to the values of Groups II, III, IV, VI, VII.

TABLE 2. *Perfusate Concentration During Liver Preservation for 24 Hours (Mean Values ±SE)*

Group	Perfusate	Lactic Dehydrogenase (U/100 ml) I	Lactic Dehydrogenase (U/100 ml) F	SGOT (U/100ml) I	SGOT (U/100ml) F	Glucose (mg%) I	Glucose (mg%) F	Osmolarity (mOsm/L) I	Osmolarity (mOsm/L) F
II	CPP	12±4.5[a,b]	95±12.3[c,d]	80±18[a]	413±96[d]	350±42[g]	295±27[h]	285±10[g]	292±8[h]
III	SGF	15±5.0[a]	61±9.6[c]	64±10[a]	396±85[f]	288±66[g]	235±61[h]	288±9[g]	295±12[h]
IV	MSGF	19±4.2	32±8.5[c]	75±33[e]	305±135	1050±45[g]	928±85[h]	580±26[g]	545±31[h]
V	MSGF with gravity flow	28±6.5[a,b]	315±42[c,d]	85±28[a]	298±86	935±72[g]	1038±86[h]	535±63[g]	515±31[h]
VI	MSGF with temperature at 7 C	15±4.3[a]	195±31.2[c,d]	68±14[a]	347±43[d]	975±83[g]	1076±72[h]	508±47[g]	480±23[h]
VII	MSGF with methylprednisolone and allopurinol	23±3.5[b]	47±14.9[d]	53±18[e]	164±39[d,f]	1012±46[g]	957±63[h]	547±3.5[g]	536±19[h]

I = Initial
F = Final

[a] $p<0.05$ when initial and final values were compared in Groups II, III, V, VI
[b] $p<0.05$ when initial values of Group II were compared to initial values of Groups V, VII
[c] $p<0.05$ when final values of Groups II, III, V, VI were compared to final values of Group IV
[d] $p<0.05$ when final values of Groups II, V, VI were compared to final values of Group VII
[e] $p<0.05$ when initial and final values were compared in Groups IV, VII
[f] $p<0.05$ when final values of Group III were compared to final values of Group VII
[g] $p<0.05$ when initial values of Groups II, III were compared to initial values of Groups IV, V, VI, VII

death. Statistical analysis with standard error and students' T test was determined for all parameters between groups of animals.

Seven groups of five dogs each (35 dogs) were studied. Group I dogs received fresh auxiliary liver transplants without preservation. Group II, the livers were perfused with CPP, group III, SGF as perfusate, group IV, SGF made hyperglycemic, hyperosmolar and hyperkalemic (MSGF), groups V to VII, MSGF was utilized as perfusate with the following changes in the system: group V, gravity flow at 12 cm H_2O was given simultaneously through both the portal vein and celiac axis, group VI, the temperature of the system was decreased from 10-12C, to 7°C, group VII, methylprednisolone 2 gm/L and allopurinol 500 mg/L were added to MSGF perfusate.

Results

Effect of Perfusate Composition, Temperature and Perfusion Technique on Fluid Loss, Flow Rate and the Chemical Composition of Perfusate During 24-hour Preservation of the Liver

Table 1 lists the perfusion characteristics at the start and at the end of the 24-hour period of liver preservation. Comparing the perfusate, the temperature, the pulsatile characteristics of the intra-arterial flow did not result in any significant difference in the flow rate during the start of perfusion. Flow rate remained reasonably stable. There was no significant decrease in flow rate during the entire period of perfusion. In fact, the only major change occurred when MSGF with added methylprednisolone and allopurinol were utilized as plasma perfusate. In this case, the flow rate increased during the 24-hour perfusion. During perfusion, there was a definite increase in the weight of the liver suggesting the formation of edema. In livers perfused with unmodified CPP or SGF or in livers perfused under gravity flow or at 7C, this ranged from 17% to 25% of the initial liver weight. However, when MSGF was utilized for perfusate with pulsatile arterial flow or MSGF with added methylprednisolone and allopurinol, the weight gain ranged only between 8% and 12%. Similarly, the perfusate fluid loss in the MSGF and MSGF with added methylprednisolone and allopurinol group was less than when the unmodified CPP and SGF was utilized or when gravity flow was utilized or MSGF at 7C.

Bile production when MSGF perfusate was utilized at gravity arterial flow was negligible. Bile production of 0.2 ml/gm of tissue occurred from livers perfused with unmodified CPP, SGF, MSGF, or MSGF at 7C. Increased bile production was noted when MSGF or MSGF with methylprednisolone and allopurinol were utilized as perfusates.

TABLE 3. *Effect of the Modification of Perfusion on the Survival of Dogs With Auxiliary Liver Preserved For Twenty-four Hours*

Groups	Perfusate	Survivors >5 days (#dogs)	Mean Survival (Days ± SE)
I	None	5/5	14.5 ± 3.5
II	CPP	1/5	2.9 ± 1.1
III	SGF	2/5	4.7 ± 1.2
IV	MSGF	4/5	11.7 ± 2.9
VII	MSGF with methylprednisolone and allopurinol	5/5	13.6 ± 4.1

Effect of Modifying Perfusate Composition and Perfusate Technique on the Chemical Composition of Perfusate During Liver Preservation for 24 Hours

Table 2 lists changes in perfusate composition which occurred during 24 hours perfusion. Unmodified CPP or SGF resulted in moderate increases in lactic dehydrogenase, SGOT, lactic acid and ammonium, whereas the

TABLE 2. *(Continued)*

Bilirubin (mg%)		Lactic Acid (mg%)		Potassium (mEq/L)		Ammonium (μg%)	
I	F	I	F	I	F	I	F
0.7±0.1	0.9±0.2	14±4.5[i]	35±8.2	4.0±0.2[g,k]	12.5±3.2[h]	285±42[a]	825±68[m]
1.0±0.1	0.9±0.05	18±2.8	31±6.7	3.9±0.3[g,k]	8.7±2.6[h]	195±55[a,l]	416±37[m]
0.6±0.2	0.4±0.1	16±4.5	34±8.5	110±6.5[g]	115±8.7[h]	315±45	425±80[m]
0.5±0.2	0.6±0.3	13±4.2[i]	54±22.9[j]	96±8.7[g]	107±10.5[h]	322±53[a]	875±41[m]
0.3±0.3	0.9±0.1	14±3.9[i]	37±12.2	87±6.2[g]	99±8.4[h]	387±46[a,l]	832±63[m]
0.5±0.1	0.7±0.2	14±4.3	26±10.7[j]	103±9.7[g]	105±11.3[h]	373±63[l]	575±98

[h] $p<0.05$ when final values of Groups II, III were compared to final values of Groups IV, V, VI, VII
[i] $p<0.05$ when initial and final values were compared in Groups II, V, VI
[j] $p<0.05$ when final values of Group V were compared to final values of Group VII
[k] $p<0.05$ when initial and final values were compared in Groups II, III
[l] $p<0.05$ when initial values of Group III were compared to initial values of Groups VI, VII
[m] $p<0.05$ when final values of Groups II, IV were compared to final values of Groups II, V, VI

osmolarity and bilirubin concentration remained stable. Modified SGF resulted in lesser increases in lactic dehydrogenase, SGOT, when compared with the unmodified perfusates. Modifying the SGF plus adding methylprednisolone and allopurinol resulted in lesser increases in SGOT, lactic acid but generally did not change the perfusate concentration when compared with MSGF without additives. Use of gravity flow rather than pulsatile arterial flow resulted in marked increases in lactic dehydrogenase and lactic acid production when compared with the other perfusion techniques. Similarly, perfusion at 7C rather than 12C resulted in greater increases in lactic dehydrogenase and lactic acid production and serum ammonium levels. There were no significant changes in glucose concentration, bilirubin concentration or perfusate osmolarity during the perfusion.

Effect of Perfusion Modification on the Survival of Dogs with Auxiliary Liver Perfused for 24 Hours

Dogs receiving transplants of fresh auxiliary livers (Group I) survived a mean of 14.5 days (Table 3). When the livers were transplanted after 24 hours of hypothermic pulsatile perfusion with CPP or SGF, there was a significant reduction in survival (Table 3). Dogs transplanted with livers perfused with modified SGF, however, had significantly greater survival and the best survivals of all comparable to the transplantation with fresh liver was obtained when 24 hours of preservation with MSGF to which methylprednisolone and allopurinol had been added was utilized. Table 4 demonstrates the effect of changing perfusate temperature and/or arterial flow on the survival of dogs with auxiliary liver transplants preserved for 24 hours. Here it is apparent that substituting gravity non-pulsatile arterial flow or lowering the temperature of the perfusate to 7C had adverse effects on the survival of the dogs when compared with the use of the MSGF itself. The best results were obtained in dogs transplanted with livers perfused for 24 hours with the MSGF to which large amounts of methylprednisolone and allopurinol were added (Group VII). All dogs survived more than five days with a mean survival of 13.5 days. The causes of death are noted in Table 5.

Dogs transplanted with auxiliary liver preserved with MSGF (Group IV) and those preserved with the similar perfusate to which methylprednisolone and allopurinol were added (Group VII) had, immediately after grafting, an initial peak in the LDH and SGOT levels. Two days later these enzymes were significantly decreased. The serum bilirubin and blood ammonium were usually in the upper

TABLE 4. *Effect of Gravity Flow and 7 C Temperature on the Survival of Dogs With Auxiliary Liver Preserved for Twenty-four Hours*

	Perfusion Variables				
Groups	Perfusate	Perfusate Temperature	Perfusion Characteristics	Survivors >5 days (# dogs)	Mean Survival (Days ± SE)
IV	MSGF	10-12 C	Pulsatile arterial flow (60 mmHg) Gravity portal flow (12 cm H_2O)	4/5	11.7±2.9
V	MSGF	10-12 C	Gravity arterial flow (12 cm H_2O) Gravity portal flow (12 cm H_2O)	3/5	7.2±2.3
VI	MSGF	7 C	Pulsatile arterial flow (60 mmHg) Gravity portal flow (12 cm H_2O)	3/5	8.1±3.4

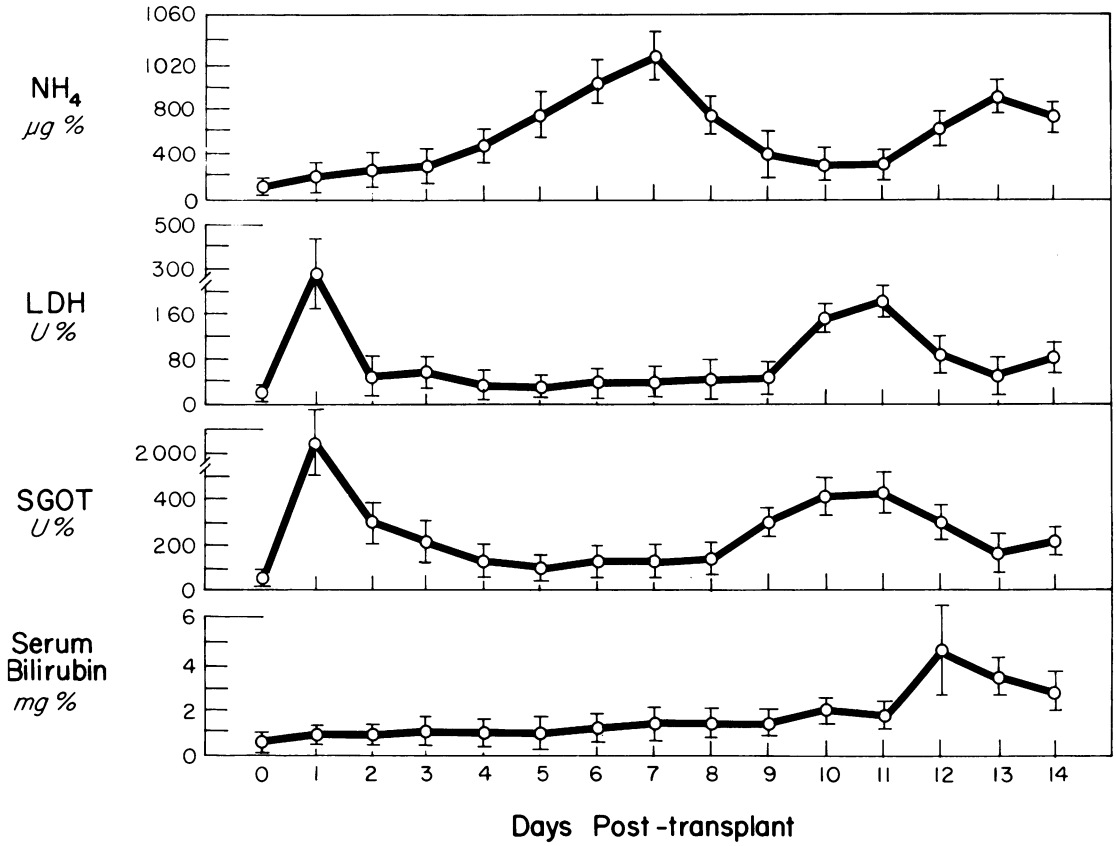

FIG. 4. Daily serum bilirubin, SGOT, LDH, and blood ammonium values (mean ± SE) in dogs transplanted with livers preserved for 24 hours with MSGF and MSGF plus allopurinol and methylprednisolone. Note an immediate rise in all values after perfusion and grafting with further decrease several days later. A second increase was observed nine days after transplantation. The blood ammonium followed a slightly different pattern.

limits in the first five days after grafting. Thereafter, the ammonium rose significantly for two days and then declined to previous levels. The SGOT and LDH began to climb eight to nine days after transplantation and decreased again two days thereafter. At the end of two weeks, all values were moderately increased (Fig. 4). Histologically there was hepatocyte preservation three weeks following transplantation with slightly central atrophy, mild bile stasis and congestion (Fig. 5).

There were no significant differences in the survival of the dogs transplanted with livers that were perfused with MSGF, pulsatile flow and 10–12C (Groups IV and VII) and the dogs transplanted with fresh liver which had no preservation (Group I). The enzymatic values (LDH, SGOT), serum bilirubin and blood ammonium posttransplantation were lower in the dogs transplanted with non-preserved livers; however, a similar pattern was observed during all post-grafting periods (Fig. 6).

Most of the dogs transplanted with livers preserved with

TABLE 5. *Cause of Recipient Death After Liver Preservation and Auxiliary Transplantation*

Cause of Death	Groups I	II	III	IV	V	VI	VII
Hepatic necrosis	1	1	0	0	1	0	0
Uncontrollable bleeding (capsular or hemorrhagic diathesis)	0	2	1	0	1	1	0
Generalized sepsis	1	1	1	0	0	1	0
Early portal vein thrombosis	0	1	1	1	1	0	0
Late portal vein thrombosis	1	0	0	1	0	1	1
Gastrointestinal bleeding	0	0	0	1	0	0	0
Small bowel necrosis	0	0	1	0	0	1	0
Celiac axis thrombosis	1	0	1	0	1	0	1
Rejection	1	0	0	1	1	0	2
Peritonitis	0	0	0	1	0	1	1

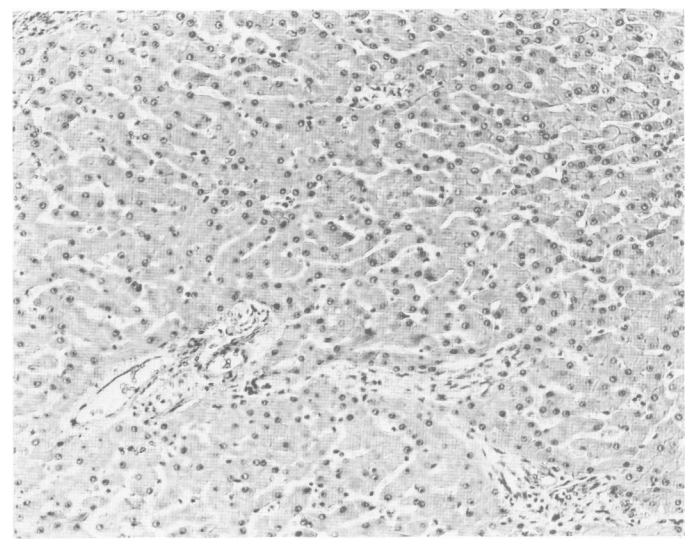

FIG. 5. Normal liver histology. Complete hepatocyte preservation after 24 hours perfusion and transplantation in one of the liver recipients of Group VII (MSGF + allopurinol and methylprednisolone) (Hematoxylin-eosin × 100).

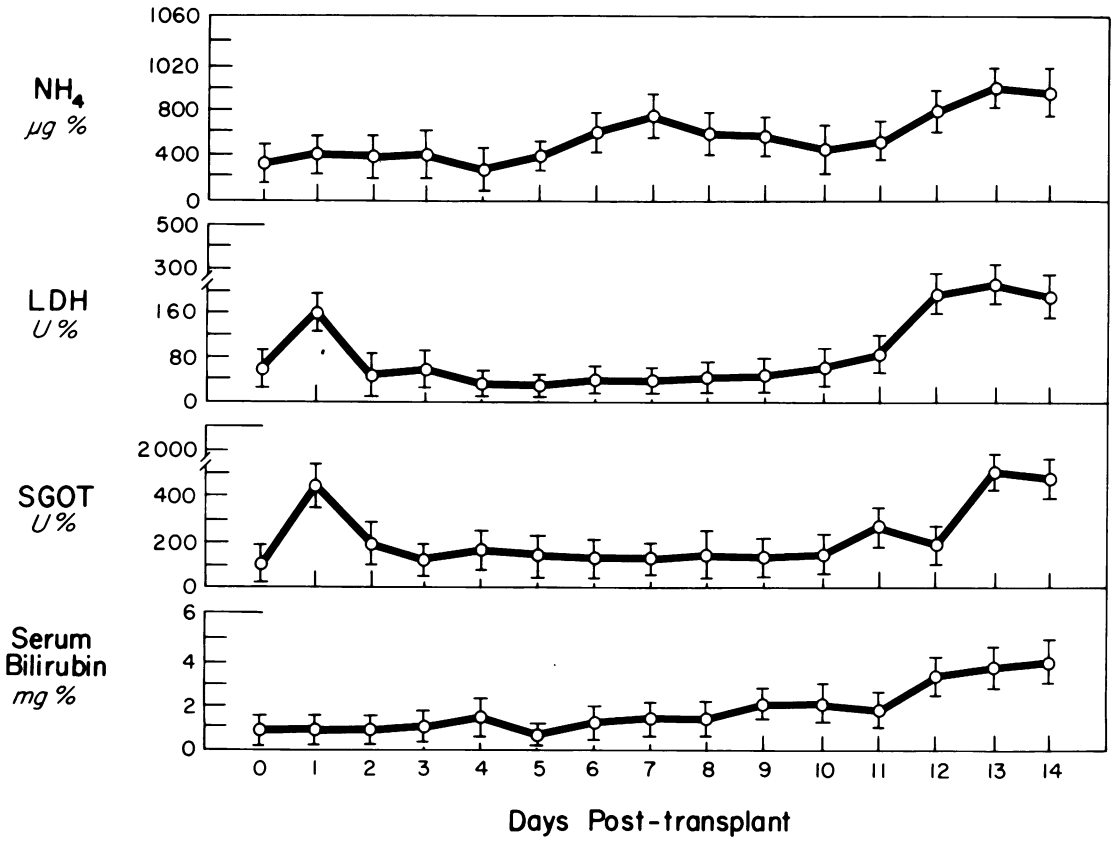

FIG. 6. Daily serum bilirubin, SGOT, LDH and blood ammonium values (mean ± SE) following transplantation of fresh livers which were not perfused prior to grafting. Note a moderate increase in all values after transplantation with further decrease two days later. A second rise was observed 11 days following transplantation. The blood ammonium followed a slightly different pattern.

CPP or SGF (Groups II, III) had a poor outcome after preservation and transplantation. These dogs had continuous rising levels of blood ammonium and serum bilirubin immediately after grafting. The LDH and SGOT ascended significantly in the first two days posttransplantation and then minimally declined to be maintained at very high levels until death (Fig. 7). Microscopically, there was massive congestion, hemorrhage, severe central atrophy and significant damage of the hepatocytes with capsular hemorrhage (Fig. 8).

Dogs transplanted with livers preserved at 7C or with gravity flow showed an initial elevation of serum LDH, SGOT, bilirubin and ammonium in the first three days after grafting. There was a decrease in the enzymatic values seven days posttransplantation with a subsequent rise three days thereafter. The serum bilirubin and blood ammonium rose again moderately before death.

Seven dogs were eliminated from the results for analysis because of technical failures which occurred during surgery and preservation. These included technical postoperative bleeding (three dogs, Groups II, III, V, one each), anesthesia complications (one dog, Group I), bronchoaspiration (one dog, Group VI), air embolii and severe acidosis during preservation (two dogs, Groups IV, VII). Four more dogs that died in the first three days after grafting were also eliminated from the study. These dogs died consecutively due to unrelated causes such as distemper (three dogs, Groups II, V, VI) and intussusception (one dog, Group III).

Discussion

Preservation of the liver offers far greater challenges than does preservation of the kidney. The present study represents a series of pilot experiments utilizing small groups of dogs to determine: 1) what flow characteristics during perfusion may be predictive of viability; 2) what chemical changes in the perfusate might be predictive of viability; 3) what modifications of perfusate composition might extend viability during perfusion; and 4) what alterations in perfusion characteristics may extend or limit viability of the perfused organ.

The basic techniques involved perfusion for 24 hours of livers which were then allotransplanted heterotopically into minimally immunosuppressed dogs with ligated portal veins. The survival of the dog was felt to depend not only on the function of the transplanted liver but also the provision, by means of the interposed transplant, for decompression of the portal circulation after portal vein ligation. If necrosis of the transplanted liver occurred due to failure to maintain viability during perfusion or rejection, the dog would die due to a combination of liver failure plus portal hypertension and intestinal congestion. It is well known that dogs do not usually survive acute portal vein ligation without portal-systemic division.[10,17,28,29] Our model of organ preservation is in no way affected by

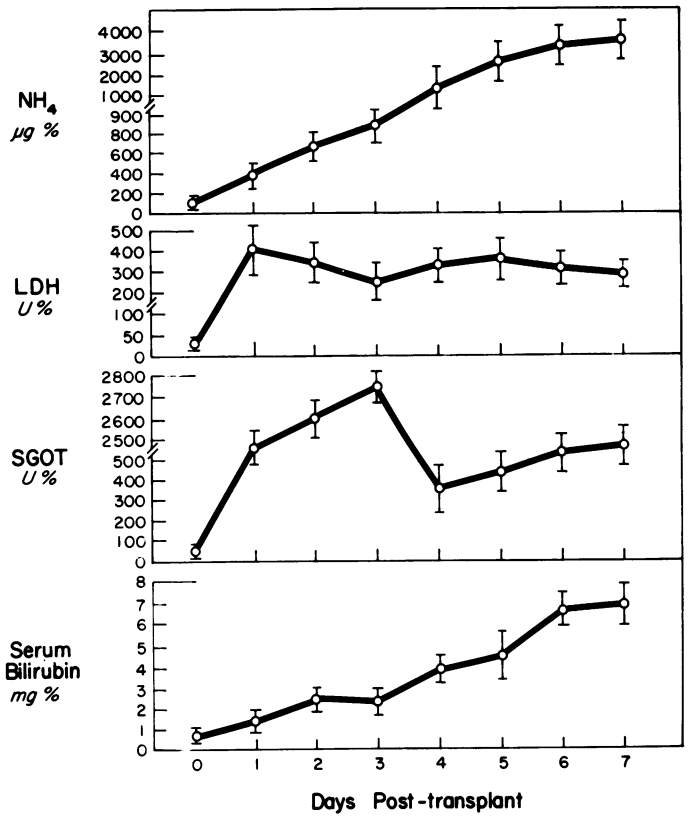

FIG. 7. Daily serum bilirubin, SGOT, LDH, and blood ammonium values (mean ± SE) in dogs transplanted with livers preserved for 24 hours with CPP and SGF. Note a continuous and significant rise in all values after preservation and transplantation. There was practically no liver function recovery after grafting.

the findings that heterotopic liver grafts atrophy with time,[14,21,34,35] or that dogs became ill after chronic portocaval diversion.[14,43,48]

Based solely on the survival of the recipient dog, it is apparent that the best perfusate consisted of MSGF, a hypertonic, hyperglycemic, hyperkalemic plasma fraction free of almost all lipoprotein, cholesterol, and fibrinogen. Only MSGF perfusion permitted recipient survival equal to the fresh transplant. Furthermore, early transplant function appeared to be as good after MSGF perfusion for 24 hours as after fresh transplantation.

MSGF was almost as good as that with methylprednisolone and allopurinol. Although one can assume that dog livers perfused with SGF or CPP are not viable enough; very few changes in the perfusate concentration during liver preservation for 24 hours hint that the MSGF or the MSGF with additives is in any way superior to CPP or SGF. Lactic dehydrogenase, SGOT, potassium and ammonium concentrations rose with all perfusates although the SGOT and lactic dehydrogenase increased less with the more successful perfusates. Measuring lactic acid, potassium and ammonium gave no assistance nor did glucose, bilirubin or osmolarity. Similarly, measuring the flow rate during the perfusion was not of great help in predicting liver viability although perfusion with MSGF with added methylprednisolone and allopurinol led to increased flow rate at 24 hours when compared with the initial flow rate. Weight gain was less with the successful MSGF perfusates and fluid loss during perfusion was minimal. Most striking was the almost double bile production when MSGF was utilized when compared with standard CPP or SGF perfusates. Thus, these findings suggest that successful perfusion with maintenance of viability of the perfused organ can be partially predicted in the failure of the lactic dehydrogenase and SGOT enzymes to increase during perfusion, failure of the flow rate to decrease and increase flow rate, a lack of weight gain, a lack of fluid loss, and marked increase in bile production. Other parameters appear less successful in predicting viability.

The mechanism by which SGF with high concentrations of potassium and glucose protected the perfused livers is not clear. It is possible that during preservation the hepatic cell membranes do not maintain stability and therefore the organ looses intracellular substances by passive diffusion. Potassium and glucose are the most important substances being extravasated during liver hypothermic preservation.[19] For this reason higher concentrations of potassium and glucose in the perfusate or extracellular space prevent a passive transport of these substances from the cells. Lie and associates[19] found that 600 mg/L of glucose and 50 mEq/L of potassium in the perfusate were the optimal concentrations for successful intermittent gravity perfusion of the porcine liver for periods up to 17 hours. The beneficial effects of SGF on perfused livers is probably related to the absence of cholesterol and fibrinogen and the relatively low values of lipoproteins in this perfusate.[37]

The addition of allopurinol to the perfusate improves *in vitro* function and posttransplant survival probably by pro-

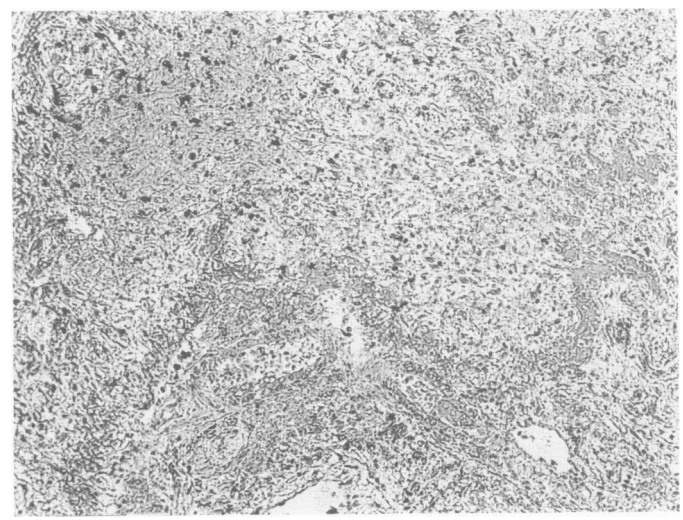

FIG. 8. There is massive congestion, hemorrhage, severe central atrophy and significant hepatocyte damage in one of the liver recipients transplanted after perfusion with CPP (Hematoxylin-eosin × 40).

ducing vasodilation and increasing the nucleotide pool with consequent conservation of energy.[38] Birbaum and colleagues[4] observed that the addition of high-energy electron bonds such as ATP, NAD, and FAD in the perfusate did not maintain the viability of transplanted dogs with liver perfused for 24 hours. Some of the signs of cellular damage during perfusion, such as potassium leak and low glucose and oxygen utilization, were reversed by the addition of ATP to the liver perfusion system.

The use of allopurinol combined with high doses of methylprednisolone (2.0 gm/L) determined a better outcome of the perfused and transplanted livers. The beneficial effect of methylprednisolone in the perfused organs is based on its action of stabilizing membrane lysosomes and improving tissue perfusion. However, regular doses of methylprednisolone (800 mg/L) routinely used in kidney preservation did not demonstrate any beneficial effect on livers perfused for 24 hours.

Belzer and his group[2] found the endothelial integrity of the sinusoids consistently damaged after 10 hours of pulsatile perfusion with cryoprecipitated plasma.[2] All porcine livers perfused for 24 hours died immediately after transplantation. Brettschneider and associates[5] perfused successfully canine livers under hypothermic perfusion and hyperbaria from eight to 25 hours. The animals transplanted with poor perfused livers died within three days after grafting with severe ischemic injury and clotting abnormalities. These problems have frequently been reported by other investigators.[8,9,11–13,15,16,20,22,30,32,33] Calne and his group,[6] with a single passage of intermittent squint perfusion through the pig's liver, were able to obtain long-term survivors after liver preservation for 12-17 hours.

Elmslie and associates[11] demonstrated that perfusion of the pig liver at 13-15C for several hours did not cause any deterioration in the ability of the rewarmed liver to excrete bromosulphalein. On the contrary, liver temperatures of 13-22C appeared to enhance the ability to excrete bromosulphalein. Castillo-Olivares and colleagues[7] found a protection of the mitochondrial respiration and the oxidative phosphorylation during hepatic preservation at 4C for three hours. Similar studies at 22C resulted in a severe impairment of the liver mitochondrial functions one hour after the beginning of preservation. The mechanism by which cold preservation protects mitochondrial respiration is based in the easy denaturation of mitochondrial enzymes due to temperature.[7] However, 4C cooling did not prevent the mitochondria from becoming uncoupled after three hours of liver preservation. It is probably that after this time cold anoxia is the main factor in determining mitochondrial deterioration. In our system, although the best results were observed in the dogs transplanted with livers perfused at 10-12C for 24 hours, there were not significant differences (p 0.08) in survival of dogs with transplanted livers that were perfused at 7C. Equally good results were obtained in livers perfused under pulsatile perfusion for 24 hours. Dogs transplanted with livers perfused with only gravity flow for the same period of time has a lower survival time with similar flow rates.

Marchioro and associates[21] reported on the competition for nutritional substrates that occurs in the presence of two livers. They concluded that the allografted organ must receive its portal supply from the intestinal venous return. We observed similar results on livers perfused for 24 hours and then transplanted with splanchnic flow diverted to the auxiliary graft. However, van der Heyde and colleagues[9,46] have shown that liver grafts deprived of portal inflow do not necessarily atrophy if the host liver is subjected to a physiological disadvantage. These results have been confirmed by Uchida and colleagues[45] and Bengochea-Gonzalez and associates.[3] Wexler and his group[48] recently performed 70% hepatectomy and bile duct ligation of dogs that had auxiliary liver transplantation without portal vein anastomosis. Their results indicated that atrophy of the grafted liver was consistently present only if the renal vein to portal vein anastomosis was thrombosed; otherwise, the grafted organs maintained similar pre-operative weights.

Twenty-four hours of liver preservation caused severe hepatic damage in the absence of a modified silica gel fraction which had high levels of potassium and glucose during perfusion. The addition of allopurinol and high doses of methylprednisolone to the perfusate allowed a complete protection of the perfused livers and all the transplanted dogs survived for long periods of time. The use of nonpulsatile flow or temperature of 7C during perfusion did not efficiently protect the liver perfused for 24 hours. Only pulsatile flow through the celiac axis and gravity flow through the portal vein with a temperature of 10-12C determined long-term survivors of all recipient dogs.

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