Forty-Eight-Hour Preservation of the Canine Liver

DAVID T. W. SUNG, M.D., JOHN E. WOODS, M.D.

From the Mayo Clinic and Mayo Foundation, Rochester, Minnesota

SUCCESS IN RENAL PRESERVATION has been extended to periods of as long as one week, with immediate life-sustaining function.[13] Success in liver preservation has been far less consistent.[1-3,5,7,11] A previous report from this laboratory has detailed experience in canine orthotopic liver transplantation after 24 hours of preservation.[9] Twelve dogs survived 5 to 70 days after transplantation. Encouraged by this success, an attempt was made to extend the period of preservation to 48 hours. This report presents the results of that experience.

Material and Methods

Thirty-eight female mongrel dogs weighing between 14 and 25 kg were used as liver donors and recipients in 19 preservation-orthotopic transplant experiments.

Sodium pentobarbital, 5 mg/kg of weight, was given intravenously for anesthesia in the earlier experiments. Later, for better control of anesthesia, a combination of sodium thiopental, halothane, nitrous oxide, and oxygen was employed in the recipient dogs. Endotracheal intubation and controlled respirations were used at all stages. The preservation system and method employed have been described previously,[9] but basically it is a modification of the renal preservation system employed in this laboratory.[14] This system provides pulsatile, hypothermic perfusion with membrane oxygenation and continuous monitoring and titration of pH to physiologic levels. For hepatic preservation, perfusion circuitry was designed so that the hepatic artery received pulsatile flow (at 80 mm Hg) and the portal vein received continuous flow (at 10 ± 2 cm H_2O). The temperature of the perfusate was maintained between 5° and 7°C over the 48-hour period of perfusion. Orthotopic liver transplantation was carried out in all 19 experiments by a technique that was previously described for the first 8 dogs.[4] In the remaining experiments, the following modifications were made:

1. Two external shunts diverting the portal and inferior vena cava blood into the right and left jugular veins separately were employed during the anhepatic period, allowing better flow than the previously used single jugular shunts.

2. The superior mesenteric artery was occluded temporarily during the period of portal vein clamping to prevent intestinal congestion.

3. Anastomosis of the donor's intrathoracic inferior vena cava and the recipient's intrahepatic inferior vena cava was adopted. The donor's suprahepatic inferior vena cava was freed from the diaphragm and was severed at a point about 2.5 cm above the diaphragm. In the recipient hepatectomy, by the use of finger dissection, the anterior branch and the main left branch of the hepatic vein were isolated and ligated. The right hepatic vein, which is actually the intrahepatic inferior vena cava, was isolated from the attached liver parenchyma and transected about 2.5 cm below the diaphragm and fashioned for anastomosis, thus giving a longer cuff (Figs. 1, 2, and 3).

Two-day-old acid-citrate-dextrose blood from the liver donor was used for transfusion in the first four dogs. Either fresh blood or fresh frozen plasma was transfused in all other dogs to provide coagulation factors and to restore blood volume. Other preoperative and postoperative management has been detailed previously.[9]

Results

Of the 19 dogs that underwent orthotopic liver transplantation after 48 hours of liver preservation, 6 were

Submitted for publication May 31, 1973.

Reprint requests: Section of Publications, Mayo Clinic, 200 First St. SW, Rochester, Minnesota 55901.

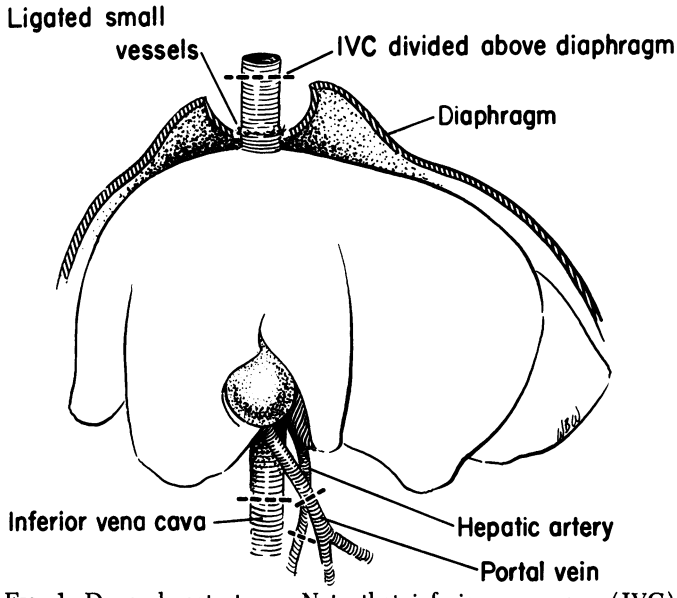

FIG. 1. Donor hepatectomy. Note that inferior vena cava (IVC) is completely isolated from diaphragm and is divided above it.

alive more than one day (Table 1). Five of the six (dogs 5, 9, 10, 11, and 15) lived from 3 to 8 days. The other 13 dogs died within 24 hours after operation.

Dogs that survived more than 3 days usually woke up within a few hours after operation and could stand up and walk in the evening, with various degrees of difficulty. They usually started to drink the next morning and had a reasonably good appetite on the second postoperative day. They remained alert and active until a day or two before death. Once they became "sick," deterioration was rapid. No attempt was made to treat them. They were sacrificed when death appeared imminent or they died during the night.

Of the last 11 dogs in whom the modified surgical technique was used and fresh blood or fresh frozen

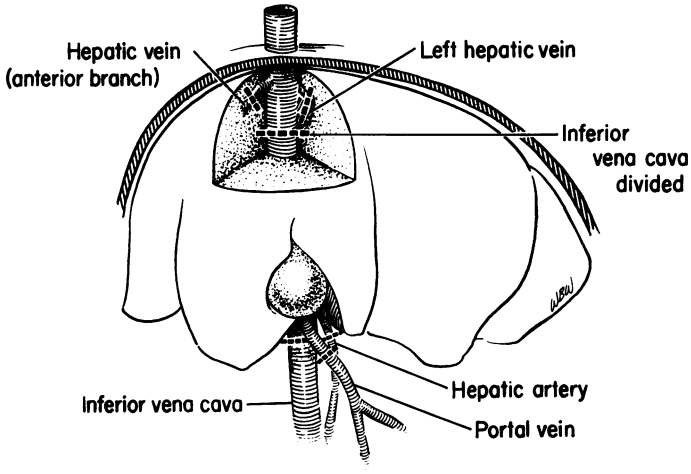

FIG. 2. Recipient hepatectomy. Note that branches of hepatic veins are dissected out within the parenchyma and are ligated and divided.

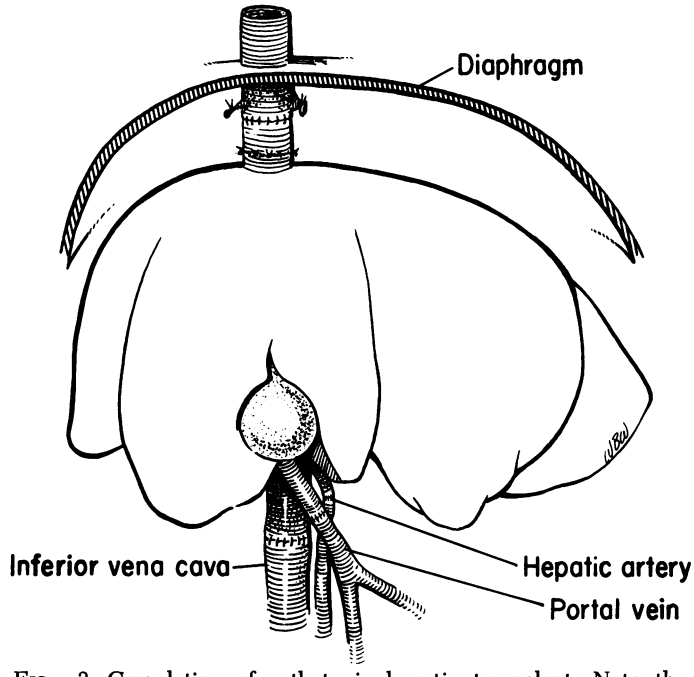

FIG. 3. Completion of orthotopic hepatic transplant. Note the length of suprahepatic segment of inferior vena cava.

plasma (or both) was administered, 4 dogs survived from 3 to 8 days (Table 2). Four of the dogs died from other immediate causes before the quality of the preserved allograft could be tested in vivo, although poor liver function may have contributed to the death. One of the dogs died from an apparent poor preservation due

TABLE 1. *Survival Time and Causes of Death of 19 Dogs Receiving Orthotopic Liver Transplants After 48 Hours of Liver Preservation*

Dog	Survival Time	Cause of Death
1	6 h	Uncontrollable oozing
2	0	Bypass failure
3	9 h	Uncontrollable oozing
4	7 h	Uncontrollable oozing
5	7+ days	Rejection
6	4 h	Didn't wake up, (?) poor liver function
7	4 h	Uncontrollable oozing
8*	7 h	Uncontrollable oozing
9	4+ days	Diffuse portal vein thrombosis, septicemia
10	3+ days	Undetermined
11	8+ days	Rejection
12	1+ days	Undetermined, (?) poor liver function
13	3 h	Pulmonary edema
14	10 h	Shock during bypass
15	6 1/2 days	Rejection
16	18 h	Damaged liver due to accident in preservation
17	8 h	Poor liver function
18	3 h	Inadequate bypass due to small jugular veins
19	5 h	Severe hypercapnia and acidosis during operation

* Pregnant dog.

TABLE 2. *Bleeding Diathesis as a Cause of Death According to Management of 19 Dogs Receiving Orthotopic Liver Transplants After 48 Hours of Liver Preservation*

Management	No. of Dogs	Uncontrollable Oozing	
		Yes	No
Old technique			
Without FB/FFP*	4	3	1
With FFP	4	2	2
Modified technique			
With FB/FFP	11	2	9
Total	19	7	12

* FB, fresh blood; FFP, fresh frozen plasma.

to the temporary occlusion of flow from the bile catheter. The remaining two dogs died from presumably poor liver function due to poor preservation.

The perfusion characteristics during 48-hour liver preservation were similar to those seen during 24-hour preservation in this laboratory[9] (Tables 3 and 4). The hepatic artery perfusion pressure, set initially at 80 mm Hg and allowed to seek its own level thereafter, generally decreased shortly after the initiation of perfusion. The pressure usually remained at its lowest level until the end of preservation. The average perfusate flow was 275 ml/min per liver at the beginning of preservation and 279 ml/min per liver at the end of preservation, with a range of 167 to 429 ml/min. There was no difference between surviving or nonsurviving livers in either perfusion pressure or perfusate flow.

Bile production in 48 hours ranged from 35 to 245 ml, and there was no relationship between surviving and bile production. Results of coagulation and liver function

TABLE 3. *Hepatic Artery Perfusion Pressure of Canine Livers Preserved for 48 Hours*

Dog	Perfusion Pressure, mm Hg			
	Initial	Highest	Lowest	Final
1	80/50	80/50	74/32	76/32
2	80/22	80/22	66/42	66/42
3	80/50	84/48	80/46	84/48
4	80/40	80/40	54/10	56/12
5*	80/42	80/42	64/24	64/24
6	80/36	80/36	70/22	74/24
7	88/44	88/44	64/36	68/34
8	80/40	80/40	46/28	64/36
9*	80/50	80/50	70/34	70/34
10*	80/42	80/42	64/28	72/22
11*	80/36	80/36	72/26	80/26
12	80/48	80/48	72/28	76/28
13	80/55	80/55	58/44	68/18
14	80/—	80/—	62/32	68/26
15*	80/50	80/50	60/28	66/24
16	80/50	80/50	65/33	65/33
17	80/50	80/50	65/30	65/30
18	80/48	80/48	64/30	67/31
19	80/40	80/40	55/17	55/17
Mean	80.4/44	80.6/44	64.5/30	68.6/28.5

* Dogs lived more than 3 days.

TABLE 4. *Perfusate Flow in Canine Livers Preserved for 48 Hours*

Dog	Flow, ml/min per Organ			
	Initial	Lowest	Highest	Final
1	250	250	300	300
2	273	273	273	273
3	429	200	429	200
4	273	273	300	300
5*	231	231	273	273
6	231	231	250	250
7	167	167	231	200
8	167	167	333	333
9*	273	273	273	273
10*	231	231	250	250
11*	333	300	333	300
12	200	200	250	250
13	375	271	375	271
14	271	231	300	231
15*	333	316	333	316
16	333	316	333	316
17	250	250	333	333
18	300	286	300	300
19	300	300	333	333
Mean	275	251	305	279

* Dogs lived more than 3 days.

studies in the five dogs that survived more than 3 days are shown in Tables 5 and 6.

The livers in these experiments did not differ significantly in gross appearance after orthotopic implantation from those previously preserved for 24 hours.[9] The chief difference in the dogs receiving livers after 48-hour preservation was that bleeding diathesis was a more serious and frequent problem.

The dogs that received fresh blood or fresh frozen plasma (or both) during operation had either no change or only a slight decrease in platelet counts and fibrinogen levels immediately after surgery; this pertained both to survivors and to nonsurvivors. The platelet counts were reduced by 30% and the fibrinogen levels by 65% in dogs that did not receive fresh blood or fresh frozen plasma. Both groups had between a twofold and a threefold increase in fibrin split products. In surviving dogs, the platelet counts showed no change immediately after operation but gradually decreased to between one-third and one-tenth of the control level in most dogs on the day of death or sacrifice. The fibrinogen levels varied. After an initial increase in fibrin split products, there was no significant change up to the time of sacrifice or death.

All dogs had a marked increase in level of serum glutamic oxalacetic transaminase (SGOT) and to a lesser extent in alkaline phosphatase immediately after surgery. The change in bilirubin level was minimal. Dogs that survived more than 3 days had a decrease in SGOT level on the second postoperative day, and this level remained low, with a subsequent increase shortly before death. The alkaline phosphatase concentration was higher on

TABLE 5. *Liver Function Studies in Dogs Surviving More Than 3 Days With Livers Preserved for 48 Hours*

	Serum Bilirugin, mg/100 ml			SGOT, U/liter			Alkaline Phosphatase, U/liter		
Survival, days	Control	Postop.	Final	Control	Postop.	Final	Control	Postop.	Final
7	0.1	0.8	2.4	25	1,114	324	25	32	2,448
4	0.2	0.3	2.1	18	848	16,200	14	32	892
3+	0.1	0.6	0.4	28	1,664	344	38	38	69
8	0.1	0.3	2.1	9	800	1,157	25	45	2,108
6 1/2	0.2	0.5	0.4	28	580	1,896	35	46	2,048

the day after surgery and remained at that level until shortly before death, when a steep rise was noted. Serum bilirubin concentrations varied, but they did not increase until a late stage.

The pathologic findings in three dogs[10] that lived 6 to 8 days were consistent with acute rejection. In the portal tracts and around the central veins, there was heavy cellular infiltration composed of mononuclear cells, immunoblasts, lymphocytes, and plasma cells. Central zonal necrosis, with evidence of regeneration, also was seen. No abnormalities were found in the hepatic arteries. Mild cholestasis was present.

Discussion

Bleeding diathesis during surgery and in the early postoperative period is the main obstacle to the success of orthotopic liver transplantation.[6,8,12] This problem is greater with the preserved liver, both for the 24- and the 48-hour liver preservation.

Subsequent to the initiation of fresh blood or fresh frozen plasma transfusions to the recipient during the transplant procedure, the incidence of uncontrollable intraoperative oozing decreased markedly. A comparison of the fibrinogen levels gives some indication as to the reason for this decrease. In dogs not receiving fresh blood or fresh frozen plasma, fibrinogen values decreased to one-third of the control levels at the end of the operation. This decrease was not observed in dogs given fresh blood products, even in the dogs that died within 24 hours. The theoretic value of fresh blood or plasma during hepatic recovery from operative and preservation injury is obvious; not only because of the decreased or absent ability of the liver to produce coagulation factors but also because the partially damaged liver may serve as a site for their destruction. Ideally, fresh blood or fresh frozen plasma (or both) should be administered during the postoperative period, until the allograft has recovered sufficiently to produce reasonable levels of endogenous coagulation factors.

Bleeding problems were further ameliorated after modification of the technique in the anastomosis of the suprahepatic inferior vena cava. In our experience, the suture lines of this particular anastomosis were the major source of continuous loss of blood in the earlier dogs in this series. Using the donor's *intrathoracic* inferior vena cava and the recipient's *intrahepatic* inferior vena cava provided a longer cuff, a softer and more elastic tissue, and a smaller vessel for anastomosis. The opening of the suprahepatic inferior vena cava below the diaphragm is unnecessarily large. Thus, the anastomosis was made technically easier and faster, and it provided a better seal between the vessels. In addition, it allowed exposure and manipulation of the anastomotic region and undersurface of the diaphragm for the checking of bleeding points after revascularization. This maneuver is impossible when two short segments of inferior vena cava are joined together between the diaphragm and the liver.

The level of SGOT during the early postoperative period seemed to be a reliable indicator of a successful transplant. A subsequent increase was noted at the time of impending loss or rejection. The level of alkaline phosphatase, as in previous experience, was consistently elevated as rejection progressed.[9] Also seen in two of the three dogs in which platelets were counted just prior to death was a significant decrease in the count, most probably secondary to the participation of platelets in the rejection reaction.

Our results represent limited success in preservation

TABLE 6. *Coagulation Studies in Dogs Surviving More Than 3 Days With Livers Preserved for 48 Hours*

	Platelets, $\times 10^3/mm^3$			Fibrinogen, mg/100 ml			Fibrin Split Products, mg/100 ml		
Survival, days	Control	Postop.	Final	Control	Postop.	Final	Control	Postop.	Final
7	82	103	310	239	239	239	384	384	192
4	200	275	72	159	159	364	96	384	384
3+	445	270	165	250	91	431	192	768	768
8	272	135	26	192	261	261	48	384	768
6 1/2	234	260	46	341	341	23	192	768	768

in that the longest survival was only 8 days, that is, until the time of rejection. In the future, immunosuppression will be implemented in order to increase survival. However, after 48 hours of preservation, orthotopically reimplanted preserved livers were able to maintain life for periods of more than 1 week. Postulated factors in the success achieved might be 1) a relatively high perfusion flow rate at 6°C, 2) high concentration of methylprednisolone in the perfusate, 3) use of methylprednisolone in the donor as a membrane stabilizer, with its parallel action of improving tissue perfusion,[13] 4) gentle handling of the tissue and meticulous hemostasis, 5) judicious use of fresh blood or fresh frozen plasma (or both) to provide the needed coagulation factors at the critical time, and 6) anastomosis of the donor *intrathoracic* inferior vena cava to the recipient intrahepatic inferior vena cava for better control of postoperative bleeding.

Summary

Nineteen experiments with 48-hour preservation of the canine liver were conducted. Five of the 19 dogs survived more than 3 days after orthotopic transplantation of the preserved liver, 3 of them until documented rejection. Modifications in technique and the use of fresh frozen plasma and fresh whole blood appeared to be of value; additional factors possibly contributed to this rather modest success.

Acknowledgment

Appreciation is expressed to N. C. J. Sun., M.D. who reviewed the histopathologic specimens.

References

1. Abouna, G. M., Koo, C. G., Howanitz, L. F., Ancarani, E. and Porter, K. A.: Successful Orthotopic Liver Transplantation After Preservation by Simple Hypothermia. Transplant Proc., 3:650, 1971.
2. Belzer, F. O., May, R., Berry, M. N. and Lee, J. C.: Short Term Preservation of Porcine Livers. J. Surg. Res., **10**:55, 1970.
3. Brettschneider, L., Daloze, P. M., Huguet, C., Porter, K. A., Groth, C. G., Kashiwagi, N., Hutchison, D. E. and Starzl, T. E.: The Use of Combined Preservation Techniques for Extended Storage of Orthotopic Liver Homografts. Surg. Gynecol. Obstet., **126**:263, 1968.
4. Cooperman, A. M., Woods, J. E. and McIlrath, D. C.: Simplified Method of Canine Orthotopic Hepatic Transplantation. Am. J. Surg., **122**:797, 1971.
5. Hinchliffe, A., Immelman, E. J., Bowes, J. B., Hunt, A. C., White, H. J. O., Johnson, M. G., Golby, M., Peacock, J. H. and Riddell, A. G.: A Transportable Apparatus for Liver Preservation. Eur. Surg. Res., **2**:427, 1970.
6. Howland, W. S., Ryan, G. M., Bettigole, R. E. and Fortner, J. G.: Coagulation Abnormalities Associated With Liver Transplantation. Surgery, **68**:591, 1970.
7. Mieny, C. J. and Myburgh, J. A.: Successful 20-Hr Preservation of the Primate Liver by Simple Cooling. Transplantation, **11**:495, 1971.
8. Perkins, H. A., May, R. E. and Belzer, F. O.: Cause of Abnormal Bleeding After Transplantation of Pig Liver Stored by a Perfusion Technique. Arch. Surg., **101**:62, 1970.
9. Petrie, C. R. and Woods, J. E.: Successful 24-Hour Preservation of the Canine Liver. Arch. Surg., **107**:461, 1973.
10. Porter, K. A.: Pathology of the Orthotopic Homograft and Heterograft. *In* Experience in Hepatic Transplantation. T. E. Starfzl, editor. Philadelphia, W. B. Saunders Company, 422 and 437, 1969.
11. Schalm, S. W., Terpstra, J. L., Drayer, B., van den Berg, C. and Veltkamp, J. J.: A Simple Method for Short-Term Preservation of a Liver Homograft. Transplantation, **8**:877, 1969.
12. Von Kaulla, K. N., Kaye, H., von Kaulla, E., Marchioro, T. L. and Starzl, T. E.: Changes in Blood Coagulation: Before and After Hepatectomy or Transplantation in Dogs and Man. Arch. Surg., **92**:71, 1966.
13. Woods, J. E.: Successful Three- to Seven-Day Preservation of Canine Kidneys: Immediate Life-Sustaining Function. Arch. Surg., **102**:614, 1971.
14. Woods, J. E., Cooperman, A. M. and Mathews, L. E.: An Effective System for Long-Term Organ Preservation. Mayo Clin. Proc., **46**:754, 1971.