Two-to-Three Day Intestinal Preservation Utilizing Hypothermic Pulsatile Perfusion

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SHORT SEGMENTS OF SMALL INTESTINE have been successfully stored without perfusion for 48 hours under hyperbaric and hypothermic conditions[6] and by perfusion with oxygenated blood for 18 hours.[1] The entire organ has been preserved for five hours by perfusion with Ringer's lactate solutions.[9,10] No systematic studies of intestinal preservation have been carried out using those methods of hypothermic, pulsatile, bloodless perfusion which have been found to be so successful in renal preservation.[2] The present study was designed to define the effects on small bowel survival of hypothermic pulsatile perfusion with a bloodless perfusate and to determine reliable functional parameters of intestinal viability during storage.

Methods

Twenty adult mongrel dogs of both sexes weighing between 15 and 24 kg were used as small bowel donors. They were anesthetized with sodium methohexital and maintained on halothane. The small intestine from the third portion of the duodenum to the ileocecal valve was resected with its mesenteric blood supply. The superior mesenteric artery was cannulated and the small intestine flushed with a cold (4°C) Ringer's lactate solution containing heparin (10,000 U/L) and procaine (1 gm/L) until the venous effluent was clear. A catheter was inserted into both ends of the intestine to drain the intestinal secretions during perfusion. The small intestine was then perfused from 48 to 72 hours (7°C, pH 7.4, pO_2 200 mmHg, pulse rate 60, initial pressure 60 mmHg) in the modified Mox-100 System (Fig.1).[12] Cryoprecipitated pooled canine plasma[2] containing Mg SO_4 (1 gm/L), regular insulin (80 U/L), PSP indicator (12 mg/L), methylprednisolone (500 mg/L), ampicillin (1 gm/L) and kanamycin (1 gm/L) was used as perfusate. In four experiments, chloropromazine (25 mg/L) was added to the perfusate. The pressure was re-adjusted during the first hour if changes occurred, but no further adjustments were made. The small bowel output through the distal ileum was monitored and fresh perfusate was added to maintain sufficient circulating volume. Pressure, flow, fluid loss, weight gain, enzymes (LDH, SGOT, B-glucuronidase), electrolytes (Na, K), osmolarity, lactic acid, pH, pO_2, oxygen consumption, oxygen delivery and vascular resistance were recorded at frequent intervals up to 72 hours. Total protein, globulin and electrolyte content of the intestinal secretions were determined every six hours.

The absorptive capacity of the small intestine during perfusion was measured by administering 10 gm of d-xylose dissolved in 100 ml of water into the cannulated proximal portion of the duodenum immediately after the start of perfusion and at 12, 24, 36, 48, 60 and 72 hours. D-xylose levels in the perfusate were determined at 15–30 minute intervals following intraluminal administration.

Following preservation for 48 hours (14 cases) to 72 hours (six cases) the entire small intestine was transplanted orthotopically into an unrelated dog according to standard techniques.[11,15] Both donor and recipient

Submitted for publication May 21, 1973.

* John and Mary Markle Scholar in Academic Medicine.

Supported by Grant #5-PO2-AM13083-04 from the United States Public Health Service.

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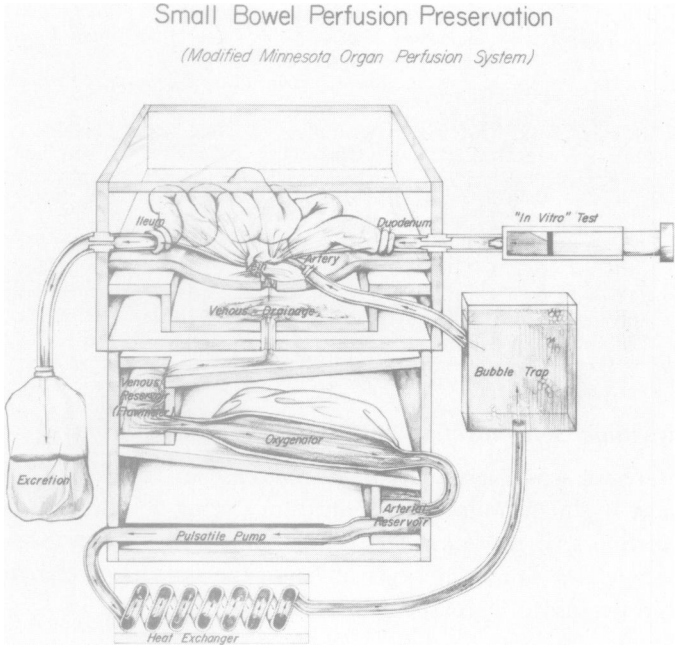

FIG. 1. Schema of the Modified Minnesota Perfusion System. The gravity flow of perfusate comes from the open superior mesenteric vein and drains through the venous reservoir and static membrane oxygenator. The perfusate then circulates through arterial reservoir, pulsatile pump, heat exchanger and bubble trap which gives flow to the superior mesenteric artery and a closed circuit is integrated.

dogs were maintained on a fluid diet for four days prior to surgery and they were treated orally with kanamycin (500 mg) and neomycin (1.0 gm) three times a day. Biopsy specimens were taken from the preserved small intestine after revascularization. Parenteral hyperalimentation using Ringer's lactate containing dextrose (59 gm/L), albumin (12.5 gm/L), vitamin C (500 mg/L), vitamin B complex (2 ml/L) and calcium chloride (0.5 gm/L) was given daily for one week in volumes of 0.5–1.5 L/day as required to maintain adequate clinical hydration. Chloramphenico (500 mgm/day) and kanamycin (1 gm/day) were given for three days and then the dosage was cut in half for the next week. Oral feeding was begun by the seventh post-operative day. Postmortem examinations were performed in all cases.

TABLE 2. *Chemical Changes in Perfusate During Long Term Hypothermic, Bloodless, Pulsatile Perfusion of the Small Bowel (Mean Values ± SE)*

Time of Perfusion (hours)	Osmolarity (mOsm/L)*	Na+ (mEq/L)	K+ (mEq/L)	Lactic Acid (mg/100 ml†)
0	285 ± 10.5	139 ± 4.3	4.2 ± 0.8	8 ± 1.5
2	287 ± 6.5	137 ± 3.7	4.0 ± 0.7	8.7 ± 0.9
6	283 ± 7.2	138 ± 3.0	4.1 ± 0.5	8.5 ± 1.0
12	290 ± 4.5	137 ± 3.0	4.3 ± 0.9	9.2 ± 0.5
18	285 ± 5.0	136 ± 2.9	4.2 ± 0.8	9.0 ± 0.7
24	282 ± 8.5	138 ± 2.5	4.6 ± 0.6	9.2 ± 1.3
30	291 ± 8.0	137 ± 2.3	4.2 ± 1.0	9.4 ± 0.9
36	292 ± 10.5	139 ± 2.5	4.7 ± 1.2	9.5 ± 0.8
48	295 ± 10.2	129 ± 4.7	5.2 ± 1.0	12.6 ± 4.3
54	305 ± 12.0	129 ± 2.5	6.2 ± 0.7	26.5 ± 3.2
60	305 ± 6.5	135 ± 4.5	7.9 ± 1.2	28 + 5.2
72	310 ± 18.5	131 ± 5.2	9.5 ± 3.2	45 ± 10.2

* Normal values = 295 ± 15 mOsm/L.
† Normal values = 0–20 mg/100 ml.

Results

Physical Perfusion Changes (Table 1)

There were few changes in flow rate, perfusion pressure or vascular resistance during the first 48 hours of perfusion. There was, however, a steady loss of perfusate into the bowel with a consequent organ weight gain. After 48 hours the resistance to perfusion rose rapidly with consequent rises in perfusion pressure and fall in flow rate (Table 1).

Changes in Perfusate (Table 2)

The perfusate osmolarity did not exhibit statistically significant changes during the total length of the experiment, despite continuous slow increase during perfusion.

TABLE 1. *Changes in Perfusion During Long Term Hypothermic, Bloodless, Pulsatile Perfusion of the Small Bowel (Mean Values ± SE)*

Time of perfusion (hours)	Flow Rate (ml/min/gm)	Perfusion Pressure (mmHg)	Vascular Resistance (dyne/sec/cm^{-5} × 10^3)	Intestinal Secretions (L)	Organ Weight Gain (% from Control)
0	0.5 ± 0.09	60 ± 0.1	55 ± 10.5	0	0
2	0.5 ± 0.08	55 ± 4.5	55 ± 12.5	0.2 ± 0.1	4 ± 0.9
6	0.5 ± 0.05	55 ± 4.2	58 ± 12.5	0.5 ± 0.1	8 ± 1.2
12	0.5 ± 0.03	55 ± 3.5	60 ± 13.5	0.8 ± 0.3	10 ± 1.5
18	0.5 ± 0.04	55 ± 4.2	58 ± 11.5	1.1 ± 0.2	11 ± 1.7
24	0.5 ± 0.07	55 ± 4.6	60 ± 12.8	1.2 ± 0.5	13 ± 2.5
30	0.45 ± 0.05	55 ± 2.6	60 ± 15.5	1.2 ± 0.6	14 ± 4.5
36	0.4 ± 0.06	60 ± 2.4	63 ± 15.5	1.8 ± 0.5	17 ± 2.1
48	0.4 ± 0.07	60 ± 8.5	64 ± 18.5	2.0 ± 0.7	18 ± 0.8
54	0.35 ± 0.1	70 ± 5.5	81 ± 20.5	2.2 ± 0.6	20 ± 2.1
60	0.25 ± 0.12	75 ± 8.0	85 ± 15.5	2.5 ± 1.9	22 ± 9.2
72	0.20 ± 0.1	85 ± 10.5	110 ± 19.5	2.8 ± 1.0	24 ± 12.3

TABLE 3. *Enzyme Changes in Perfusate During Long Term Hypothermic, Bloodless, Pulsatile Perfusion of the Small Bowel (Mean Values ± SE)*

Time of Perfusion (hours)	LDH (Units/100 ml)*	SGOT (Units/100 ml)†	B-gluronidase (Units/100 ml)‡
0	35 ± 15.5	25 ± 10.5	75 ± 15.5
2	38 ± 10.2	28 ± 5.5	95 ± 10.2
6	40 ± 8.5	30 ± 4.5	100 ± 8.5
12	35 ± 6.5	34 ± 6.5	105 ± 9.5
18	45 ± 10.5	35 ± 8.5	100 ± 15.0
24	70 ± 14.3	45 ± 6.5	108 ± 10.2
30	65 ± 15.2	46 ± 5.3	110 ± 8.7
36	72 ± 10.5	48 ± 10.3	115 ± 10.2
48	75 ± 25.0	85 ± 12.4	145 ± 18.3
54	200 ± 13.5	175 ± 8.5	148 ± 20.5
60	275 ± 10.3	180 ± 6.5	150 ± 40.5
72	235 ± 25.5	185 ± 25.5	150 ± 35.5

* Normal values = 40–80 U/100 ml.
† Normal values = 0–40 U/100 ml.
‡ Normal values = 80–120 U/100 ml.

The sodium concentration in the perfusate was normal for the first 48 hours of perfusion, only falling slightly thereafter. The potassium concentration rose rapidly after 48 hours. Lactic acid concentration was also stable for the first 48 hours, but became very high after that time (Table 2).

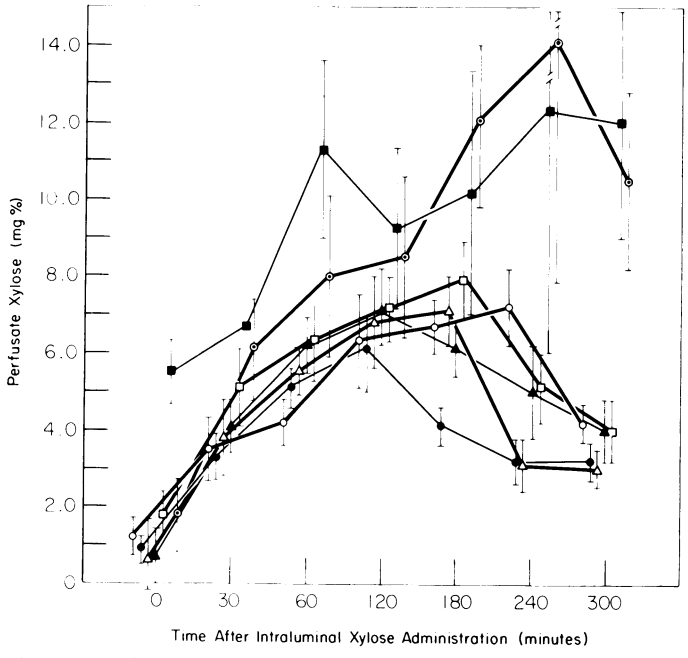

FIG. 2. Perfusate d-xylose values after intraluminal administration. Each line represents the pentose levels in the perfusate at various intervals after beginning of perfusion.

○——○ 0 hours perfusion
●——● 12 hours perfusion
△——△ 24 hours perfusion
▲——▲ 36 hours perfusion
□——□ 48 hours perfusion
■——■ 60 hours perfusion
⊙——⊙ 72 hours perfusion

TABLE 4. *Chemical Changes in Intestinal Secretions During Long Term Hypothermic, Bloodless, Pulsatile Perfusion of the Small Bowel (Mean Values ± SE)*

Time of Perfusion (hours)	Total Proteins (gm/100 ml)	Globulins (gm/100 ml)	Na+ (mEq/L)	K+ (mEq/L)
6	0.9 ± 0.3	0.4 ± 0.2	10 ± 2.5	2.9 ± 0.7
12	2.1 ± 0.9	1.2 ± 0.5	20 ± 9.7	4.2 ± 3.2
24	4.5 ± 1.2	3.2 ± 1.2	38 ± 6.5	6.5 ± 1.5
36	6.7 ± 1.0	4.5 ± 0.9	47 ± 8.5	4.5 ± 1.2
48	8.5 ± 2.0	6.3 ± 1.5	67 ± 10.3	8.2 + 3.2
60	10 ± 5.2	7.9 ± 5.2	70 ± 10.2	7.3 ± 5.2
72	12 ± 3.2	10 ± 2.4	75 ± 14.2	10.6 ± 4.2

Enzyme Concentration within the Perfusate (Table 3)

There was a gradual steady increase in LDH, SGOT, and B-glucuronidase concentration during the first 36 to 48 hours of preservation. When the perfusion was prolonged for longer periods of time, the enzyme levels in the perfusate increased markedly.

D-xylose Absorption during Perfusion (Fig. 2)

The absorption pattern of d-xylose from the lumen into the perfusate did not change during the first 48 hours of perservation. Longer periods of preservation allowed the d-xylose to rapidly enter the perfusate.

Intestinal Secretations During Perfusion (Table 4)

The volume of intestinal secretions increased gradually during perfusion as did the content of total protein, globulin, sodium and potassium. In particular the potassium concentration in the intestinal secretions increased markedly after 48 hours of perfusion.

Oxygen Consumption, Delivery and pH Perfusion Changes (Table 5)

The pH, pCO_2, A-V O_2 difference, oxygen delivery and consumption did not change during the first 36 hours of perfusion. From 36 to 48 hours a minimal decrease in all parameter was observed. After 48 hours, acidosis, hypocapnia and low oxygen consumption became increasingly severe as perfusate flow decreased and vascular resistance rose.

Changes after Transplantation

Orthotopic intestinal allotransplantation was carried out in six intestines perfused for 72 hours. Immediately after revascularization there was gross evidence of serosal and mesenteric hemorrhage. There was no microscopic evidence of mucosal damage (Fig. 3). These recipient dogs survived a mean of 24 ± 10.5 (SE) hours. Death was not apparently related to gross breakdown of the transplanted intestine. The animals died with mesenteric venous thrombosis, septicemia, mesenteric hemorrhage or ischemic necrotic bowel.

TABLE 5. *Metabolic Changes in Perfusate During Long Term Hypothermic Bloodless Pulsatile Perfusion of the Small Bowel (Mean Values ± SE*

Time of Perfusion (hours)	pH	pCO_2	A-V O_2 Diff. (vol. %)	O_2 Delivery (ml/min/gm)	O_2 Consumption (ml/min/100 gm)
0	7.39 ± 0.02	40 ± 2.5	40 ± 3.5	1.0 ± 0.03	1.6 ± 0.08
2	7.38 ± 0.01	39 ± 1.2	39 ± 2.1	1.0 ± .05	1.6 ± 0.06
6	7.39 ± 0.05	38 ± 0.9	38 ± 3.2	1.0 ± 0.01	1.6 ± 0.09
12	7.38 ± 0.09	38 ± 1.1	38 ± 2.0	1.0 ± 0.07	1.55 ± 0.07
18	7.38 ± 0.07	38 ± 1.7	38 ± 1.9	1.0 ± 0.09	1.55 ± 0.05
24	7.38 ± 0.05	38 ± 2.6	38 ± 4.1	1.0 ± 0.06	1.5 ± 0.10
30	7.37 ± 0.09	37 ± 2.0	37 ± 4.5	0.98 ± 0.08	1.5 ± 0.09
36	7.36 ± 0.07	34 ± 4.3	37 ± 3.2	0.94 ± 0.06	1.45 ± 0.08
48	7.36 ± 0.1	32 ± 3.6	36 ± 8.5	0.94 ± 0.09	1.4 ± 0.25
54	7.32 ± 0.1	30 ± 2.7	30 ± 6.5	0.84 ± 0.1	1.05 ± 0.09
60	7.30 ± 0.09	25 ± 4.5	25 ± 9.5	0.75 ± 0.09	1.00 ± 0.25
72	7.30 ± 0.09	22 ± 4.5	20 ± 5.5	0.65 ± 0.1	0.95 ± 0.32

Intestinal transplantation was carried out after 48 hours of perfusion in 14 dogs. Immediately after revascularization, there were signs of active tone and peristalisis with erythema of the serosa, but no petechiae or hemorrhages in the mesentery. Biopsy revealed minimal interstitial inflammation. These fourteen recipients survived a mean of 6.0 ± 2.5 (SE) days, but died of peritonitis with sepsis, intestinal obstruction or venous thrombosis. Histological study did not reveal clear cut evidence of rejection (Fig. 4).

Discussion

Belzer and his associates[3] discovered that the success of long-term hypothermic pulsatile perfusion of the kidney depended on a low perfusate concentration of lipids and lipoproteins. When these substances were present in the perfusate an increase in vascular resistance with a consequent fall in perfusate flow occurred. Such substances can now be removed by cryoprecipitation of the plasma perfusate.[3] Twenty-four to 48 hour hypothermic pulsatile perfusion of kidneys using cryoprecipitated plasma is now

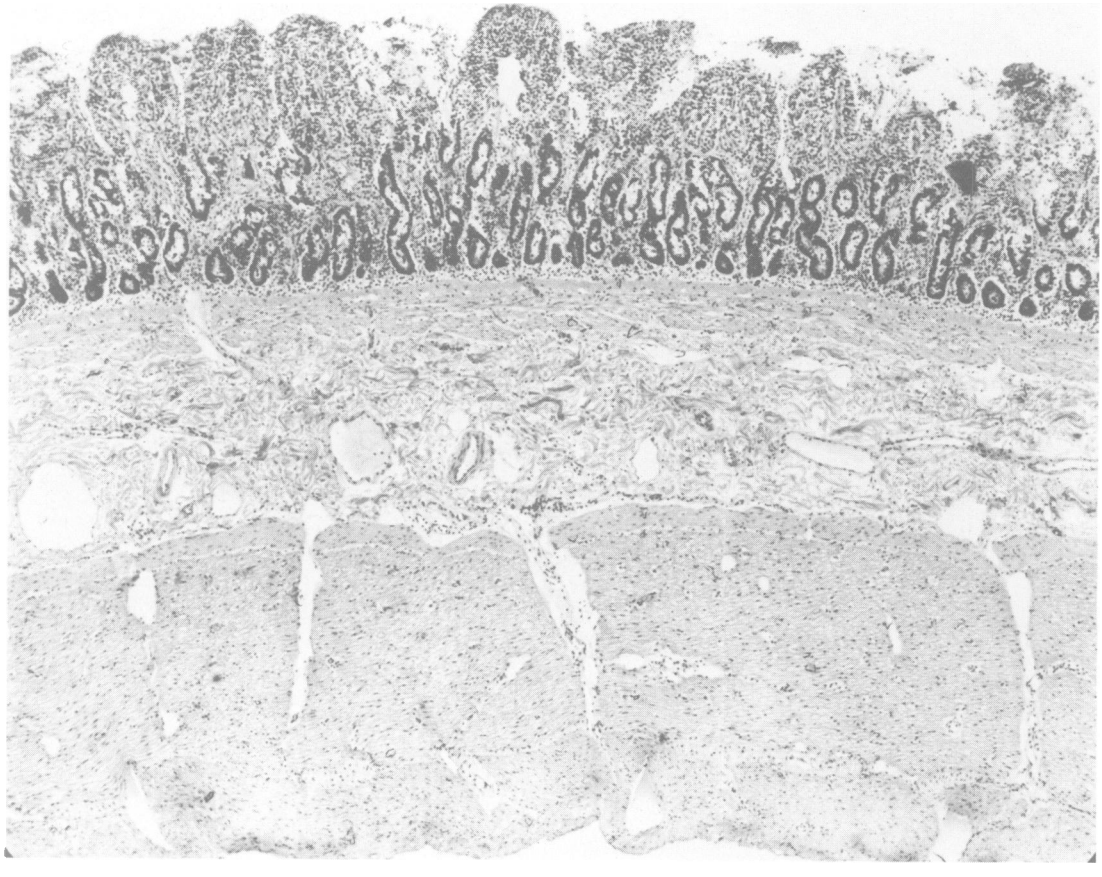

FIG. 3. (×160) Microscopic changes following 72 hour preservation and immediate transplantation. Some edema of the submucosa is present but there is no perivascular lymphocytic infiltration. The mucosa is intact but there are some lymphocytes and plasma cells in the mucosa itself. There are also dilated lymphatics present.

routine in most transplantation centers. The present studies demonstrate that the principles of renal preservation apply as well to small intestinal preservation. Forty-eight hours of hypothermic pulsatile perfusion does not seem to severely impair metabolic function, or cause gross changes in the mesentery or intestine. When transplanted the intestine survived an average of six days. Longer periods of perfusion (72 hours) are associated with changes in vascular resistance, decreased flow, poor oxygen consumption and chemical signs of cellular death. After transplantation the 72-hour grafts did not survive more than 24 additional hours. The failure of preservation after 48 hours could be predicted by any of a number of changes in the physical or chemical characteristics of the perfusate. The simplest test of viability, however, appears to be the rise in perfusion pressure and decreased flow. This parameter has also been found to be the simplest and best parameter of organ viability in renal preservation studies as well.[3] The presence of spontaneous contractions are of little value as indicator of viability. In preparations[9] with perfusates containing dog red blood cells, muscle activity was obtained when oxygen consumption ranged from only 0.26 to 0.44 ml/100 gm per minute—just barely enough to keep the smooth muscle alive. Others[4,5] studying serotonin release in isolated segments obtained similar results.

Ruiz and associates[14] found statistically significant decrease in d-xylose absorption in small intestine preserved for 48 hours under hypothermia and hyperbaric oxygen. A delay in the start of absorption was observed in the preserved groups. This was related to the time it took the small intestine to achieve normothermia. We initially thought that d-xylose absorption from the lumen into the perfusate might be a good indicator of viability. The opposite effect was seen so that rapid appearance of xylose in the perfusate appeared to be an indicator of altered intestinal permeability.

A number of problems remain. Despite the absence of gross and microscopic signs typical of rejection, and in total absence of gross intestinal perforation, all dogs died in an average of seven days even when the perfusion was carried out for only 48 hours. The simplest explanation for death is the absorption of toxins from the damaged intestine. Thus, submicroscopic changes in the intestinal viability must have occurred which, while not rendering the bowel immediately non-viable, ultimately led to the death of the recipient. The use of intestinal antiseptics were designed to reduce the absorption of endotoxins from the bowel until recovery of the transplanted segment could occur. There are several factors, however, which interfere with the recovery of the bowel. The first is the lack of lymphatic drainage from the trans-

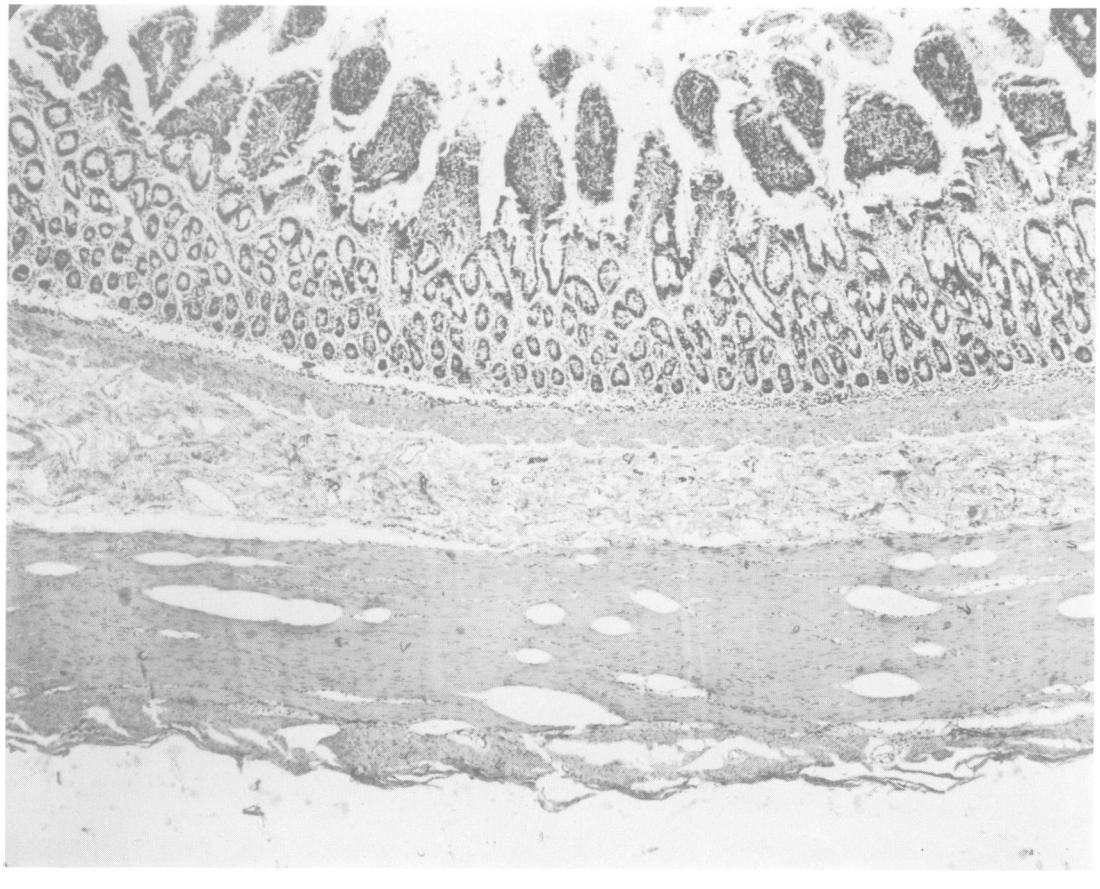

FIG. 4. (×160) Microscopic changes following 48 hour preservation. The mucosa is intact and shows no inflammatory infiltrate. The submucosal tissue shows no edema and no evidence of perivascular cuffing by inflammatory cells. The underlying muscle layers are intact except for some fixation artifact.

planted small bowel. Ruiz and associates[15] have shown that lymphatics regenerate poorly in allografted transplanted bowel. The second factor is probably the early changes of rejection which may further increase intestinal permeability.

This failure to achieve longer survival suggests that a better test of viability and ultimate transplantability during perfusion might be the presence of endotoxin or other bacterial products within the perfusate. Sensitive tests for endotoxin are currently available[13] and the appearance of endotoxin in the perfusate might be a suitable warning that the endotoxin has passed through the damaged small bowell wall.

Whatever the best indicator of viability, the present model can serve as an excellent experimental model to study the effects of various additives, new artificial perfusates, and to study newer methods designed to prolong viability in isolated preparations of intestine. For example, chloropromazine and methylprednisolone have been shown to extend the period of safe renal perfusion, but we noted no difference in intestinal preservation. Allopurinol is another additive designed to increase the tolerance of preserved organs to ischemia. Allopurinol, other adrenergic blocking drugs,[7–9,16] and plasma substitutes are currently being investigated.

Summary

Canine small intestine was preserved by hypothermic pulsatile perfusion for periods up to 72 hours. All physical and chemical studies represented acceptable parameters of viability; the simplest test of viable bowel, however, were changes in perfusion pressure and flow rate. Signs of organ distress during preservation did not occur until after 48 hours of perfusion. Recipients of 48 hours preserved bowel survived a mean of 6.0 ± 2.5 (SE) days after transplantation. Signs of organ distress were observed after 48 hours and bowel transplanted after 72 hours preservation did not survive longer than 24 hours.

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