Survival of Small Intestine Following Excision, Perfusion and Autotransplantation

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IN A PREVIOUS report from this laboratory,[10] a method was described by which segments of canine sigmoid colon were excised, connected to a model perfusion system and perfused for periods of 5 hours. In these experiments the gross and microscopic appearance of the perfused bowel, its arteriovenous oxygen difference and its ability to produce mucus suggested that these bowel segments remained viable throughout the *in vitro* perfusion. The ultimate test of viability, however, is survival and function of the excised, perfused bowel segments following reimplantation in the donor animal. The present report summarizes the result of a series of experiments in which bowel segments were autotransplanted as the final test of the perfusion system in maintaining *in vitro* organ viability.

Results

Large Bowel Segments. In an initial series of experiments, the canine sigmoid colon was excised with intact inferior mesenteric artery and vein and connected to the perfusion system. Using a perfusate consisting of heparinized, fresh autologous blood and Ringer's solution, perfusion was carried out under normothermic conditions for 5 hours. In ten experiments the perfused sigmoid segments met many criteria of viability; the bowel appeared pink and moist, had tone and peristaltic movements; the arteriovenous oxygen saturation difference was in the 23–25% range; the histological appearance of periodic biopsy showed normal architecture.

Following 5 hours of colon perfusion, attempts were made to transplant the bowel segments. In no instance was the procedure technically feasible due to the extremely small calibre and fragility of the inferior mesenteric artery and vein.

Small Bowel Segments. In a second series of experiments conducted at room temperature the small intestine and its superior mesenteric artery and vein were used. Initially the entire small intestine was excised and perfused. The optimal perfusion flow rate was 120 to 150 cc./minute.

Once again the excised, perfused intestine grossly and microscopically appeared viable. Arteriovenous oxygen saturation, production of enteric juice and peristaltic movements seemed consistent with anatomic and functional integrity.

Following perfusion periods of up to 5 hours, autotransplantation was carried out. Reanastomosis was technically easy due to the relatively large caliber of the superior mesenteric artery and vein.

Since the small intestine plays so vital a role in digestion, severe physiologic disturbance were anticipated following autotransplantation of the small intestine. Ballinger [2] suggests that denervation of the small intestine results in a high incidence

Submitted for publication May 15, 1967.

This research was supported by a grant of the Damon Runyon Fund.

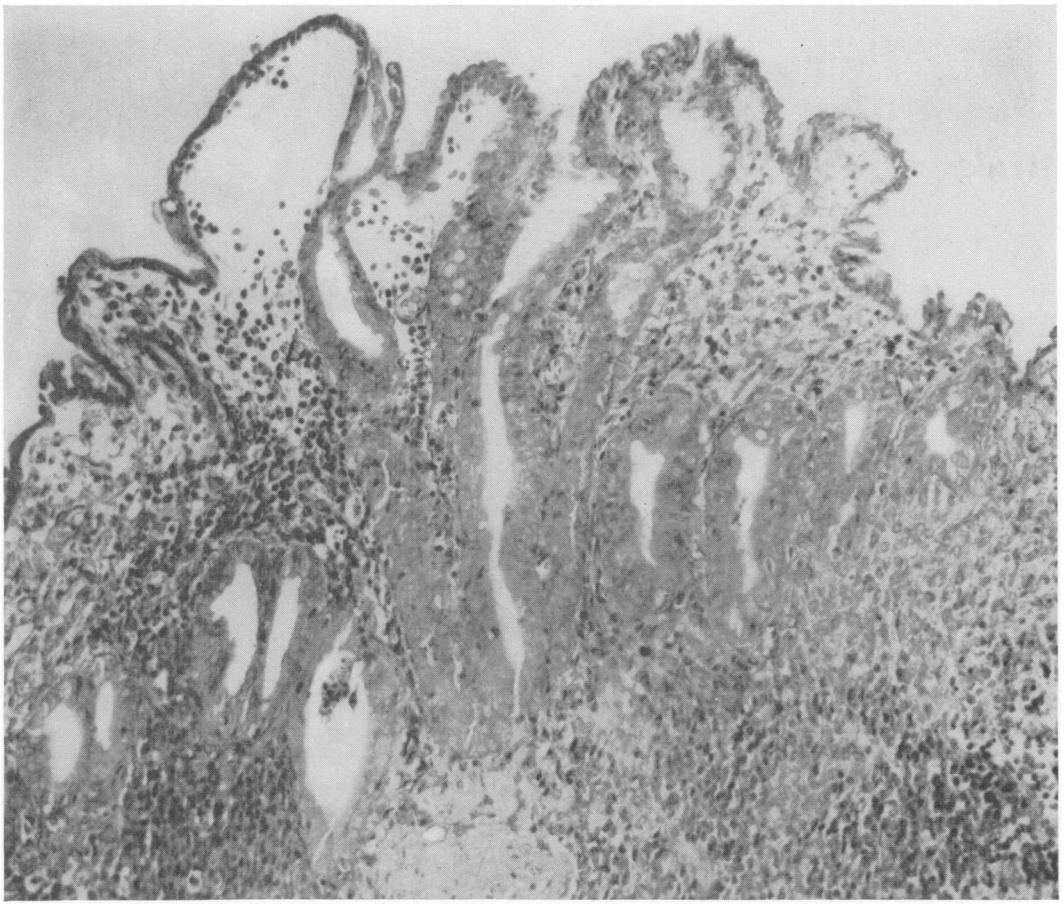

FIG. 1. Microphotograph of normal small intestinal organ culture (×240).

of postoperative diarrhea with malabsorption and a high death rate. The interruption of lymphatics and lacteals and any mucosal damage could also be expected to contribute considerably to morbidity and mortality.

With these considerations in mind a series of 25 small intestinal experiments were carried out. Seven animals died from technical failures such as laceration of mesenteric vessels, incompatibility of homologous blood, hypoxia from over medication, or poor airway. Eighteen animals recovered from the perfusion-autotransplantation only to die 1 to 5 days later. In no instance could death be attributed to small intestinal failure *per se*. Death was nearly always due to thrombosis of the superior mesenteric vein at the anastomotic site, the clot being propagated a short distance, 1.0 to 1.5 cm. Occasionally there was a thrombus in the superior mesenteric artery. Several deaths could be attributed to peritonitis and sepsis following leakage of the end-to-end small bowel anastomosis.

Beginning with the twenty-sixth experiment, anticoagulation of the postoperative animal was carried out, whereas in earlier experiments only the perfusate had been anticoagulated. Prothrombin time of the dog's blood was determined preoperatively and once daily postoperatively. During the first 24-hour period heparin was administered, followed by coumadin on the opera-

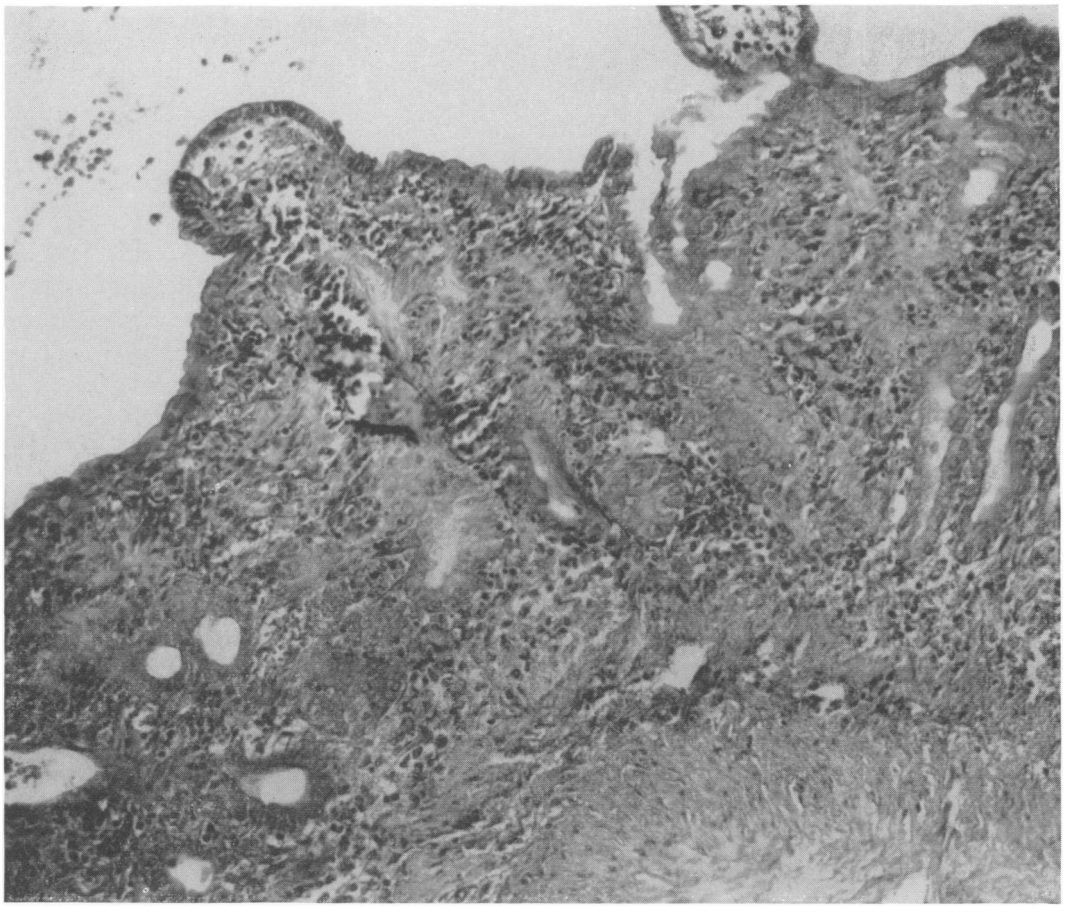

FIG. 2. Microphotograph of organ culture of small intestine after four hours perfusion at room temperature (×240).

tive and succeeding days. In the beginning poor control of anticoagulation resulted in death from intraperitoneal hemorrhage. Ten experiments were required before a proper dosage of anticoagulant drug could be established, the margin of error in anticoagulation of dogs apparently being much narrower than in humans. The dosage regimen finally evolved for animals weighing between 18 to 23 Kg. is as follows: 2.5 mg. of coumadin on the day of operation, followed by daily maintenance dose of 1.0 to 1.25 mg.

In addition to more refined anticoagulation, other improvements in method were instituted. A greater effort was made, for example, to avoid hypotension in the dog throughout the day of operation. Fluid and electrolyte balances were carefully maintained; the pH of the perfusate was kept in the 7.2 to 7.3 range. Eventually an improved perfusate was developed; in early experiments homologous bank blood diluted with Ringer's solution was used; later a four-part perfusate was found to be more effective: 1) fresh heparinized autologous blood (150 cc.); 2) Tis-u-sol * (balanced salt solution, 100 cc.); 3) low molecular weight dextran 10% (50 cc.); 4) Dextrose 50% (20 cc.). Added to the perfusate is 250,000 units of aqueous penicillin. With this new perfusate, edema of the specimen

* Tis-u-sol supplied by Baxter Laboratories, Inc., Morton Grove, Illinois.

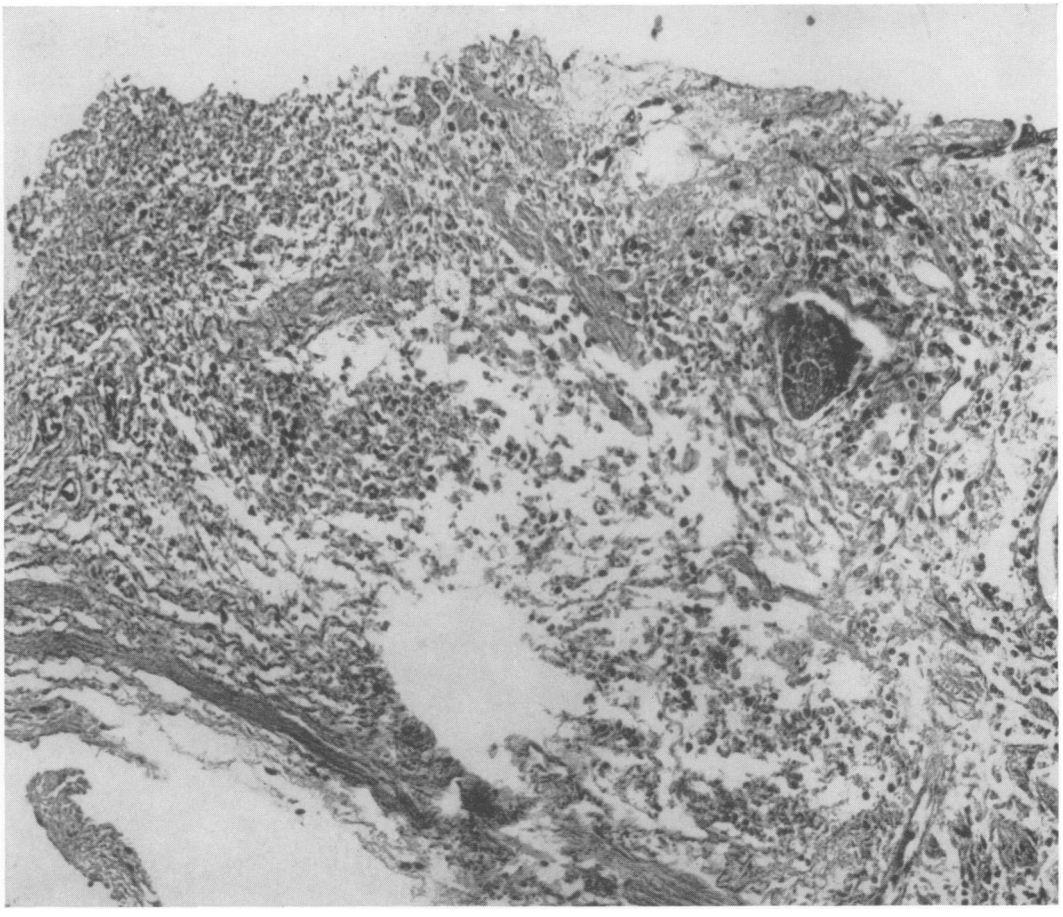

FIG. 3. Microphotograph of organ culture of small intestine kept in N/S and room temperature for four hours (×240).

decreased sharply as indicated by specimen weights before and after perfusion.

With these improvements, seven animals survived 2 or more weeks following autotransplantation; one animal is alive 3 months and one 6 months following perfusion-autotransplantation of its small intestine.

Diarrhea usually began 2 to 3 days following operation and continued for several weeks, during which time intravenous or hypodermoclysis fluid supplement was required. Penicillin and streptomycin were given during the first postoperative week to minimize secondary infection. Diarrhea usually stopped after 2 weeks, presumably due to regeneration of the mucosa and reestablishment of absorptive function.

A major technical improvement was added in later experiments with the use of the Nakayama anastomosis clamp,[9] an ingenious device which allows for quick, faultless small vessel anastomosis utilizing metal clips instead of suture material. With this device, shorter segments of intestines and small caliber vasculature were excised and reanastomosed with diminished adverse effect upon intestinal function.

Organ Culture and Radioisotope Studies. Organ cultures were performed by Merchant's method.[8] A piece of tissue approximately 1 × 2 mm. was placed into

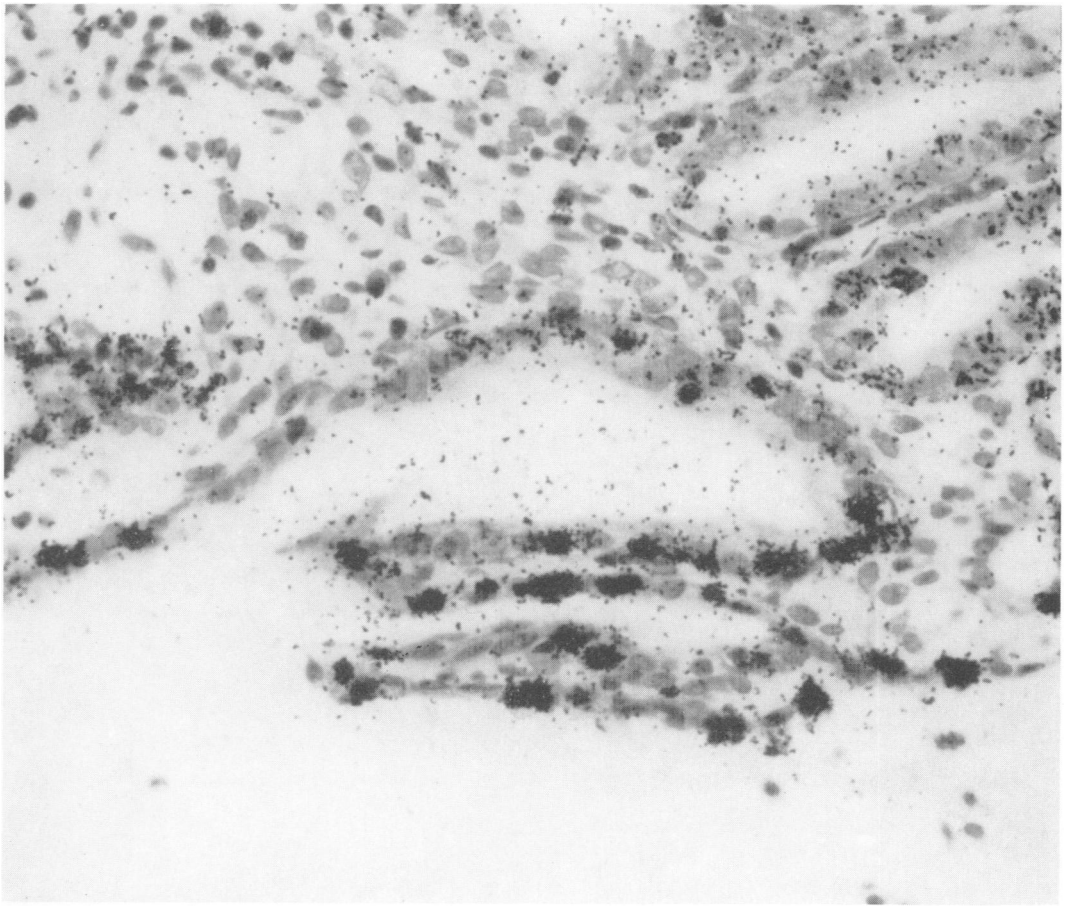

FIG. 4. Uptake of Tritiated Thymidine in normall intestine (×1,600).

1.0 ml. of culture media 199 * and incubated at 37° C. for 24 hours under a continuous gas flow (95% O_2 and 5% CO_2). Following the 24-hour culture, histologic slides were made by the usual technic.

In addition, uptake of tritiated thymidine [3] was studied by the following technic: Bowel tissue was incubated for 3 hours in 1.0 ml. of medium 199 containing 1.0 μg. of H^3 thymidine ** under conditions identical with the organ cultures. Following incubation, the histological slides and autoradiograms were coated by Nuclear Trach Emulsion *** using the dipping method. The preparations were kept at 4° C. for 1 week and the slides were developed and stained with hematoxylin.

Figure 1 represents a 24-hour organ culture of normal, intact canine small intestine. The histologic characteristics are those of normal tissue. These cultures act as a control for comparison with subsequent organ and tissue cultures. This is clearly viable tissue.

Figure 2 represents canine small intestine culture 4 hours after excision-perfusion at room temperature. The histologic characteristics are similar to those of the normal control cultures with excellent preservation of viable tissue.

Figure 3 represents an organ culture of canine small intestine which was excised

* Microbiological Associates Inc., Bethesda, Maryland.

** Specific activity 1.9 c./mmole Schwarz Bioresearch.

*** Kodak Type NTB2.

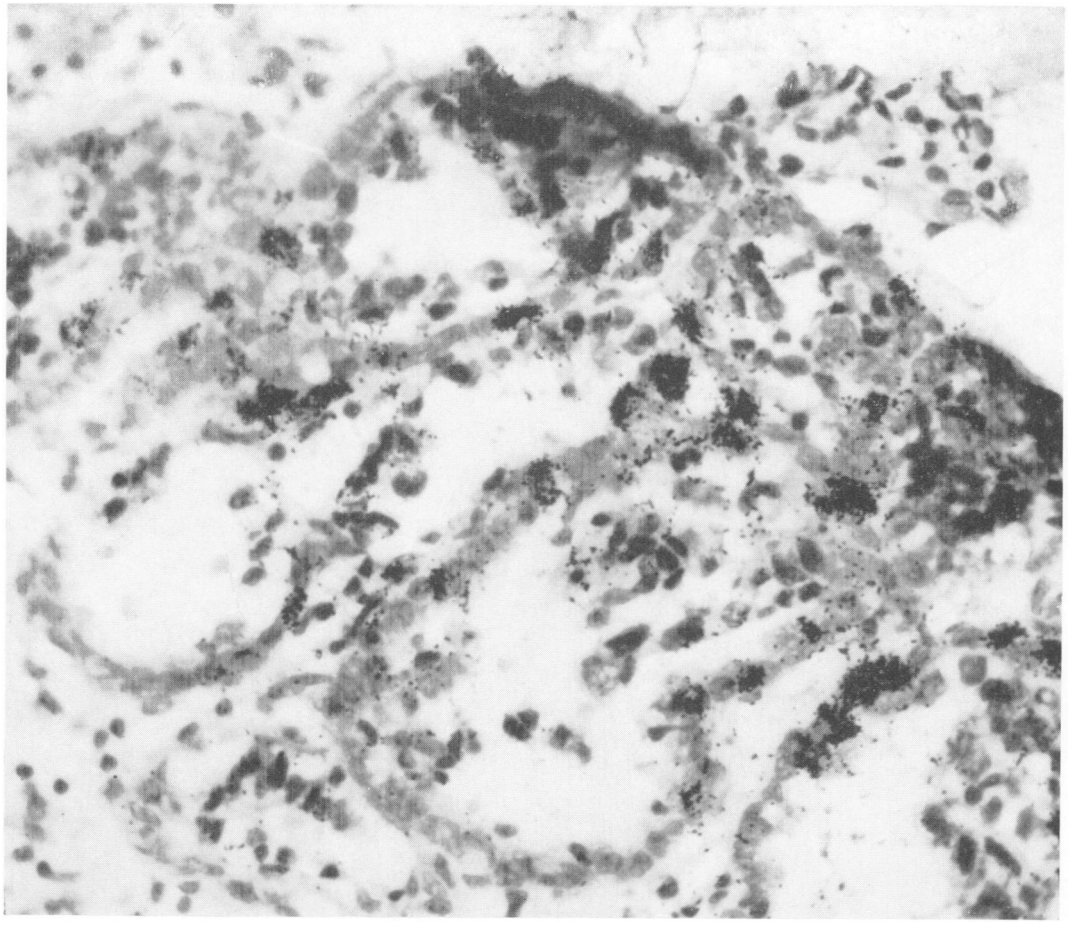

FIG. 5. Uptake of Tritiated Thymidine in small intestine perfused four hours after excision at room temperature (×1,600).

and left in saline at room temperature without perfusion for 4 hours. There is extensive autolysis of tissue with loss of the normal bowel architecture. This tissue must be considered non-viable.

Figure 4 demonstrates the uptake of tritiated thymidine in intact, normal canine small intestine. This radioautograph is a normal control.

Figure 5 demonstrates the uptake of tritiated thymidine of canine small intestinal culture after excision-perfusion. This study shows a slight increase of uptake, consistent with active, viable tissue.

Figure 6 shows complete absence of tritiated thymidine uptake in a canine small intestinal culture four hours after immersion in saline at room temperature without perfusion. This tissue must be considered non-viable.

Discussion

Through these experiments a major objective has been achieved, i.e., establishing the ability of the excision-perfusion method to maintain an *in vitro* environment compatible with organ viability and subsequent *in vivo* function. The ultimate test of this system was survival and good function following autotransplantation. This has now been clearly demonstrated.

Lillehei and his group have added the hyperbaric chamber to their previous hypothermic method of prolonged organ pres-

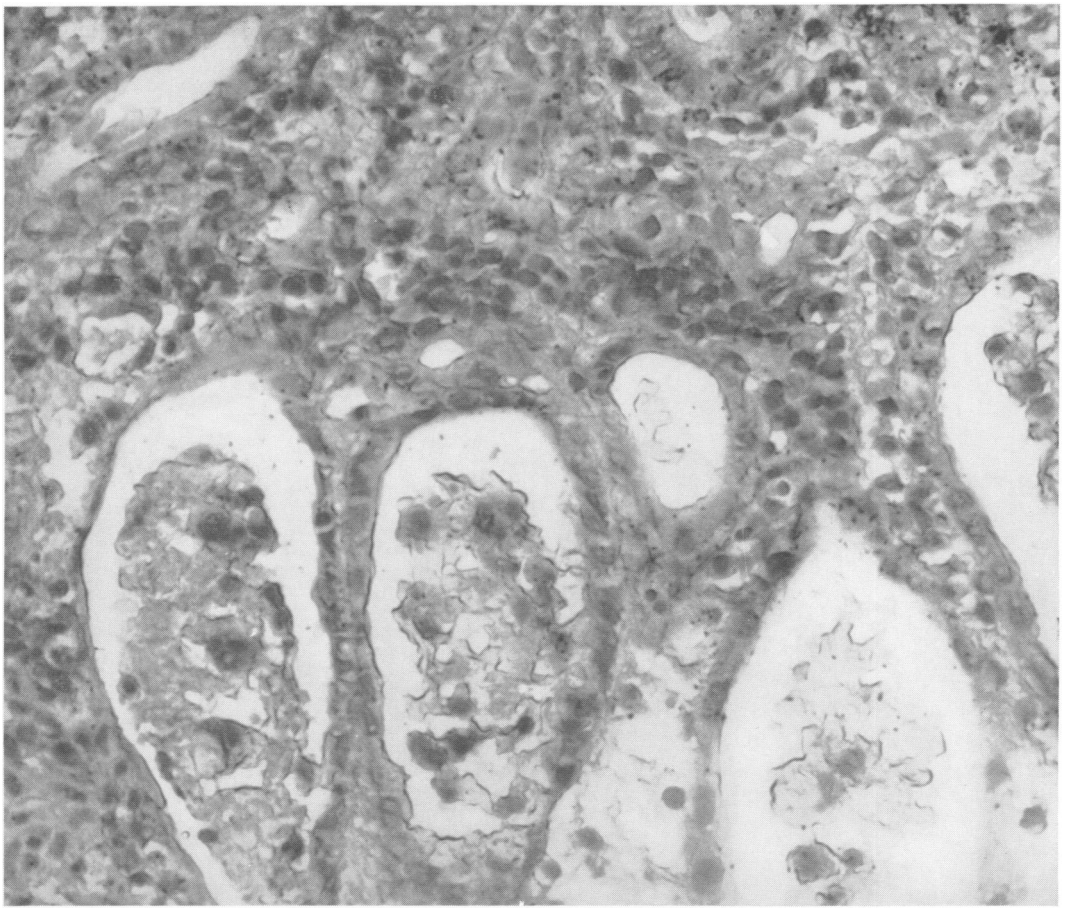

FIG. 6. Uptake of Tritiated Thymidine in small intestine kept in N/S and room temperature for four hours (×1,600).

ervation.[4-7] Using 7.9 atmospheres of O_2 at 4° C. they have preserved dog kidney, heart and small intestine for periods of up to 48 hours. In some experiments they homotransplanted the small intestinal segments to the neck of recipient dogs and claim a 90% "success." They describe "success" as tissue viability as determined by gross and microscopic study of the small intestine for up to four days. No organ culture studies were done. They have not successfully autotransplanted these segments. Their work establishes a histologic viability but does not test physiologic integrity of the reimplanted tissue.

In one study[2] the influence of chlorpromazine was found to depress metabolism in bowel segments thus reducing cellular damage which usually follows prolonged hyperbaric-hypothermic storage.

Austen and McLaughlin[1] recently concluded that *in vitro* non-pulsatile blood flow to isolated small intestine results in impaired metabolic function and gross changes in the bowel and mesentery within 6 hours. With pulsatile flow these changes did not occur after perfusion of up to eighteen hours.

Summary

The object of this research was to establish the ability of a specially designed model perfusion system to maintain the

viability of canine intestinal segments *in vitro* and following autotransplantation.

Excision and perfusion of canine sigmoid colons did not prove to be difficult. Autotransplantation of the colons, however, was consistently attended by technical failure due to the extremely small caliber of the inferior mesenteric vessels. In subsequent experiments small intestinal segments and their larger caliber superior mesenteric artery and vein were anastomosed without technical difficulty.

In 25 experiments, isolated segments of dog small intestine were perfused for up to five hours prior to autotransplantation. The first 15 experiments failed due to a variety of factors including superior mesenteric artery and vein thrombosis, sepsis or postoperative distemper. Better controlled anticoagulation, more careful water and electrolyte balance, use of antibiotics and better selection of experimental animals resulted in a significant improvement in survival. With greater familiarity with the technic of small vessel anastomosis, shorter segments of small intestine were eventually used. With less bowel being removed there was a concomitant decrease in post autotransplantation diarrhea and debility.

A number of long-term post autotransplantation survivals establishes the perfusion method as an effective means of maintaining the viability of excised intestinal segments for periods of at least 5 hours.

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