JOURNAL OF SURGICAL RESEARCH 46, 142–146 (1989)

Long-Term Nutritional Function of Orthotopic Small Bowel Autotransplants

SESHADRI RAJU, M.D.,[1] HITOSHI FUJIWARA, M.D., JAMES B. GROGAN, PH.D., AND JAMES L. ACHORD, M.D.

Departments of Surgery-Transplantation and Medicine, University of Mississippi Medical Center, Jackson, Mississippi 39216-4505

Submitted for publication August 4, 1987

Nutritional function following autografting of the entire small bowel was followed in 11 dogs for 12 months. Although positive nitrogen balance was recovered within a few weeks, the animals did not achieve their preoperative body weight for up to 6 months. Fat and D-xylose absorption remained depressed and never fully recovered. Hematocrit and serum iron did not normalize until the second postoperative month or later. Abnormal serum albumin and albumin/globulin ratio persisted for 12 months. When the mesenteric vein was anastomosed to the vena cava rather than to the portal vein, this group had more severe abnormalities in body weight, hematocrit, total protein, and serum albumin, in addition to a significant rise in liver enzymes. These findings represent a discouraging portent for the functional utility of small bowel transplants. © 1989 Academic Press, Inc.

INTRODUCTION

Experimental orthotopic small bowel transplantation is a technically formidable procedure with a high perioperative mortality [1–4]. Animals undergoing this procedure appear to die from metabolic, infectious, and preservation-related causes in the post-transplantation period. Many of these avoidable problems were identified and corrected in our laboratory, resulting in an experimental model with over 70% long-term survival (unpublished data). With this improved survival, it was possible to undertake meaningful long-term nutritional studies after orthotopic small bowel transplantation. An autotransplantation model was chosen to determine the nutritional sequelae of neural, lymphatic, and vascular division without the confounding influence of transplant rejection and immunosuppression on recipient animals. It has previously been reported by Ballinger *et al.* [5] that substantial nutritional disturbances could be expected from lymphatic and neural division alone.

[1] To whom correspondence and reprint requests should be addressed at University of Mississippi Medical Center, Department of Surgery-Transplantation, 2500 North State Street, Jackson, MS 39216-4505.

METHODS

Eleven adult mongrel dogs with orthotopic total small bowel autotransplantation were chosen for preoperative and postoperative nutritional studies. Data covering 12 months after surgery are reported.

Technique

Several modifications were introduced to reduce the infectious, metabolic, and preservation-related complications and mortality attendant upon total small bowel transplantation. With the modifications, over 70% of the animals undergoing autotransplantation survived long term. Briefly, dogs were given 2 g of oral neomycin daily for 3 days and food was withheld for 24 hr prior to surgery. Under pentobarbital anesthesia with endotracheal intubation and mechanical ventilation, a laparotomy was performed through a midline incision. The entire small bowel from the distal part of the duodenum to an inch proximal to the ileocecal valve was isolated on a mesenteric vascular pedicle. After systemic heparinization, isolated gut was resected, flushed with 500 to 700 ml of cold heparinized lactated Ringer's solution as previously described, and further cooled by intraluminal irrigation of the same solution with added kanamycin (0.5 g/liter). Poorly perfused ends of the transplant were resected (usually about 15 cm at either end) before reimplantation. The graft was revascularized by anastomosis to the mesenteric vessels for portal drainage ($n = 6$) or to the abdominal aorta and inferior vena cava in the end-to-side fashion for vena caval drainage ($n = 5$). Bowel continuity was restored by an end-to-end anastomosis of the recipient duodenum and ileum to the upper and lower ends of the transplant, respectively, by a single-layer anastomotic method. Cold ischemic time averaged approximately 80 min. A 15-cm segment of proximal jejunum was isolated with intact vascular pedicle and the ends were exteriorized for monitoring purposes (Thiry-Vella loop). The peritoneal cavity was irrigated profusely with warm saline to combat hypothermia, and liberal fluid replacement was instituted. No blood was transfused either during or after surgery. Animals were treated with 2 g oral neomycin daily for 4

0022-4804/89 $1.50

days after surgery. Parenteral antibiotics (cephalosporin) and fluids were administered for 5 days, at which time oral feedings were resumed. An anti-diarrheal agent, such as Lomotil, was administered for 1 month postoperatively in case of severe diarrhea.

Nutritional Parameters

Dogs were weighed preoperatively and at regular intervals following transplantation. Blood samples were collected to determine hematocrit, hemoglobin, total serum protein, serum albumin, serum globulin, total cholesterol, triglyceride, SGPT, SGOT, LDH, and serum iron at regular intervals during the 12-month period of observation.

D-Xylose absorption tests were performed according to the method of Hawkins [6]. After fasting overnight, 0.5 g/kg of D-xylose with 300 ml of water was administered through a stomach tube. Blood samples were drawn at 0, 20, 40, 60, 90, and 120 min afterward for spectrophotometric analysis [7]. Nitrogen balance was determined by feeding dogs specific amounts of dry food (500 g/day). Output was collected from metabolic cages for 5 days (2 days in some instances) for averaging. Nitrogen content was determined by the standard Kjeldahl method. Fecal fat analysis was done by the method of Van de Kamer *et al.* [8]. Fat analysis was performed preoperatively and at regular intervals postoperatively starting at 2 weeks. Fat excretion was determined from 5-day collection of feces and expressed as the average daily excretion (in grams) over each collection period.

Statistics

The unpaired Student t test or the Wilcoxon group comparison was utilized.

RESULTS

During the 12-month follow-up period, three dogs died, two due to strangulation and one due to an undetermined cause.

Postoperatively all animals lost weight, some losses exceeding 10% of the preoperative weight. There was no sign of recovery until the fourth postoperative month when a gradual restoration of body weight began (Fig. 1). The portal venous drainage group (PVD) tended to maintain their postoperative weights better than the vena caval drainage group (CVD); the differences were not statistically significant, however. The mean weight of PVD group at 12 months finally attained the preoperative level, whereas in the CVD group, the animals weighed less than the preoperative level.

Hemoglobin, Hematocrit, and Serum Iron

There was a sharp decline in hemoglobin, hematocrit, and serum iron during the first postoperative month as could be expected from surgical blood loss. There was a slow recovery thereafter. Preoperative values were reached between 4 and 6 months after surgery (Table 1). Again, the PVD group tended to fare better than the CVD group in regaining these hematological parameters.

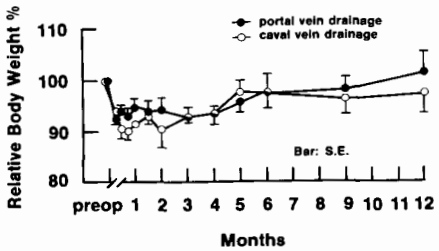

FIG. 1. Body weight changes after total orthotopic small bowel transplantation. Note poor recovery of caval drainage group.

Nitrogen Balance

All animals had positive nitrogen balance (2.9 ± 1.3 g N/day over 5 days) preoperatively (Fig. 2). Two weeks after transplantation three of five CVD dogs were in negative nitrogen balance (0.5 ± 2.2 g N/day), whereas all PVD dogs had recovered positive balance (3.2 ± 2.3 g N/day). The lower value for CVD dogs was accounted for by their significantly lower intake (266 ± 46 g vs 421 ± 79 g/day in PVD, $P < 0.005$). All dogs eventually regained positive balances concomitant with the recovery of appetite and food intake. However, some negative balances were observed after 1 month during episodes of diarrhea despite a normal food intake.

Serum Proteins

Total serum protein levels were decreased significantly on the 10th postoperative day (Table 1). In the portal venous drainage group, the total serum protein level attained the preoperative value by 6 months after surgery. However, the serum albumin in both groups remained below normal for the entire 12-month study period after surgery, such that the recovery of total serum protein was attributable to an increase in serum globulin during the postoperative period. As a result, the albumin globulin ratio remained abnormal for the entire 12 months postoperatively. The CVD group showed even more severe abnormalities in protein hemostasis. Preoperative total serum protein values were not restored in this group until 12 months after surgery. Essentially, there was no recovery of serum albumin levels postoperatively in this group.

Carbohydrate Absorption

Preoperative D-xylose absorption tests showed predictable peak values around 60 min after administration. The mean peak value within 2 hr was 72.8 ± 12.0 mg/ml (Table 1; Fig. 3). Postoperatively, in all animals both peak levels and areas under the curves were persistently lower than those preoperatively and never fully recovered. At

TABLE 1

Hematological and Serum Chemistry Parameters after Total Orthotopic Small Bowel Transplantation

Number of dogs	Group	Preoperative	10 day	20 day	30 day	45 day	2 month	3 month	4 month	6 month	9 month	12 month
	PVD:	6	6	6	6	6	6	6	5	5	4	4
	CVD:	5	5	5	5	5	5	5	5	4	4	4
Hemoglobin (g/dl)	PVD	13.9 ± 1.4	10.2 ± 1.9[a]	11.2 ± 2.2[a]	11.8 ± 1.1[a]	12.3 ± 1.7	12.2 ± 1.8	12.9 ± 1.4	13.1 ± 2.0	14.6 ± 1.3	15.2 ± 2.3	14.6 ± 1.6
	CVD	13.8 ± 1.9	9.7 ± 0.5[a]	11.5 ± 1.5	11.7 ± 2.6	11.3 ± 2.0	11.5 ± 1.4	12.3 ± 1.1	11.6 ± 2.4	13.0 ± 1.3	14.2 ± 1.7	13.1 ± 1.7
Hematocrit (%)	PVD	43.8 ± 4.9	32.2 ± 5.3[a]	36.8 ± 6.6	38.2 ± 3.0[a]	39.0 ± 6.0	40.3 ± 5.7	41.8 ± 5.1	41.8 ± 4.0	46.4 ± 4.7	46.1 ± 6.0	45.5 ± 6.2
	CVD	44.6 ± 7.1	31.8 ± 2.2[a]	36.6 ± 4.0	37.2 ± 7.8	35.7 ± 6.4	37.0 ± 3.8	39.4 ± 3.0	38.0 ± 6.9	40.8 ± 3.8	43.2 ± 4.6	40.3 ± 4.9
Serum iron (mcg/dl)	PVD	138 ± 59	100 ± 38	66 ± 22[a]	89 ± 41	103 ± 27	99 ± 44	139 ± 55	133 ± 45	136 ± 14	161 ± 44	172 ± 78
	CVD	159 ± 96	108 ± 68	104 ± 31	80 ± 22	77 ± 19	84 ± 60	107 ± 46	99 ± 39	106 ± 31	145 ± 18	123 ± 50
Total protein (g/dl)	PVD	6.5 ± 0.5	5.7 ± 0.4[a]	5.9 ± 0.8	5.7 ± 0.7[a]	5.6 ± 0.5[a]	5.8 ± 0.8	5.9 ± 0.6	6.5 ± 0.5	6.8 ± 0.8	6.1 ± 0.5	7.1 ± 1.2
	CVD	7.1 ± 0.8	6.0 ± 0.6[a]	5.9 ± 0.8[a]	5.8 ± 0.5[a]	5.8 ± 0.5[a]	5.7 ± 0.7[a]	6.1 ± 0.6[a]	5.9 ± 0.3[a]	5.7 ± 0.3[a,*]	5.9 ± 0.8	7.0 ± 1.1
Albumin (g/dl)	PVD	3.1 ± 0.2	2.6 ± 0.2[a]	2.5 ± 0.3[a]	2.4 ± 0.2[a]	2.5 ± 0.2[a]	2.4 ± 0.1[a]	2.4 ± 0.3[a]	2.6 ± 0.3[a]	2.6 ± 0.3[a]	2.8 ± 0.1	2.6 ± 0.3[a]
	CVD	3.2 ± 0.4	2.6 ± 0.3[a]	2.5 ± 0.4[a]	2.6 ± 0.3[a]	2.5 ± 0.4[a]	2.4 ± 0.5[a]	2.5 ± 0.4[a]	2.5 ± 0.4[a]	2.5 ± 0.3[a]	2.4 ± 0.3[a,*]	2.5 ± 0.4[a]
A/G Ratio	PVD	0.91 ± 0.16	0.82 ± 0.16	0.73 ± 0.14	0.77 ± 0.14	0.83 ± 0.17	0.76 ± 0.25	0.68 ± 0.16[a]	0.69 ± 0.22	0.65 ± 0.15[a]	0.87 ± 0.10	0.67 ± 0.24
	CVD	0.87 ± 0.37	0.76 ± 0.15	0.79 ± 0.14	0.84 ± 0.14	0.80 ± 0.29	0.78 ± 0.36	0.77 ± 0.14	0.77 ± 0.27	0.81 ± 0.22	0.69 ± 0.09	0.63 ± 0.23
SGPT (IU/liter)	PVD	30 ± 11	37 ± 19	29 ± 16	19 ± 5	18 ± 6	18 ± 8	22 ± 6	22 ± 10	28 ± 8	34 ± 18	34 ± 12
	CVD	30 ± 9	51 ± 15[a]	239 ± 265[a,*]	72 ± 48[a,*]	31 ± 13	25 ± 9	29 ± 7	40 ± 25	37 ± 13	28 ± 9	32 ± 3
SGOT (IU/liter)	PVD	33 ± 9	36 ± 5	25 ± 6	32 ± 10	37 ± 9	34 ± 13	37 ± 10	34 ± 12	40 ± 11	41 ± 13	34 ± 6
	CVD	34 ± 9	56 ± 21*	104 ± 103*	35 ± 7	31 ± 7	32 ± 3	34 ± 12	51 ± 23	38 ± 6	40 ± 7	41 ± 7
LDH (IU/liter)	PVD	111 ± 51	324 ± 212[a]	168 ± 34[a]	247 ± 210	322 ± 269	238 ± 210	264 ± 165	224 ± 134	277 ± 187	286 ± 32	81 ± 17
	CVD	115 ± 98	595 ± 405[a]	265 ± 174	189 ± 89	171 ± 41	169 ± 77	241 ± 203	424 ± 442	165 ± 65	244 ± 89	152 ± 80
Cholesterol (mg/dl)	PVD	157 ± 38	126 ± 25	124 ± 32	127 ± 31	112 ± 20[a]	123 ± 21	113 ± 34	117 ± 17	119 ± 28	138 ± 32	128 ± 21
	CVD	144 ± 27	139 ± 31	119 ± 34	141 ± 39	135 ± 28	119 ± 16	137 ± 41	142 ± 25	119 ± 25	126 ± 13	138 ± 32
Triglyceride (mg/dl)	PVD	38 ± 21	52 ± 21	34 ± 26	39 ± 6	54 ± 22	49 ± 17	32 ± 19	31 ± 8	35 ± 7	53 ± 26	46 ± 24
	CVD	52 ± 29	50 ± 9	44 ± 10	44 ± 15	65 ± 19	39 ± 14	38 ± 17	50 ± 26	49 ± 14	80 ± 22	40 ± 10
Peak xylose (mg/dl)	PVD	72.4 ± 12.5	50.1 ± 5.5[a]	45.5 ± 9.4[a]	40.7 ± 11.8[a]	51.1 ± 10.8[a]	34.0 ± 9.9[a]	40.5 ± 23.0[a]	45.3 ± 10.5[a]	46.2 ± 12.4[a]	53.4 ± 11.7[a]	42.8 ± 12.5[a]
	CVD	73.3 ± 13.0	39.4 ± 8.1[a,*]	45.1 ± 16.2[a]	45.5 ± 11.0[a]	36.6 ± 14.0[a]	39.2 ± 11.9[a]	35.5 ± 9.6[a]	43.9 ± 6.5[a]	50.4 ± 2.8[a]	46.1 ± 10.6[a]	49.1 ± 17.5[a]

Note. Values are means ± SD.

[a] Means statistical significance versus preoperative value of the same group.

* Means $P < 0.05$ between PVD and CVD groups at the same period. Tests were performed by an unpaired t test or a Wilcoxon group comparison (SGPT, SGOT, and LDH).

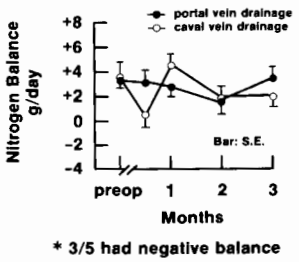

FIG. 2. Nitrogen balance after total orthotopic small bowel transplantation.

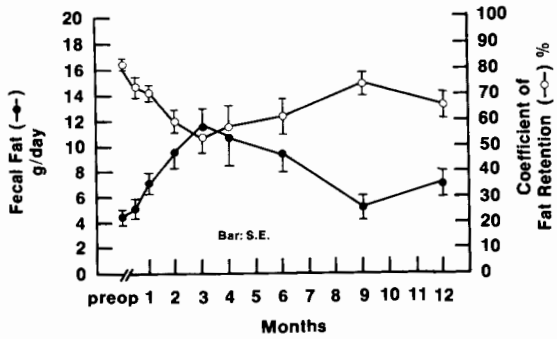

FIG. 4. Fecal fat excretion and fat absorption after small bowel autotransplantation.

10 days postoperatively, there was a 40 to 50% reduction in peak values of xylose. There was a further reduction in peak values for up to 2 months postoperatively (Table 1), which correlated roughly with the pattern of fecal fat excretion and changes in body weight (Figs. 4 and 1, respectively). Gradual recovery began thereafter, but even at 12 months mean peak values were still reduced by about 40% from preoperative levels. There was a significant difference between the PVD and the CVD groups at 10 days postoperatively but not thereafter.

Fat Absorption and Excretion

There was a reduction in serum cholesterol levels postoperatively which plateaued at 2 months after surgery. Even at 12 months after transplantation, serum cholesterol levels tended to remain below preoperative levels (Table 1). Serum triglyceride levels showed no significant change postoperatively (Table 1). Fecal fat determined by 5-day collection averaged 4.7 ± 1.7 g/day preoperatively. Following autotransplantation, fat excretion increased progressively for 3 months by which time there was a mean increase of 3.7 times (range, 1.7 to 6.8) in fat excretion compared to preoperative values (Fig. 4). There was gradual improvement in fat excretion after 3 months, but even at 12 months fat excretion remained abnormal. Mean fat content of dry food was 5.35 g/100 g, for four determinations (range, 4.6–7.3 g/100 g). From this value, the coefficient of fat retention could be calculated as depicted in Fig. 4.

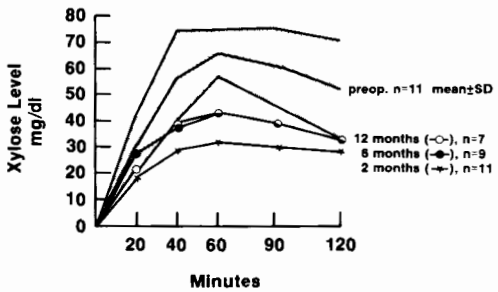

FIG. 3. Xylose absorption in small bowel autotransplantation at various intervals after transplantation. Preoperative values had not been restored even after 12 months following transplantation.

There was no difference in PVD and CVD groups in fat excretion or absorption.

Liver Enzymes

The CVD group showed a significant increase in both GOT and GPT during the first postoperative month. Four of five animals in the CVD group showed a recurrence of serum GPT increase between 4 and 5 months after surgery. Except for sporadic increases, animals with PVD did not show serum enzyme elevations postoperatively (Table 1).

DISCUSSION

The extent of nutritional abnormalities seen in this group of autotransplanted dogs is surprising and was unexpected. Carbohydrate, protein, and fat metabolism was shown to be significantly affected and remained abnormal for the 12-month period of observation after transplantation. The bases for these persistent abnormalities are intriguing and largely unknown. For instance, xylose absorption is known to be affected by stomach-emptying time, the rate of absorption, the duration of absorption, and the rate of xylose metabolism after absorption [9]. It is improbable that there is altered metabolism of xylose in these autotransplanted animals. The basis of abnormal xylose absorption curves must be sought among altered motility and/or absorption patterns [10] following autotransplantation. Denervation of the gut that occurs with autotransplantation could explain some of these abnormalities, since Ballinger *et al.* [5] has previously documented similar nutritional abnormalities with denervation of the gut alone without vascular division or replantation. These experiments were in fact prompted by the latter's observation that abnormalities of gut function were noted after autotransplantation [5]. Even though cold ischemia was utilized during replantation in our experiments, significant enterocyte damage and enzyme hyposecretion may well have played a role in these abnormalities. During the autotransplant procedure approximately 45 cm of small bowel was "lost" due to ex-

cision of unperfused ends of the graft and also from construction of the Thiry-Vella loop. Loss of absorptive surface from this relatively small length "lost" is unlikely to have been the basis of the noted abnormalities.

The persistently increased fat excretion was again unexpected and surprising. Certainly, lymphatic disruption could have explained decreased fat absorption in the early postoperative period. It has been established by a variety of methods that lymphatic reconnection to the small bowel autotransplant occurs within a few weeks after surgery [11–13]. Therefore, the persistence of steatorrhea must be explained by problems with other essential elements of fat absorption, such as mucosal cell integrity, bile salt concentrations, and pancreatic secretions; none of these were studied in this experiment. Since oral cyclosporine absorption is dependent upon the integrity of fat absorption [1, 14], the observed steatorrhea will pose a problem for administration of this drug in allotransplanted animals requiring immunosuppression.

It was clear from this investigation that vena caval drainage was inferior to portal venous drainage of autotransplanted small bowel. As a general rule, the former group evinced a greater degree and persistence of nutritional abnormalities. Elevation of enzymes in the CVD group can be explained by abrupt and significant reduction of portal vein blood flow because of the surgical procedure. It is well known that total diversion of portal flow (Eck fistula) carries a high mortality in the dog. Also, the deleterious effect of portacaval shunt on long-term nutritional function has been recognized [15]. If technically feasible, therefore, portal venous drainage should be preferred to vena caval drainage in small bowel transplantation.

The wide spectrum of nutritional abnormalities depicted denotes a discouraging portent for allotransplantation of small bowel. Malfunction of allotransplanted gut has been recognized by others [16] but has been generally attributed to chronic rejection. The experiments reported here demonstrate that even without the deleterious effects of acute, subacute, or chronic rejection, and without the additional complications imposed by immunosuppression, autotransplantation of the entire small bowel results in abnormal digestive and absorptive performance. For reasons of satisfactory immunosuppression and avoidance of rejection, there is evidence (unpublished observations) to indicate that allotransplantation of the small bowel should be limited to the smallest length necessary to support nutrition. From the extent of nutritional abnormalities witnessed following autotransplantation, it is clear that significant reductions in small bowel length for allotransplantation will not be possible. In addition, decreased cyclosporine absorption in allotransplantation could be expected. Even if small bowel allotransplantation can overcome these difficulties, abnormal nutritional performance should be expected. Despite successful allotransplantation, continued hyperalimentation (enteral or intravenous) in the postoperative period would appear to be required.

ACKNOWLEDGMENT

This work was supported by NIH Grant No. SAT 5 R01 AM32249-02.

REFERENCES

1. Reznick, R. K., Craddock, G. N., Langer, B., Gilas, T., and Cullen, J. B. Structure and function of small bowel allografts in the dog: Immunosuppression with cyclosporin A. *Canad. J. Surg.* **25:** 51, 1982.
2. Craddock, G. N., Nordgren, S. R., Reznick, R. K., *et al.* Small bowel transplantation in the dog using cyclosporine. *Transplantation* **35:** 284, 1983.
3. Pritchard, T. J., and Kirkman, R. L. Small bowel transplantation. *World J. Surg.* **9:** 860, 1985.
4. Kirkman, R. L. Small bowel transplantation. *Transplantation* **37:** 429, 1984.
5. Ballinger, W. F., II, Christy, M. G., and Ashby, W. B. Autotransplantation of the small intestine: The effect of denervation. *Surgery* **52:** 151, 1962.
6. Hawkins, K. I. Pediatric xylose absorption test: Measurement in blood preferable to measurement in urine. *Clin. Chem.* **16:** 749, 1970.
7. Roe, J. H., and Rice, E. W. A photometric method for the determination of free pentoses in animal tissues. *J. Biol. Chem.* **173:** 507, 1948.
8. Van de Kamer, J. H., ten Bokkel Huinink, H., and Weyers, H. W. Rapid method for the determination of fat in feces. *J. Biol. Chem.* **177:** 347, 1949.
9. Sammons, H. G., Morgan, D. B., Frazer, A. C., Montgomery, R. D., Philip, W. M., and Phillips, M. J. Modification in the xylose absorption test as an index of intestinal function. *Gut* **8:** 348, 1967.
10. Stanley, J. C., Brink, B. E., and Fry, W. J. Experimental intestinal ischemia: Absorption studies following gradual celiac and superior mesenteric artery occlusion. *J. Surg. Res.* **14:** 133, 1973.
11. Kocandrle, V., Houttuin, E., and Prohaska, J. V. Regeneration of the lymphatics after autotransplantation and homotransplantation of the entire small intestine. *Surg. Gynecol. Obstet.* **122:** 587, 1966.
12. Ruiz, J. O., Uchida, H., Schultz, L. S., and Lillehei, R. C. Problem in absorption and immunosuppression after entire intestinal allotransplantation. *Amer. J. Surg.* **123:** 297, 1972.
13. Goott, B., Lillehei, R. C., and Miller, F. A. Mesenteric lymphatic renegeration after autografts of small bowel in dogs. *Surgery* **48:** 571, 1960.
14. Mackenzie, R., Nordgren, S., Lossing, A., *et al.* Cyclosporin A absorption in canine small intestinal transplantation. *Transplant. Proc.* **14:** 646, 1982.
15. Fisher, B., Lee, S., Fedor, E. J., and Levine, M. Intestinal absorption and nitrogen balance following portacaval shunt. *Ann. Surg.* **167:** 41, 1968.
16. Diliz-Perez, H. S., McClure, J., Bedetti, C., *et al.* Successful small bowel allotransplantation in dogs with cyclosporine and Prednisone. *Transplantation* **37:** 126, 1984.