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THE EFFECT OF DENERVATION ON INTESTINAL PERMEABILITY AND FUNCTION FOLLOWING SMALL INTESTINAL TRANSPLANTATION

By

Shengtao Yao 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE**

in

Experimental Surgery

Department of Surgery

Edmonton, Alberta

Fall, 1997

University of Alberta

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Degree: Master of Science

Year This Degree Granted: 1997

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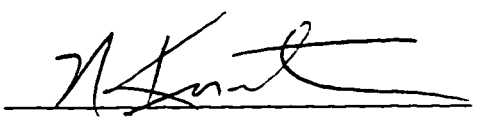

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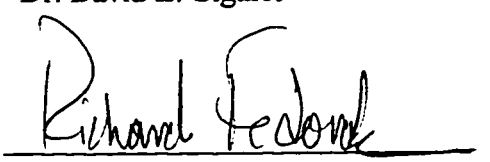

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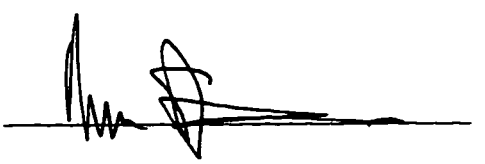

Dr. Bruce R. Stevenson

Sept. 30, 1997

DEDICATION

This text is dedicated to my parents **Rui-Min** and **Xiou-Fang** who have always provided unfailing support and encouragement and to **Rong** for her continuous love and support.

ABSTRACT

Previous studies have demonstrated reduction in active and passive nutrient absorption, and increase in intestinal permeability following small intestinal transplantation. Accordingly we hypothesised that the reduction in nutrient absorption is due to an increase in intestinal permeability caused by denervation. **Method:** Male Lewis rats were randomly assigned to three groups (26 rats for each group): sham, denervation and transplantation. The permeability to inulin was assayed at 2 weeks (n=10), 6 weeks (n=10), and 6 months (n=6) in Ussing chambers. Tyramine was applied for the confirmation of the denervation status. Hypersensitivity to epinephrine after denervation was also assessed. **Results:** There was an increase in permeability to inulin in all three groups at 2 weeks postoperation. By 6 weeks, the permeability in the sham group had normalized, but the permeability to inulin in both denervation and transplantation groups remained increased. There was no difference in permeability to inulin among the three groups by 6 months. In the denervation and transplantation groups, there was evidence of denervation, with decreased sensitivity to adrenergic neurotransmitter release by tyramine at 2 week, 6 week, and 6 month studies. There was no hypersensitivity to epinephrine through out the period of study. **Conclusion**: The denervation following small intestinal transplantation is associated with increase in permeability, and the increased permeability recovers with time. This does not appear to have a significant impact on nutrient transport in the long term.

ACKNOWLEDGEMENTS

I wish to express sincere thanks to my supervisors: Dr. David L. Sigalet and Dr. Norman M. Kneteman.

I would like to thank Dr. Richard N. Fedorak and Dr. Karen Madsen for their advice and help throughout this study, and Dr. Lin-fu Zhu for his help with microsurgical transplant procedures.

I also greatly appreciate the support I have received from Rong Huang during the preparation of the thesis and thank her for statistical advice.

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Chapter I

INTRODUCTION

Short bowel syndrome is the clinical manifestation of a fundamental reduction in the functional intestinal absorptive surface area with consequent malabsorption [1]. Most commonly, it results from massive resection of the small intestine as treatment for necrotizing enterocolitis, mesenteric vascular accidents, inflammatory bowel disease, and trauma [2]. Prior to the development of total parenteral nutrition (TPN), the long-term prognosis for patients with short-bowel syndrome was very poor.

I. Total Parenteral Nutrition

Patients with short bowel syndrome are presently managed with total parenteral nutrition. However, there are many disadvantages to using TPN as a long term treatment. First, TPN is expensive [3]. In 1978 at the Cleveland Clinic, TPN cost $ (US) 21,000 for each patient managed at home for the first year. This included patient training, nondisposable equipment, maintenance supplies, and follow-up including pharmacist resupplies, physician examination, and monthly laboratory tests. In total it would cost $ 19,700 per patient per year for the following years. The cost would be $ 74,000 per year for each patient if in hospital. Second, TPN requires long-term venous access which greatly limits the lifestyle of the patients and their family [4]. Finally the most important disadvantage associated with TPN, especially in infants and children, is TPN associated cholestasis and secondary liver dysfunction [5]. Prolonged use of TPN may result in severe cholestasis in 30 to 42% of all

infants receiving TPN. TPN is also primarily supportive, not a curative, option for the patients with short bowel syndrome [1]. Several studies have shown that 50% of TPN patients are children who still have a reasonably high mortality, which is significantly associated with the complications of TPN related liver failure [62]. The mortality in adults from liver failure with short-bowel syndrome is less and is estimated to be 5-10%. The long-term prognosis of children with short-bowel syndrome is also considered to be worse than in adults. This appears to be related to growth problems, vascular access difficulty, and problems with psychosocial development [89].

The recently growing experience in both animal and human small intestinal transplantation gives the hope for a definitive alternative for the patients with short bowel syndrome and will allow them to resume totally oral nutrition. Since rejection and graft-vs-host-disease (GVHD) still remain as the major obstacles, immunological aspects of small intestinal transplantation have been extensively studied [6-14]. However, the functional capacity of the small intestine following transplantation is rarely investigated.

II. The Development of Small Intestinal Transplantation

As early as 1959, when transplantation of other organs was performed, the notion of transplantation of small intestine as a potential therapy for short bowel syndrome patients was investigated in laboratory animals by Lillehei et al. in Minnesota [2,66]. They used standard microsurgical techniques that were based on the original work of Carrel in the 1900s to construct vascular anastomoses in small intestinal transplantation. The surgical techniques for small intestinal transplantation, as well as graft preservation, were developed by this group

[67]. They demonstrated that the procedure was technically feasible. However, the survival time for dogs undergoing orthotopic allotransplantation of the small intestine was only 8-15 days [68].

Despite the rather poor experimental results in the 1960s and early 1970s, seven cases of human small intestinal transplantation were performed and reported by 1972 [69,70]. The first two cases were performed by Detterling and associates at the Boston Floating Hospital in 1964 [67]. In 1967, Lillehei et al. performed a cadaver donor transplantation of jejunum, ileum, and right colon into a 46-year-old woman [71]. In 1968, Okumura et al. performed the same operation on a 34-year-old woman [67]. Oliver et al. transplanted the entire small intestine and right colon into a 35-year-old man with Gardner's syndrome in 1969 [72]. In 1971, Alican et al. reported a mother-to-child transplantation of one metre of ileum in a 10-year-old child [73]. Fortner et al. also performed a sister-to-sister (HLA identical) transplantation of 1.7 metre segment of ileum in a 37-year-old woman in 1972 [74]. This was the only one who had partial graft function. All of the cases were failed, most due to either acute rejection or technical deficiencies. One patient lived for 76 days, taking nutrition through the transplanted intestine for part of this time, before dying of systemic infection associated with rejection [70].

Those poor results, and the introduction of total parenteral nutrition in the early 1970s, meant that few further attempts at small intestinal transplantation were made. The introduction of cyclosporin by Borel in 1974 [67] and the demonstration of its specific effects in experimental transplantation in 1976 [75] led to a flurry of clinical trails in kidney and later in liver and other organ transplantation in humans [67].

With the promising results obtained in kidney, liver, and heart transplantation utilizing cyclosporin, ten known intestinal and four combined liver-small intestinal transplantations were performed with cyclosporin in the 1980s [76-79]. However, all of the clinical attempts at small intestinal transplantation to this point were unsatisfactory although several groups have demonstrated cyclosporin to be effective in small intestinal transplantation in rats, pigs and dogs [6-8,10,90,101,116].

In 1989, the first successful liver/small intestinal transplantation was carried out by Grant et al. in London, Ontario, Canada [79]. The patient had an early rejection episode. He took a normal diet shortly after the transplantation [85]. Four further transplantations were performed in this series and three of the five patient were well with functioning grafts [86].

In 1990, Starzl et al. carried out the first of a series of small intestinal and liver/small intestinal transplantations using the new immunosuppressive drug tacrolimus (FK 506) which had previously been shown to be effective in liver transplantation [87]. With the introduction of tacrolimus, another significant step was made toward a substantial improvement in the outcome after small intestinal transplantation. The most recent results (1989-1994) reported by several major centres indicated that patient survival, which was 25% in the cyclosporin era, has improved to 67% since the introduction of tacrolimus, while graft survival has increased from 19% to 57%. Other new and more specific immunosuppressive agents which are currently being under investigation in combination with cyclosporin or tacrolimus are mycophenolate mofetil (RS-61443), rapamycin, deoxyspergualine, and brequinar sodium.

Todo et al. reported 30 cases of small intestinal transplantation in early 1990s from the same centre [88]. 8 of the 9 small intestinal transplantation recipients were alive from 8

to 15 months. 13 of the 17 combined liver and small intestinal transplant patients were alive from 5 to 30 months posttransplantation. All of the 13 surviving recipients, except one, were completely free from total parenteral nutrition. Abdominal multi visceral transplantations were performed in 4 patients as well. All of the 4 patients were alive with functioning grafts from 3 to 15 months. From 1989 through 1994, 100 intestinal transplantations, combined intestine/liver transplantations, and intestine as part of a multi visceral transplant procedure were performed [89].

III. The Animal Models Used in Small Intestinal Transplantation

1. Dog

In 1902, Carrel reported the first vascularized organ transplant and stated that it would be possible to transplant portions of small intestine into the neck of dogs [90]. Since the anatomy of the mesenteric circulation in dog has little differences from man except in nomenclature of the vessels, Lillehei et al. started to investigate small intestinal transplantation on a dog model in 1959 [66]. They established a technique for autografting of the intestine. They isolated and divided the superior mesenteric vessels and replaced the small intestine by only suturing the superior mesenteric vessels and anastomosing the small intestine to the duodenum proximally and the colon distally. By using the dog model, they observed that the intestine could tolerate up to 5 hours ischemia *in vivo* if cooled to 5^0c. They found regeneration of the lymphatic connections between the graft and the central lymphatic systems 3 weeks after transplantation. They also studied rejection by placing isolated loops of intestine in the neck to observe graft rejection [2,66,71].

During 1960s, immunosuppressive drugs such as azathioprine, cyclophosphamide, vinblastine sulfate, antilymphocytic globulin, prednisone, and steroids were used in small intestinal transplantation in the dog model. Their effects, however, were limited [91-93]. In the 1970s, cyclosporin was first used on a small intestinal transplantation dog model by Reznick et al. [94]. Later on, other groups used dogs to demonstrate that cyclosporin prolonged the survival of small intestinal allografts [6-8]. D'Alessandro et al. found that the combination of RS-61443 and cyclosporin significantly prolonged canine intestinal allograft survival [13].

Dog models were also used to observe absorptive functions after small intestinal transplantation as well [95,96]. The results indicated that the impaired absorption following small intestinal transplantation was due to intestinal denervation and was related to bacterial overgrowth [95,96].

The preservation of intestine was also investigated in dog models. The experiments showed that cooling the intestine with iced saline sponges could retard ischemia induced histological changes in the intestine [97].

2. Pig

The pig is biologically much closer to man than other animals [98] and digestion in the pig is similar to the human [99]. Both Ricour and Pritchard reported small intestinal transplantation on pig models and examined the effects of cyclosporin on immunosuppression using different surgical techniques [9,100]. As a consequence, they had different results. Ricour's group anastomosed the graft with superior mesenteric artery to aorta and superior mesenteric vein to infra renal vena cava, and found that cyclosporin as a single agent as well

as when used with supplemental therapy could not prevent rejection of small intestinal grafts in pig. However, Pritchard et al. used cyclosporin with drainage of the superior mesenteric vein into the portal circulation and found survivals from 100 to 200 days without graft rejection. There were other groups studying the technical problems and immunosuppressive agents in the pig model of small intestinal transplantation [101-104]. Balen et al. investigated the techniques of a multi visceral (liver, duodenum, and pancreas) transplantation model on pigs [105]. The pig model of small intestinal transplantation was also used to examine the new immunosuppressive drug tacrolimus [106]. Sigalet et al. investigated segmental intestinal transplantation in the pig as a model for small intestinal transplantation using live related donors for clinical practice in the future [175].

3. Other Animals

There were also other animal models used in small intestinal transplantation. Zhong et al. developed a mouse intestinal transplantation model [107-109]. They anastomosed the donor aorta to the recipient aorta and donor portal vein to the recipient infra renal vena cava. This small intestinal transplantation model was used to study the immunology of intestinal grafts at the molecular level. Rabbits were also used to investigate the prevention of ischemic effects on anastomosis by Dockendorf et al. who found that wrapping the anastomosis with a vascularized omental pedicle could augment blood flow [110].

4. Rat

Certain studies of experimental transplantation demand the use of inbred animals. Cost and animal source are additional considerations that have resulted in studies being carried out on small-sized and low-cost animals, such as rats [111].

In 1971, Monchik and Rusell first published a technique of transplanting the entire small intestine in an auxiliary way with venous out-flow into the infra renal vena cava in rats [111]. They also established the standard for initial investigation of immunosuppression on the rat model. Based on Sakai and Fukuda et al.'s finding that the rejection pattern of intestinal graft was altered by draining its venous blood into portal vein [112,113], Kort et al. anastomosed the mesenteric vein to the recipient's portal vein in the rat to reduce the rejection of the graft [114]. This was also considered a better physiological posttransplant state [115]. There were other techniques investigated on rat models such as combined small intestine and colon transplantation [2,116], using recipient renal pedicle for the venous connections [2,117], and using the superior mesenteric artery as the arterial connection [2,118]. Rat models were used to investigate the prevention of leakage from intestinal anastomoses by an omental wrap [119,120]. Zhong et al. developed two new models of combined liver and small intestine transplantation in the rat [121]. Kellnar et al. also used fetal rat intestinal transplantation as therapy for the short bowel syndrome in the rat model [122]. They showed that in the rat model fetal small intestine can be an actual functioning substitute for normal small intestine.

The rat models have been widely used in investigating immunosuppression and related problems. Since one of the key factors for small intestinal transplantation is immunosuppression, many groups have investigated the effects of cyclosporin on small intestinal transplantation in the rat model after this drug was introduced in the 1970s [11,12,90,111,123,124]. The results have shown that cyclosporin can successfully control both rejection and graft-versus-host disease after small intestinal transplantation. However,

cyclosporin has many adverse effects on the functions of both the graft and host tissues [125]. It effects nutrient absorption by reducing active glucose uptake at the cellular level due to impacts on sodium/glucose transport and impairment of insulin release by pancreatic islets, and also damages kidney [126-128]. The newer immunosuppressive agent tacrolimus has also been studied in rat small intestinal transplantation [14,129-131]. The studies provided evidence that rejection of small intestinal allografts could be successfully prevented and treated with tacrolimus. Several methods were used to detect the rejection phenomenon of grafts in the rat model, including endoscopic examination, intestinal fatty acid-binding protein (I-FABP) content in serum, and hyaluronan accumulation in the lamina propria of the graft [132-134].

The digestive and absorptive function of small intestinal graft was evaluated in rat models of small intestinal transplantation [135]. These studies indicated that the absorptive function for water, electrolytes, and protein following small intestinal transplantation was normal [136]. However, the transport of glucose and absorption of fat were reduced. These may be attributed to denervation [137]. There have also been studies on absorption of Vitamins such as Vitamin A in the rat model [138].

Other works with the rat model of small intestinal transplantation were reported. These included preservation of the small intestinal graft, regeneration of sympathetic activities in small intestinal transplantation, and other studies [139].

IV. The Application of Small Intestinal Transplantation in Humans

After more than three decades of experimental and clinical investigation, several different procedures of small intestinal transplantation have been performed in man: isolated small intestinal grafts, combined liver and small intestinal grafts, and multi visceral grafts [88]. Up to 1996, about 185 clinical small intestinal transplants (including isolated small intestine, combined liver/small intestine, and multi visceral transplantation) were reported by centres in the United States, Canada, and Europe [79,80-84].

1. Isolated Small Intestinal Transplantation

The first two human small intestinal transplants were performed at Boston Floating Hospital in 1964 by Detterling et al. and never reported in detail [70]. On April 5, 1967, Lillehei and colleagues performed a small intestinal transplantation on a 46-year-old woman. The standard microsurgical techniques, based on the original work of Carrell in the 1900s, were used to construct vascular anastomoses [66]. An end-to-side superior mesenteric-ileal venous anastomosis and an end-to-side superior mesenteric artery-iliac artery anastomosis were performed. Then a jejunostomy and colostomy with the proximal and distal ends of the graft were performed. The restoration of intestinal continuity was planned at a later date. Unfortunately, the patient died of shock 12 hours after the transplantation [66]. Since then, there have been dozens of attempts at clinical small intestinal transplants [70,71,73,140,141]. In most clinical cases of small intestinal transplantation, the grafts were initially placed out of continuity with the native intestine in order to avoid anastomotic breakdown. The ends of the graft were either both exteriorised as stomas, or the proximal end was anastomosed to the native jejunum and the distal end was brought out as a stoma [77,82,142,143]. The latter

techniques allowed normal upper gastrointestinal secretions and enteral nutrition to flow through the graft which could improve mucosal nutrition [144]. The graft was put into continuity with the native intestine at a second operation at a later date.

2. *Combined Liver and Small Intestinal Transplantation*

It has been known for many years that combining liver transplantation with grafting of another organ reduces the risk of rejection of the second organ, such as kidney [145]. In addition, long term TPN leads to steatosis and liver failure [62]. Combined liver and small intestinal transplantation is partially based on those consequences [146].

The first successful combined liver and small intestinal transplantation in man was performed by Grant et al. on November 13, 1988 [79]. The donor jejunum, ileum, liver, and abdominal aorta along with the superior mesenteric artery and coeliac artery were isolated. The liver and the small intestine were flushed in situ with 0.9% NaCl containing 2.1% mannitol, removed *en bloc*, and stored for 90 minutes at 4°C. The graft was placed in an orthotopic location. The donor and recipient infrarenal vena cava were joined end to end. The end of the donor aortic conduit was anastomosed to the side of the recipient infrarenal aorta, the end of the recipient portal vein to the side of the portal vein of the graft. The recipient's distal large intestine was oversewn. The end of the donor jejunum was anastomosed to the recipient's duodenum. The end of the graft ileum was exteriorized as an ileostomy. The patient's liver enzymes, bilirubin concentrations, and prothrombin times returned to normal range 2 months posttransplantation. Enteral feeding gradually increased 1 week postoperation. The patient maintained a normal weight on an unrestricted oral diet 8 months posttransplantation.

Subsequently, there have been several attempts at combined human liver and small intestinal transplantation. Combined liver and small intestinal transplantation was initially performed in patients with liver failure resulting from long term total parenteral nutrition [79], but after several successful cases, the combined liver and small intestinal transplant has now been proposed for and used in the same kind of patients with normal liver function [86].

3. Abdominal Multi Visceral Transplantation

Abdominal multi visceral transplantation is performed for patients who have abdominal malignancies or extensive thrombotic disorders involving both the celiac axis and superior mesenteric artery or possibly the entire splanchnic system, or severe motility disorders that involve the entire gastrointestinal tract [79,147].

Starzl reported ten multi visceral transplantation cases for treatment of abdominal malignancies in 1989 [148]. All of the patients received stomach, liver, duodenum, pancreas, and small intestine. The donor operation was based on the standard multiple organ procurement procedure [158]. The entire abdominal aorta was mobilized, and ligated, and all branches were divided except the superior mesenteric artery and coeliac axis. The mesenteric and other posterior parietal connections, including the afferent fibres to coeliac and superior mesenteric ganglia, were severed. The stomach was transected at the espophagogastric junction. The colon was transected in the descending or sigmoid portions. After perfusion, the vena cava was transected above and below the liver, and the aorta was cut free well above the coeliac axis. For the recipient, the visceral attachments to the posterior parietes were severed, and the coeliac axis and superior mesenteric artery were ligated and divided. The stomach was divided leaving a small cuff at the diaphragm for subsequent anastomosis to the

donor stomach. The vena cava was severed above and below the liver. The liver, stomach, pancreas, spleen, small bowel, colon, and omentum were then removed *en bloc*. The donor organs were removed from the ice bath, positioned and revascularized. The vena cava was anastomosed above and below the liver. The aortic graft was attached to the abdominal aorta by an end to side anastomosis, and the specimen was arterialized. The gastrointestinal continuity was reestablished with a gastrogastrostomy proximally and end colostomy distally. Eight of the ten patients were alive 3-9 months after transplantation. The eight surviving patients ate well enough to support their nutritional needs. All patients had normal or nearly normal liver functions. None of the surviving patients required insulin at any time, however, several exhibited severe pancreatitis.

There were also other reports of multi visceral transplantation in humans. Starzl and Williams et al. each performed multi visceral transplantation in two infants. Among the 4 cases, one infant was alive 193 days after transplantation, and one was surviving 109 days posttransplantation [148,149].

4. Small Intestinal Transplantation with Bone Marrow Transplantation

Bone marrow cells recovered from the same donor as the intestine can be infused intravenously into the recipient during the transplantation. The rationale for simultaneous bone marrow infusion in solid organ transplantation was from the observations that donor leukocytes from transplanted organs had migrated and survived throughout the body of the recipient for as long as three decades [159]. The major counter argument regarding the infusion of bone marrow with small intestinal transplantation is the risk of graft-vs-host-

disease (GVHD). There has been no reported direct evidence of GVHD in the studies carried out by Starzl's group to date [160].

The two main problems with clinical small intestinal transplantation are rejection and graft-versus-host disease. The technical problems of small intestinal transplantation were largely solved during previous research [2]. Before cyclosporin become available, all nine patients who had undergone small intestinal transplantation died of either technical failure or rejection and graft-versus-host disease. After cyclosporin was introduced, the survival period of patients having a small intestinal transplantation has significantly increased. Tacrolimus, an immunosuppressive agent, has been equally successful in both monotherapy and in combination with azathioprine and corticosteroids [150-152]. Although there have been a number of successful small intestinal transplants in recent years, most have been associated with postoperative complications, often due to infection which were likely related to heavy immunosuppression [58]. The responsible microorganisms included bacteria, fungi, and viruses. Among the viruses, cytomegalovirus and Epstein-Barr virus associated B-cell lymphomas attributed to the most losses. There have also been other failures which could be traced back to surgical or management errors [58]. The technical surgical errors included intestinal anastomotic leakage, hepatic artery thrombosis, biliary anastomotic leakage, and cerebral infarction associated with intraoperative cardiac arrest. The management errors included overdosing or underdosing of immunosuppressive agents. However, the recipients were extremely ill, some of them were in advanced stages of liver failure at the time of surgery. In some American centres transplantation is now offered to patients before they

develop life threatening complications of total parenteral nutrition, and a corresponding reduction in posttransplantation morbidity is anticipated [76].

V. Physiology of the Intestinal Epithelium

The epithelium of the small intestine is located at a strategic interface since the intestinal lumen is in continuity with the external environment. Material absorbed from the lumen must first traverse the epithelium to gain access to the mucosal blood and lymph vessels for distribution to more distant tissues.

The two major functions of the intestinal epithelium are to absorb large quantities of water, electrolytes, and nutrients that are presented to the intestinal lumen and to limit the diffusion of molecules that are selectively concentrated on each side of the epithelial cells [15,16]. This latter selecting property of the epithelium is referred to as permeability [16,17,18].

1. The Structure and Function of Intestinal Epithelium

The mucosa of the small intestine consists of three distinct layers [19]. The muscularis mucosae, the deepest, contains a thin sheet of smooth muscle cells and separates the mucosa from the submucosa. The lamina propria, the middle layer, consists of blood vessels, lymph vessels, small unmyelinated nerve fibres and various connective tissue cellular elements. The epithelium consists of crypt (secretory), and villous (absorptive) cells [19].

Epithelium forms a semipermeable boundary and acts to regulate the transit of electrolytes and molecules [20]. There are two regulatory components: the epithelial cells (transcellular pathway) and the space around the epithelium (paracellular pathway). The major

permeability pathway across the epithelium is the paracellular pathway. It contributes more than 85% of passive permeation even for molecules as small as ions [60].

The space between individual epithelial cells must be sealed so that transepithelial osmotic and electrical gradients are maintained [21]. There are several different intercellular junctions at the apical end of intercellular borders to serve adhesive and permeability barrier functions between the individual epithelial cells (Figure 1) [20, 21, 22]. Gap junctions create intercellular pores which allow communication of small molecules between adjacent cells; desmosomes and adherent junctions act as an adhesive function; tight junction or zona occludens, the apical most of these intercellular junctions, is composed of variable numbers of points or "kisses" at which the plasma membranes of adjacent cells come to very close contact [21-24]. Since other junctions cannot restrict free diffusion of macromolecules within them, tight junctions remains the major paracellular pathway barrier.

2. The Absorptive Function

The intestinal absorption of simple sugars and amino acids is coupled to Na^+ transport and facilitated by a high Na^+ concentration on the mucosal side of the intestinal epithelial cells [22, 26-28]. Glucose and Na^+ share the same carrier molecule into the epithelial cells (Figure 2) [27]. Since the concentration of intracellular Na^+ is lower than the concentration of Na^+ on the mucosal surface of the cell, and the intracellular compartment is at least 40 mv negative with respect to the mucosal solution, Na^+ moves into the cell along its concentration gradient and the electrodiffusion gradient [29,30]. When Na^+ binds to the carrier molecule, the carrier molecule increases its affinity for glucose. After both Na^+ and glucose are bound to the carrier molecule, the carrier undergoes a conformational change during which both molecules are

transported across the membrane. Glucose moves with the Na^+ and is released into the cell. A steady state of low Na^+ concentration within the cell is maintained by the activity of the Na^+/K^+ ATPase at the basolateral membrane. The accumulation of glucose in the cell raises the overall internal glucose concentration above that of the extracellular fluid at the serosal surface, so that glucose diffuses into the interstitium and then to the capillaries. Absorption of amino acids follows the same pattern as glucose transport. The transport of Na^+ from lumenal to serosal side of epithelial cells creates an osmotic effect leading to a flow of water in the same direction across the tight junction [22,31,32]. The active glucose transport not only increases the rate of fluid absorption but in addition increases the width of the tight junction also, thus increasing permeability to large solutes and promoting the absorption of nutrients by solvent drag [17,33-35]. Thus, alterations in intestinal permeability probably reflect changes in permeation through the intercellular junctions [25,29,30,35].

Small intestinal permeability can be affected by various factors such as celiac sprue, rheumatoid arthritis, Crohn's disease, and rejection following small intestinal transplantation [37,53-56]. The increasing intestinal permeability caused by the above factors would favour the Na^+ reverse flux from serosal side to mucosal side of the epithelium, thus decreasing the osmotic effect from the lumenal to serosal side of the epithelial cell. Consequently, the net Na+, water, and nutrients absorption would be reduced [50,51].

3. The Measurement of Permeability

Intestinal permeability can be detected by changes in the penetration of poorly absorbed water soluble molecules, and by tight junction morphological studies [25].

A. Inert Solutes

To assess intestinal permeability studies, poorly absorbed probes are administered orally. Since small quantities of probes penetrate across the epithelial cells, their excretion rate in the urine over the following period of time is used to measure their penetration through the intestinal epithelial barrier [16,18]. It is important that the absorbed probes can be rapidly and fully excreted by the kidney without metabolic depletion and endogenous synthesis [36]. The poorly absorbed probes also can be used in a Ussing chamber to assess the intestinal epithelial permeability directly [176]. A variety of probes have been used for permeability assessment including mannitol, rhamnose, lactulose, 51Cr-EDTA, 99mTc-DTPA, and PEG 400 [16,18,38-40].

Transmucosal penetration of these probes is mainly through two different routes: through the transcellular pathway (rhamnose), and through the paracellular pathway or tight junction (lactulose, 51Cr EDTA, 99mTc-DTPA, inulin, and PEG 400).

Mannitol is a widely used water soluble probe with a molecular weight of 182 Daltons and cross sectional diameter of 0.67 nm. The mechanical mannitol permeability of intestinal epithelium is mediated by passive diffusion and solvent drag and related to net water flux [37]. Mannitol passes mainly through the paracellular pathway [41,42]. It is not hydrolysed by intestinal enzymes, has no significant mediated absorption, and is fully excreted unchanged in urine [43,44].

Lactulose (mol wt. 340) resists degradation by intestinal disaccharidases, and less than 0.5% is absorbed. The absorbed lactulose is excreted totally in urine after oral administration [39,44]. Lactulose penetrates intestinal mucosa through the paracellular pathway [41,45]. The

slight absorption of 99mTc-Diethylenetriaminopentaacetic acid (DTPA) (mol wt. 393) also occurs along the paracellular pathway [46]. 99mTc-DTPA cannot be absorbed by the normal intestinal mucosa. It is excreted in urine after oral administration [40].

Inulin is a big probe with a molecular weight of 5000 Daltons [47]. It crosses the epithelium through the paracellular pathway only, using aqueous channels [47].

B. Electron Microscopy

The structural changes of tight junction width, the thickness of paracellular gaps, and particle size can be analysed using electron microscopy [48, 49].

By freeze fracture electron microscopy, the tight junction appears as a set of long parallel, linear fibrils with a meshwork of interconnecting strands [49]. Since a single strand is shared by adjacent cells and transjunctional molecular flow must pass through strands or gaps in strands, the number of strands determines the tight junction permeability [52].

The tight junction is highly dynamic and capable of structure reorganization [180]. The relationship of tight junction morphology to permeability could be resolved on molecular analysis of junction elements such as ZO-1, ZO-2, cingulin, and occludin [181-184].

4. The Effects of Intestinal Permeability

Intestinal permeability can be affected by various factors such as celiac sprue, rheumatoid arthritis, Crohn's disease, nonsteroidal anti-inflammatory drugs (NSAID) induced enteritis, rejection after small intestinal transplantation, etc [37, 53-56]. Since the Na/Glucose transport system could modulate the width of the tight junction [17], Cyclosporin A may indirectly affect the intestinal permeability by affecting the activity of the Na/Glucose cotransportor [54, 57].

The increasing intestinal permeability caused by the above factors would favour the Na^+ reverse flux from serosal side to mucosal side of the epithelium, thus reducing the net Na^+, water, and nutrient absorption [50, 51].

VI. The Innervation of Small Intestine

Small intestine is richly supplied with nerves. The autonomic nervous system of small intestine consists of three divisions: the sympathetic, parasympathetic, and enteric. These nerves form a complex communication network and an effector system that plays an important role in the control of intestinal function [62].

1. The Enteric Division

The enteric division of the autonomic nervous system is an independent, and integrative system that differs in structure and function from the sympathetic and parasympathetic divisions of the autonomic nervous system [63]. It consists of the myenteric plexus which is located between the longitudinal and circular muscle layers and the submucosal plexus which lies within the submucosa (Figure 3). Myenteric and submucosal plexuses are ganglionated and are interconnected into a single functional system. Nerve fibres from the ganglionated submucosal plexus extend into the mucosa to form a nonganglionated mucosal plexus adjacent to the muscularies mucosae, surrounding the crypts and subjacent to the villous cells. The submucosal plexus functions to control and coordinate absorptive and secretory function, blood flow, and contractility of the muscularis mucosae. Most of the myenteric neurons project to the muscularis externa and are probably involved in the generation of specific motility patterns.

In order for neurons to have an influence on the epithelium, neurotransmitters must be released to the enterocytes. Some neurotransmitters such as acetylcholine, vasoactive intestinal peptide (VIP), peptide histidine isoleucine (PHI), substance P, cholecystokinin (CCK), somatostatin, neuropeptide Y (NPY), and catecholamines (norepinephrine or epinephrine) have direct effects on the epithelia. Other neurotransmitters such as 5-Hydroxytryptamine (5-HT) and opioid peptides affect mucosal function indirectly by acting on submucosal motor neurons that innervate the epithelial cells [63]. There are receptors specific for each chemical messenger presenting on the enterocytes in order for neurotransmitters to influence mucosal function. Acetylcholine inhibits active chloride absorption or stimulates active chloride secretion, or both. VIP and PHI cause an increase in short-circuit current that is associated with an inhibition of sodium and chloride absorption or chloride secretion, or both [65]. Substance P acting on enterocytes increases transmural electrical PD and short-circuit current that increases chloride secretion. Somatostatin and NPY decrease short-circuit current and transmural PD by acting directly on enterocytes. They stimulate sodium and chloride absorption by increasing neutral sodium chloride influx at the brush border membrane. Norepinephrine or epinephrine reduces short-circuit current and transmural PD, stimulates sodium, chloride, and water absorption, and decreases bicarbonate secretion. 5-HT increases short-circuit current and transmural PD due mainly to chloride secretion [60]. Opioid peptide decreases short-circuit current and transmural PD by an increase in sodium and chloride absorption that is mediated by stimulation of a neutral sodium and chloride uptake mechanism at the brush border membrane or a reduction in chloride conductive secretory pathway, or both [63].

2. *The Extrinsic Division*

The central nervous system monitors sensory information from the intestine and sends commands via the sympathetic and parasympathetic nervous system to the enteric ganglia, or possibly directly to mucosal cells to modulate activity of the effectors [63]. Preganglionic parasympathetic fibres originate in the lower medulla oblongata and are carried by vagal efferent fibres to the small intestine. All of the preganglionic parasympathetic fibres terminate on intrinsic neurons. Preganglionic sympathetic fibres that supply the intestine arise from neurons in the intermediolateral spinal columns between the fifth thoracic and the second or third lumbar segments, and pass through the sympathetic chain without synaptic relay to synapse in prevertebral sympathetic ganglia (Figure 4). Postganglionic fibres from the celiac and superior mesenteric ganglia enter the intestinal wall in the mesenteric nerves and are prevalent around the epithelial crypts and ramify among neurons of the submucous plexus. Sensory receptors within the small intestinal wall continuously monitor the digestive state of the intestinal tract and transmit this information to integrative circuitry in the ganglia. Sensory information from the small intestine is processed in the central nervous system. Command signals from the central nervous system are transmitted along sympathetic and parasympathetic efferent pathways to the intestinal tract [64].

Autonomic nerve fibres influence epithelial fluid and electrolyte transport with the sympathetic fibres increasing absorption (decreasing secretion) and the parasympathetic fibres decreasing absorption (increasing secretion). The sympathetic nervous system may play an important role in regulation of the intestinal absorptive function. The sympathetic fibres may inhibit secretory nerve reflexes via an activation of α_2-adrenergic receptors that influence

directly the transporting epithelium [65]. The extrinsic nerves control of mucosal function may be exerted in two principally different ways. The extrinsic nerves may innervate mucosal structure directly or may control mucosal function indirectly by influencing intramural nervous reflexes. Impairment of intestinal innervation could affect mucosal absorptive function such as diabetic diarrhea in streptozocin-induced diabetic rats [56].

VII. Hypothesis of the Study

Following small intestinal transplantation, both denervation and lymphatic disruption are inevitable. Since lymphatic reconstitution occurs 4-6 weeks following transplantation, the fat absorption and lymphatic drainage dysfunction would be limited to within a few weeks postoperation [59]. Experiments have shown that intestinal denervation leads to diarrhea, weight loss, and abnormal intestinal motility [56]. Since reinnervation after small intestinal transplantation occurs at least 6 months postoperation, denervation may have a major impact on intestinal nutrient absorption following transplantation.

We hypothesized that denervation following small intestinal transplantation would alter intestinal permeability and nutrient absorption.

To examine the hypothesis, we developed surgical denervation and transplant models in rats to assess the effect of the denervation component of intestinal transplantation on intestinal function.

Chapter II

MATERIALS AND METHODS

I. Animals

1. Animal Care

Male Lewis rats weighing between 250g and 300g were obtained from Charles River Canada, St. Constant, Province of Quebec and were housed in Plexiglass cages. Animals had free access to food and water. During the study period, the animals were fed with standard laboratory chow. The guidelines of the Canadian Council of Animal Welfare were followed for animal care. Day/night cycles were 12 hours, and the temperature was maintained at 20 ± 2^{0}C. The experimental protocol was approved by the Animal Welfare committee of the University of Alberta.

2. Experimental Group

The animals were randomly assigned to three treatment groups: a sham operation group, a surgical denervation group and a small intestinal transplantation group. Each group included 26 animals. The permeability to inulin study, assessment of denervation by tyramine, and epinephrine dose-response studies after denervation were performed at 2 weeks, 6 weeks and 6 months postoperation. 10 animals were studied from each group at 2 weeks and 6 weeks postoperation. 6 animals were studied from each group at 6 months postoperation.

3. Pre-, During and Postoperation Care

The animals were fasted for 12 hours before the operations. The animals were under general anaesthesia (2% halothane and 98% oxygen) via face mask and were kept at 37^{0}C on

a heating pad during the operations. All the surgical procedures were carried out under sterile conditions. The animals were housed in individual Plexiglass cages after operation. Animals received Cefazolin 40 mg/kg SQ pre-operation and q 8/h × 1 day postoperatively. Animals also received Buprenorphine 0.05-1 mg/kg SQ q 12/h × 48 hours postoperation as needed. Animals were allowed free access to water, and food was reintroduced 12 hours after operation. Survival was 100% in the control group, and exceeded 90% in the denervation and transplantation groups.

II. Surgical Procedures

1. Sham Operation

After animal was anaesthetized, the small intestine was visualised via an abdominal midline incision. The proximal and distal small intestine was identified by recognition of the ligament of Treitz and ileocecal valve. The rest of the small intestine was packed in a saline-moistened gauze. The small intestine was divided roughly 2 cm beyond the ligament of Treitz proximally and 2 cm above the ileocecal valve distally, after the blood vessels have been ligated with 6-0 suture and divided. For the anastomosis of jejunum, two stay sutures with 6-0 silk were put at both sides of the anastomosis as anchors. A 1 cm in length piece of macaroni was inserted into the intestinal lumen to sustain the anastomosis. The jejunum was anastomosed end-to-end using 14-16 interrupted sutures with 6-0 silk. The ileum anastomosis followed the same method. The abdominal wound was closed using running 3-0 Dexon for peritoneum and muscle layers and running 4-0 Dexon for skin. 20 ml saline was given subcutaneously after the operation.

2. Surgical Denervation

The midline incision was performed after the animal was anaesthetised. The ligament of Treitz and the terminal ileum were identified. The rest of the small intestine was packed in a saline-moistened gauze. The small intestine underwent transection 2 cm beyond the ligament of Treitz proximally and 2 cm above the ileocecal valve distally after blood vessels had been ligated with 6-0 suture and divided. The small intestinal mesentery, lymphatic vessels, and the tissue up to and including the adventitia of the superior mesenteric artery (SMA) and superior mesenteric vein (SMV) were carefully divided. The small intestine was now completely isolated, maintaining continuity only through the SMA and SMV. The small intestine was then reanastomosed in continuity in the same fashion as the sham operation group. The peritoneum, muscle layers, and skin of the abdominal wound were sutured with running 3-0 Dexon and 4-0 Dexon. The animal was given 20 ml of saline subcutaneously after the closure of the wound.

3. Small Intestinal Transplantation

One stage orthotopic small intestinal transplantation was performed using microsurgical techniques as previously described (60,61). Lewis to Lewis syngeneic transplantation was performed in order to eliminate immune interaction.

A. Donor Operation

After anaesthesia was induced in the donor animal, a midline incision from the xyphoid to the pubis was performed. Packing the small bowel with saline-moistened gauze, the large intestine was transected in the middle of the descending colon after ligation with 6-0 silk. The left colic, middle colic, right colic, and ileocolic arteries and veins were isolated and ligated.

The descending colon, transverse colon, ascending colon, cecum, and 2 cm of distal ileum were removed. After the ligament of Treize was identified, intestinal transection was performed between the duodenum and jejunum. All tributaries to the portal vein including the gastric and splenic veins were ligated with 6-0 silk. The superior mesenteric vein was separated from the pancreas by individually ligating the venous tributaries from the organ. The right renal artery was isolated, ligated with 6-0 silk, and divided. The aorta at the origin of the superior mesenteric artery was freed of surrounding tissue. The entire small intestine was completely isolated and attached only to its vascular pedicle including the portal vein and infrarenal aorta. With the small bowel retracted to the right, the retroperitoneum was entered. The aorta above the left renal artery was isolated and a PE-90 catheter was introduced into the aorta. The animal was systemically heparinized using 300 units heparin in 1 ml saline injected via the penile vein. The aorta was ligated proximal to the take off of the superior mesenteric artery with 4-0 silk. The graft was flushed with 4-5 ml iced Ringer's solution through the catheter which had been previously placed in the aorta. The portal vein was divided midway between the pancreas and the porta hepatis. A clear effluent from the portal vein was produced after the flush. The superior mesenteric artery with a cuff of aorta was resected by dividing the aorta above and below the origin of the the superior mesenteric artery. The graft was removed from the donor and stored in iced Ringer's solution at 4^0C.

B. Recipient Operation

After being anaesthetised with 2% halothane, the recipient animal was opened by a midline incision and the intestine of the recipient was packed in a saline-moistened gauze and retracted to the animal's left. The recipient's infrarenal vena cava and aorta were isolated

distal to the left renal artery. The aorta and infrarenal vena cava were cross-clamped by Hartman curved haemostatic mosquito forceps. The donor bowel was brought into the right abdominal cavity for engraftment.

The infrarenal vena cava (IVC) was punctured using a 30G needle and flushed free of the remaining blood with saline. The punched hole was enlarged to the size of the donor's portal vein. The portal vein of the small intestinal graft and the recipient's IVC were sutured in end to side fashion using a running 10-o silk suture. The preparation of the donor aorta and the recipient's aorta for end-to-side anastomosis followed the same procedures as the venous anastomosis. One-way-up suturing was used to anastomosis aorta to aorta using running 10-0 sutures [179]. The Hartman curved haemostatic mosquito forceps was then carefully removed and a sponge was gently pressed on the top of the anastomoses for a few minutes or until there was no obvious bleeding from the anastomoses. To compensate for blood loss, 4 ml Ringer's solution was injected via the penile vein. The graft was packed in a saline-moistened gauze. The recipient's small intestine was removed from 2 cm beyond the ligament of Treitz proximally to 2 cm above the ileocecal valve distally after ligation with 6-0 suture and division of all its connecting intestinal blood vessels. The proximal end of the small intestinal graft was anastomosed to the left jejunum of the recipient and the distal end of the small intestinal graft was anastomosed to the left ileum of the recipient by end to end anastomoses using 6-0 silk suture using the procedure described in the sham operation group. The graft was placed into the abdominal cavity. The peritoneum and muscle layers of the abdominal wound were sutured with 3-0 Dexon suture and the skin was closed with 4-0

Dexon suture. The animals were given 20 ml of saline subcutaneously. Water was given 4 hours later and food was reinduced 24 hours postoperation.

III. Studies on Ussing Chamber

1. Preparation for Ussing Chamber

Once the animal was euthanised by cardiocentesis with overdose of pentobarbital (65mg/kg), the abdomen was quickly opened and the jejunum and ileum (15 cm from each) were removed. The jejunal and ileal segments were flushed with 10 ml ice-cold normal-Ringer's solution and incubated with Ringer's solution of the following composition (mM): Na, 143; K, 5; Mg 1.1; Ca, 1.25; HCO_3, 25; Cl, 123; and $HPO_4 + H_2PO_4$, 1.95 with pH 7.4 while gassed with 5% CO_2 in 95% O_2. The specimens were cut open along the mesenteric border with fine scissors. Segments of 2-3 cm were mounted on a chamber. The serosa and the muscle layer were stripped with caution by using micro-forceps. The top of the Ussing chamber was replaced and quickly attached to a Ussing chamber holder. Then the chamber was connected with the reservoir. Agar bridges were used to connect the chamber with the electric detector. The mucosal side of the reservoir was filled with 10 ml Ringer's solution and the serosal side with 10 ml of 1 mM inulin dissolved in Ringer's solution and gassed with 5% CO_2 in 95% O_2 at 37^{0}C. The circulating water bath was turned on to ensure a uniform 37^{0}C temperature in all the reservoirs. 20 mM of fructose was added to each side of the reservoir for offering energy to the tissue. Stabilization was assured by monitoring Isc changes for 20 minutes, the permeability and denervation studies were performed. After the

permeability and denervation studies were performed, response to 20 mM D-glucose was used to determine the tissue integrity.

2. In Vitro Permeability Study

8 Ussing chambers were used to measure the permeability to inulin for each rat (4 for jejunum and 4 for ileum). After the tissues were mounted on the chambers as described above, 5 μCi ^{3}H-inulin from Dupont was added to the serosal side of the reservoir. After a stabilization period of 20 minutes, two 100 μl samples from the serosal side, "hot" side, and one 1 ml sample from the mucosal side, "cold" side, were taken and pipetted into 7 ml scintillation vials as the first (basal) samples. Volume was replaced with the same amount of Ranger's solution. The second samples, 1 ml, were taken from the mucosal side 10 minutes later, replacing with the same amount of Ranger's solution. After another 10 minute flux, two 100 μl samples from the serosal side and one 1 ml sample from the mucosal side were taken as the last samples. All the samples from the cold side were filled with 4 ml of Beckman's Ready Gel Cocktail and mixed by shaking. The samples from the hot side were filled with 900 μl Ringer's solution and then filled with 4 ml of Beckman's Ready Gel Cocktail and mixed by shaking. The samples were counted for radioactivity of tritium on a scintillation detector.

3. Tyramine Response Study

Intestinal short-circuit current (Isc) response to additional tyramine is caused by the release of stored epinephrine and norepinephrine [153]. After the permeability study was performed, four chambers were used for the tyramine response study (2 for jejunum, and 2 for ileum). The intestinal short-circuit current were recorded at basal. 1 mM theophylline was added to the serosal reservoir to simulate the tissues to maximize the Isc response 15 minutes

before tyramine was added [154]. The Isc was read and recorded. 50 μM pargyline, a monoamine oxidase inhibitor, was also added to the serosal reservoir 10 minutes before tyramine to prevent tyramine metabolism by cytoplasmic monoamine oxidase [153]. After 10 μM tyramine was added to the serosal reservoir, Isc was measured. 5 mM D-glucose was then added to both sides of the reservoirs to determine the tissue integrity.

4. Epinephrine Dose-Response Study

Epinephrine stimulates active NaCl absorption and reduces the secretion of HCO_3 and Cl^-, thus reducing the Isc [56]. The Isc response to additional epinephrine was determined in both jejunal and ileal mucosa. After permeability to inulin was performed, another 4 chambers were used for epinephrine dose-response study (two for jejunum, and two for ileum). 10^{-9} M, 10^{-8} M, 10^{-7} M, 10^{-6} M, and 10^{-5} M epinephrine were added sequentially to the serosal reservoir with 5 minute intervals. The Isc response was recorded before and after adding the different concentrations of epinephrine. 20 mM D-glucose was added to both sides of the reservoir to determine the tissue integrity at the end of the study.

IV. Statistical Analysis

Data were reported as mean ± SEM, and statistical analysis was performed by paired student T-test for the Isc response to tyramine. The permeability studies were tested by ANOVA and epinephrine dose-response studies were tested by Nonparametric K-related sample test with $p<0.05$ taken as being statistically significant

Chapter III

RESULTS

In general, the animals from all groups recovered from the operation well. Weight gain, feed intake, and activity were identical in all groups. Survival was 100% in the sham group, and exceeded 90% in the denervation and transplantation groups.

I. Intestinal Permeability

At two weeks postoperation, inulin fluxes (nmol/cm^2/hr) from serosal to mucosal side in jejunum and ileum were no different among the three groups (Figure 5). The inulin flux in jejunum and ileum in the sham group were 12.2 ± 0.5 and 12.6 ± 0.9. Inulin fluxes were 11.4 ± 0.7 in jejunum and 11.3 ± 0.7 in ileum in the denervation group. In the transplantation group, jejunum inulin flux was 11.1 ± 0.8 and ileum inulin flux was 12.9 ± 0.8. However, by 6 weeks inulin fluxes (nmol/cm^2/hr) in the sham group dropped from 12.2 ± 0.5 to 8.5 ± 0.5 in jejunum and from 12.6 ± 0.9 to 9.4 ± 1.0 in ileum ($p<0.05$). In contrast, inulin fluxes in jejunum and ileum in both the denervation group (13.5 ± 0.9, 15.1 ± 1.0) and the transplantation group (13.8 ± 1.7, 14.9 ± 1.5) still remained at an elevated level (Figure 6). By 6 months postoperation, the permeability to inulin in both jejunum and ileum was at the same level among the three groups (Figure 7). The inulin fluxes (nmol/cm^2/hr) in the sham group were 14.6 ± 0.8 in jejunum and 15.5 ± 0.6 in ileum. In the denervation group, the inulin fluxes in jejunum and ileum were 15.5 ± 1.2 and 13.8 ± 0.7. The inulin fluxes were 14.7 ± 1.5 and 15.1 ± 0.9 in jejunum and ileum in the transplantation group.

II. Tyramine Response Study

Two weeks postoperation, Isc responses ($\mu A/cm^2$) to tyramine among sham operation, surgical denervation, and transplantation groups in both jejunum and ileum were as shown in Fig 8,9 and Table 1. Isc responses to tyramine in both jejunum and ileum in the sham group (Δ-13.1 ± 1.2, Δ-17.7± 2.4, $p<0.05$) were significantly different compared to the denervation group (Δ2.1 ± 0.6, Δ1.3 ± 1.6, p=N.S.) and transplantation group (Δ-0.8 ± 0.7, Δ0.3 ± 2.5, p=N.S.). Six weeks postoperation, Isc responses followed the same pattern as 2 weeks postoperation as shown in Fig 10, 11 and Table 2. There were also significant Isc decrease ($p<0.05$) to tyramine both in jejunum (Δ-14.5 ± 1.6) and ileum (Δ-32.45 ± 3.66) in the sham group. Isc responses were not altered in jejunum and ileum in both the denervation group (Δ3.1 ± 1.0, Δ2.0 ± 0.99, p=N.S.) and the transplantation group (Δ-3.9 ± 1.3, Δ-6.5 ± 2.3 p=N.S.) in contrast with the sham group (Δ-14.5 ± 1.7, Δ-32.5 ± 3.7, $p<0.05$). Six months postoperation, denervation effects on Isc response to tyramine persisted in jejunum and ileum in the denervation group (Δ-2.5 ± 2.3, Δ1.1 ± 1.3, p=N.S.) and the transplantation group (Δ-8.6 ± 1.2, Δ-5.8 ± 2.6, p=N.S.) in comparison with the sham group (Δ-12.6 ± 1.9, Δ-22.7 ± 2.7, $p<0.05$) (Figure 12, 13 and Table 3).

III. Epinephrine Dose-Response Study

The response relationships between the concentration of exogenously added epinephrine and Isc among the three groups in both jejunum and ileum at 2 weeks, 6 weeks, and 6 months postoperation are shown in Figures 14 - 19. There were significant Isc ($\mu A/cm^2$) responses to epinephrine at the concentration of 10^{-7} M in jejunum and ileum of the

sham group at 2 weeks (Δ12.6 ± 2.2, Δ7.0 ± 2.1), 6 weeks (Δ5.8 ± 1.7, Δ12.1 ± 2.4), and 6 months (Δ6.9 ± 1.2) postoperation. An exception was the response of ileum at 6 months where epinephrine at 10^{-6} M (Δ5.86 ± 1.7) was required. The significant responses to epinephrine in jejunum of the denervation group were 10^{-7} M at 2 weeks (Δ3.82 ± 0.8) and 10^{-6} M at both 6 weeks (Δ7.0 ± 2.6) and 6 months (Δ10.8 ± 1.7). The epinephrine responses in ileum of the denervation group were at 10^{-6} M at 6 weeks (Δ7.1 ± 2.3) and 10^{-5} M at 2 weeks (Δ11.1 ± 3.2) and 6 months (Δ8.3 ± 2.6) postoperation. There was no significant response to epinephrine until the concentration was 10^{-6} M in both jejunum (Δ6.4 ± 1.3) and ileum (Δ7.3 ± 1.8) in the transplantation group at 2 weeks postoperation. The significant response to epinephrine in the transplantation group at 6 weeks in both jejunum (Δ9.5 ± 1.1) and ileum (Δ8.6 ± 3.2) was at 10^{-5} M. The response to epinephrine at 6 months postoperation in the transplantation group was at 10^{-7} M in jejunum (Δ9.7 ± 1.7) and 10^{-6} M in ileum (Δ11.2 ± 1.3). Overall, there was no hypersensitivity to epinephrine dose response in denervation nor transplantation groups in either jejunum or ileum at 2 weeks, 6 weeks, and 6 months postoperation when compared to epinephrine dose response in the sham group.

Chapter IV

DISCUSSION

Small intestinal transplantation only reestablishes the blood circulation and the continuity of intestine. Complete transection of both nerves and lymphatics is an inevitable consequence of transplantation. Since lymphatic reconstitution occurs 4-6 weeks following transplantation, the fat and lymphatic drainage dysfunction should be limited to a few weeks postoperation [137,154]. Following small intestinal transplantation, since reinnervation occurs 6 months or more postoperation [155], we believe denervation may play a major part in alteration of intestinal nutrient absorption following transplantation.

I. Confirmation of the Models.

The intestinal autonomic nervous system is composed of parasympathetic, sympathetic, and enteric divisions [63]. After small intestinal transplantation, the apparent morphological integrity of the intrinsic nervous system remains intact and the function is largely preserved [161]. However, the extrinsic nerves are completely disrupted as a consequence of transplantation. Although both parasympathetic and sympathetic innervation is lost following transplantation, the results are consistent with a loss of sympathetic input, by transection or chemical ablation of sympathetic nerves [162, 163]. Tyramine has an indirect sympathetic effect on intestinal transport that is by way of a tyramine-releasable norepinephrine pool in the intestinal mucosa [164]. The norepinephrine acts as an endogenous neurotransmitter that increases net absorption of sodium, chloride, and water and inhibits

chloride and bicarbonate secretion, thus tyramine can decreases Isc in the normal innervated small intestine. For the confirmation of the denervation models used in this study, the Isc response to tyramine was measured. In the sham group there was a significantly increased Isc response to tyramine in both jejunum and ileum compared with the tyramine response in the denervation and transplantation groups at 2 weeks and 6 weeks postoperation which did not change appreciably by the 6 month period (Figure 8 -13 and Table 1 - 3). This implies that there is ongoing change in the adrenergic transmitter stores in both the denervation group and the transplantation group during this period of time. The results indicate ongoing denervation effects in the denervation and transplantation groups. There was no difference between the denervation and transplantation groups, confirming a similar degree of denervation in both denervation and transplantation groups. Although the jejunal and ileal Isc responses to tyramine at 6 months in the transplantation group showed a trend to decrease (Figure 12, 13 and Table 3), there was no statistically significant difference in values before and after applying tyramine. These results are consistent with the observations of Kiyochi et al. that morphologically extrinsic sympathetic reinnervation required at lest 6 months following small intestinal transplantation [155].

II. Hypersensitivity to Epinephrine

Previous investigators have shown that sympathetic innervation both inhibits intestinal secretion and mediates absorption via α_2-adrenergic receptors [165]. Norepinephrine inhibits release of substances that cause secretion such as vasoactive intestinal peptide, substance P, or serotonin. Norepinephrine acting at α_2-adrenergic receptors on the terminals would

suppress release of transmitters responsible for fast and slow synaptic transmission within the ganglia [165]. These functions serve to shut down the synaptic circuits and reduce ongoing activity of submucosal nerves and thereby increase absorption of ions and water from the intestinal lumen. The effect of additional epinephrine on intestine mucosa could be reflected by Isc changes. The denervation hypersensitivity to additional epinephrine is due to increased numbers of postsynaptic α_2-adrenergic receptors. Chang et al. showed denervation hypersensitivity in a streptozocin-induced denervation model [56]. However, the epinephrine dose response in our study did not show hypersensitivity in either the denervation group or the transplantation group through out the study (Figure 14 - 19). The Isc response to epinephrine in jejunum and ileum in both the denervation group and the transplantation group is at 10^{-6} M or 10^{-5} M concentration. The concentration for Isc response to epinephrine in sham group was mainly at 10^{-6} M in jejunum and 10^{-7} M in ileum. Taguchi et al. also reported that there was no change in either the membrane receptor population or the contractile properties of the muscle following small intestinal transplantation [166]. There are two possibilities which could explain how the denervated intestine adapts to the denervation changes.

First, pharmaceutical-induced denervation may impair both extrinsic and enteric nervous systems. The models of denervation and transplantation in our study only impair the extrinsic nervous system while the enteric nervous system still remains intact. The enteric division of the autonomic nervous system is an independent, integrative system that differs in structure and function from the sympathetic and parasympathetic divisions of the autonomic nervous system [64]. The enteric nervous system has an extensive autonomy and

continues to control the intestinal function after extrinsic nerve fibres are severed. Shen et al. demonstrated that the distribution of enteric nervous fibres containing vasoactive intestinal polypeptide, enkephalin, substance P, and somatostatin was visibly unchanged in the transplanted small intestine [167]. Because of the significance of the coexistence of extrinsic and enteric nervous systems, the enteric nervous system may compensate for the loss of the extrinsic nervous system. Also, pharmaceutical-induced denervation may cause denervation of adrenergic neuron cell bodies, whereas , the surgical denervation only interrupts the nerve fibres. Thus there was no appearance of denervation hypersensitivity in either denervation or transplanted groups.

There is likely no change in the epinephrine receptor population of the epithelium after denervation and transplantation. There is no lack of epinephrine in the tissue after operation. Although adrenergic neurons are thought to be almost entirely extrinsic, the levels of norepinephrine in rat jejunum, once reduced after extrinsic denervation, returned to control levels within 45 days [172]. Taguchi et al. also showed that myogenic activity of intestinal smooth muscle was unaltered by transplantation and responses to cholinergic and adrenergic agonists were similar in graft and control, indicating no change in the membrane receptor population [173]. In addition, Osihi et al. reported low dose norepinephrine had no effect on ileum flux of water or electrolytes even in neurally intact dogs [174].

However, the epinephrine dose-response in this study did not reach the plateau. We could not demonstrate the maximal Isc response to epinephrine. Hypersensitivity in both denervation and transpant groups may appear with higher epinephrine concentrations.

III. The Permeability Study

Ballinger et al. reported that diarrhea and weight loss following small intestinal transplantation disappeared between 4 and 6 months postoperation [168]. The observation is consistent with our permeability study results which show the increase in permeability to inulin at 6 weeks in both denervation and transplantation groups, and its recovery towards the level of the sham group by 6 months postoperation (Figure 6-7). In review of the interesting correlation between evidence of denervation noted in both denervation and transplantation animals, and the increase in the permeability to the large molecular weight probe inulin, our studies in this regard were originally prompted by attempts to use permeability markers as a measure of rejection [169]. The crux of the current investigations was to determine if the previously described changes in nutrient transport observed after transplantation could be explained by denervation induced alteration in permeability.

The intestinal permeability can be affected by various factors such as celiac sprue, rheumatoid arthritis, Crohn's disease, nonsteriodal anti-inflammatory drugs (NSAID) induced enteritis, rejection after small intestinal transplantation, etc [37,53-56]. Since the Na/Glucose transport system could modulate the width of the tight junction [17], Cyclosporin A may indirectly affect the intestinal permeability by affecting the activity of the Na/Glucose cotransportor [54,57].

The results of our investigations reflect the complex interplay between postoperative trauma and the effects of changes induced by transplantation and denervation status on intestinal permeability phenomena. There is clearly a generalized increase in intestinal permeability of both jejunum and ileum in all three groups at 2 weeks postoperation (Figure

5). It was demonstrated that TNF increases small intestinal permeability [156]. Abe et al. reported that TNF concentrations reached highest peak at 14 days post peritoneal operation [157]. The high level permeability in the sham group at 2 week postoperation may be caused by the high level of TNF resulting from the operation at 2 weeks earlier. The changes in permeability following intestinal transplantation in this study have shown the effect of denervation on intestinal function. The permeability defect caused by the surgical procedure had resolved by 6 weeks postoperation. In contrast, the permeability in the transplantation and denervation groups demonstrated increased jejunal and ileal permeability which persisted through out the six week study (Figure 6).

Intestinal permeability, one of the two physiological functions of intestinal epithelium, is the regulation of the transit of electrolytes and molecules maintained by tight junctions at the apical end of intercellular borders of intestinal epithelium [20-22]. Following small intestinal transplantation, many factors can alter intestinal permeability such as rejection, GVHD, reperfusion, and immunosuppressive drugs [53, 54, 57]. The increasing intestinal permeability would favour the Na^+ reverse flux from serosal side to mucosal side of the epithelium, thus reducing net Na^+, water, and nutrient absorption. In reviewing the interesting correlation between evidence of sympathetic denervation noted in both the denervation and transplantation animals, and the increase in permeability to the large molecular weight probe inulin at 6 week postoperation in jejunum and ileum, it would seem that the changes may be due to alteration in the tight junction. It is interesting to note that previous workers have shown that sympathetic denervation causes its effects on ion transport using cyclic AMP as a second massager [15]. As cyclic AMP reacts with the cytoskeleton of the intestinal

epithelium, it may have a specific role in the orientation of tight junctional intramembranous strands thereby regulating paracellular ion flow [170]. We would therefore suggest that the changes in permeability noted herein may be induced by a similar mechanism. There is some evidence, however, indicating that morphological changes in tight junction do not always occur in conjunction with functional changes. Fedorak et al. reported there was no detectable alteration in the ultrastructure of the tight junction under electron microscopic study in the pancolitis rat model even though the ileal permeability to mannitol, inulin, sodium, and chloride increased significantly [177]. The results of increasing permeability in this study imply that surgical denervation after small intestinal transplantation alters intestinal function in addition to temporal surgical procedure effect.

Changes in intestinal permeability are related to many factors as mentioned above. One proven factor known to alter intestinal permeability is 'aging'. Katz et al. showed that the permeability to PEG 900 was 1-1.3% in 5-15 weeks old rats and increased to 1.8-2.4% in 8-24 months old rats [178]. With respect to the present study, increased permeability in control animals may have been associated with the aging process. In the control group, permeability rose from 8.5 (jejunum) and 9.4 (ileum) nmol/cm^2/hr at 6 weeks postoperation [10 week old rats] to 14.6 (jejunum) and 15.5 (ileum) nmol/cm^2/hr at 6 months postoperation [8 month old rats]. Although this increasing trend was also present in both denervation and transplant groups throughout the 6 month recovery period, the effect was not as dramatic as was observed in the control group.

Permeability defects resulting from denervation following small intestinal transplantation are unlikely to have significant effects for a long time frame. The results of

permeability to inulin in both jejunum and ileum in our study showed there was no difference among the three groups (Figure 7) at 6 months postoperation. The defect in permeability appeared that had become apparent in the denervation and transplantation groups at 6 weeks postoperation had improved at 6 months postoperation. These results are consistent with the clinical observations that diarrhea and weight loss following small intestinal transplantation disappeared between 4 and 6 month postoperation [36].

Material absorbed from the intestinal lumen must first traverse the epithelium and then diffuse to the mucosal blood and lymph vessels. The effect of denervation on permeability of the intestine may also occur between the blood stream and the epithelium. It is well known that sympathetic nerve stimulation reduces intestinal capillary filtration coefficient and capillary pressure by increasing precapillary resistance [171]. The reduction of intestinal capillary filtration coefficient and capillary pressure increases the net force favouring the removal of absorbed fluid by the capillaries and thereby enhances water, electrolyte and nutrient absorption. Thus lack of sympathetic nerve stimulation in the denervation and transplantation groups would reduce the osmotic and electrical gradients which is the force for water and nutrient absorption crossing the epithelial cells. The lack of sympathetic nerve stimulation may also contribute to the increased permeability in both jejunum and ileum in the denervation and transplantation groups at 6 weeks postoperation. During this period, the denervated and transplanted intestine may be influenced by changes in blood flow caused by the sympathetic denervation of mesenteric arteries. Although the reinnervation of the intestinal tract occurs at least 6 months after transplantation, extrinsic sympathetic reinnervation begins in the arterial anastomosis by 3 weeks [155]. Kiyochi et al. reported on

sympathetic reinnervation of rat jejunum and ileum following small intestinal transplantation. They showed that the jejunal arteries began to be innervated by sympathetic fibres at 3 weeks, and that the densities of the reinnervated fibres were about 18%. Reinnervation rates were 30% at 6 weeks, and could reach 73% at 6 months after transplantation. The sympathetic nerve densities of the ileal arteries followed the same pattern, though the densities at 3 weeks, 7%, and 6 weeks, 26%, were lower than those in the jejunum. The earlier reinnervation of the mesenteric arteries may contribute to the improvement of permeability at 6 months postoperation in both denervation and transplantation groups although the sympathetic reinnervation of the small intestine occurred at least 6 months following small intestinal transplantation.

In conclusion, the denervation following transplantation of the small intestine did not demonstrate hypersensitivity to epinephrinein in concentration between 10^{-9} and 10^{-5} M. Denervation increases the intestinal permeability to inulin at 6 weeks postoperation, but the defect in intestinal permeability recovered at 6 months after transplantation. This indicates that small intestinal transplantation is functionally feasible. However, the increased permeability to large molecules at 2 weeks and 6 weeks may also be associated with an increased permeability to endotoxin. This may secondarily result in up regulation of the immune response and may help to explain the major difficulties with the control of rejection after small intestinal transplantation.

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Figure 1. Intercellular Junctions

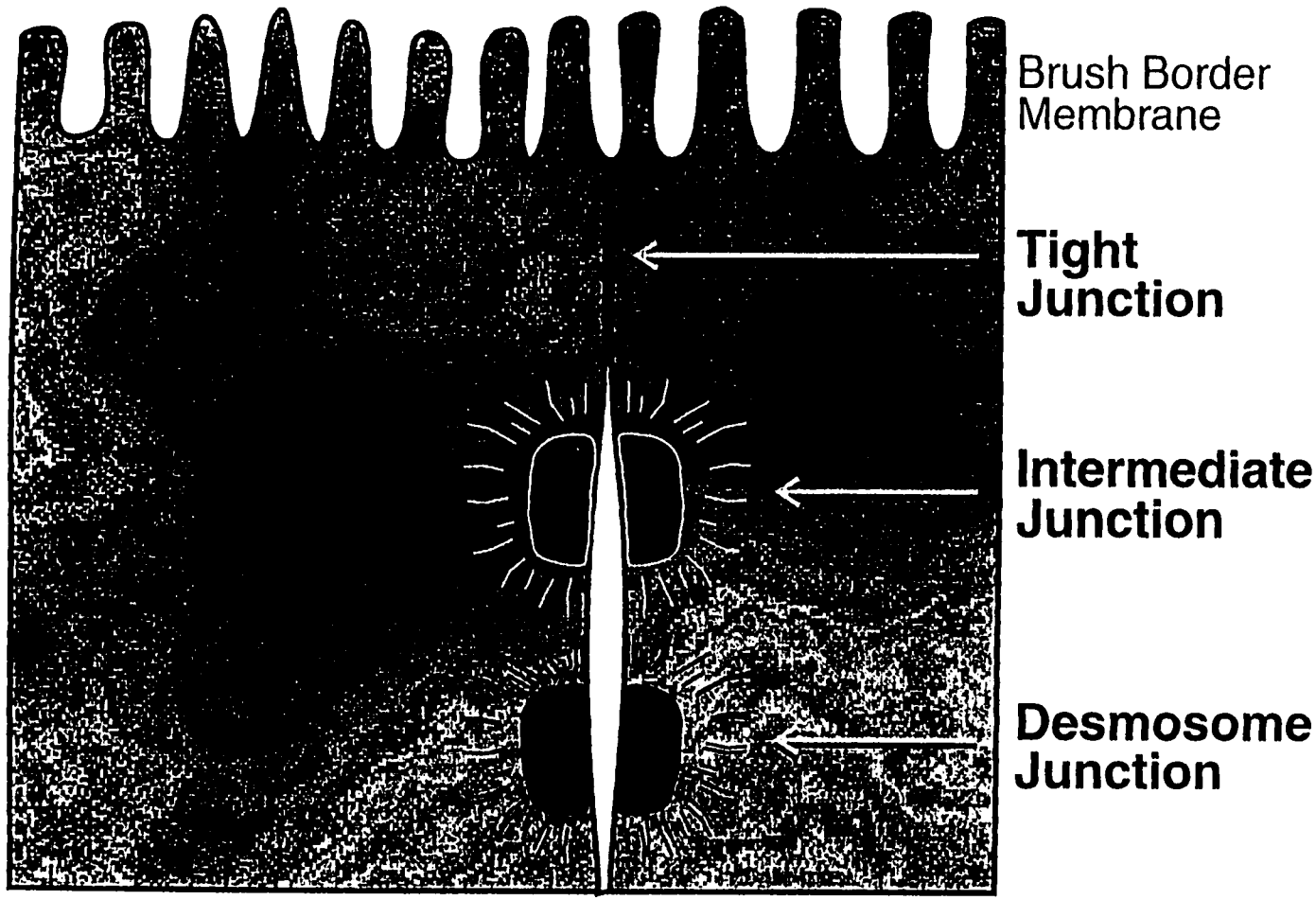
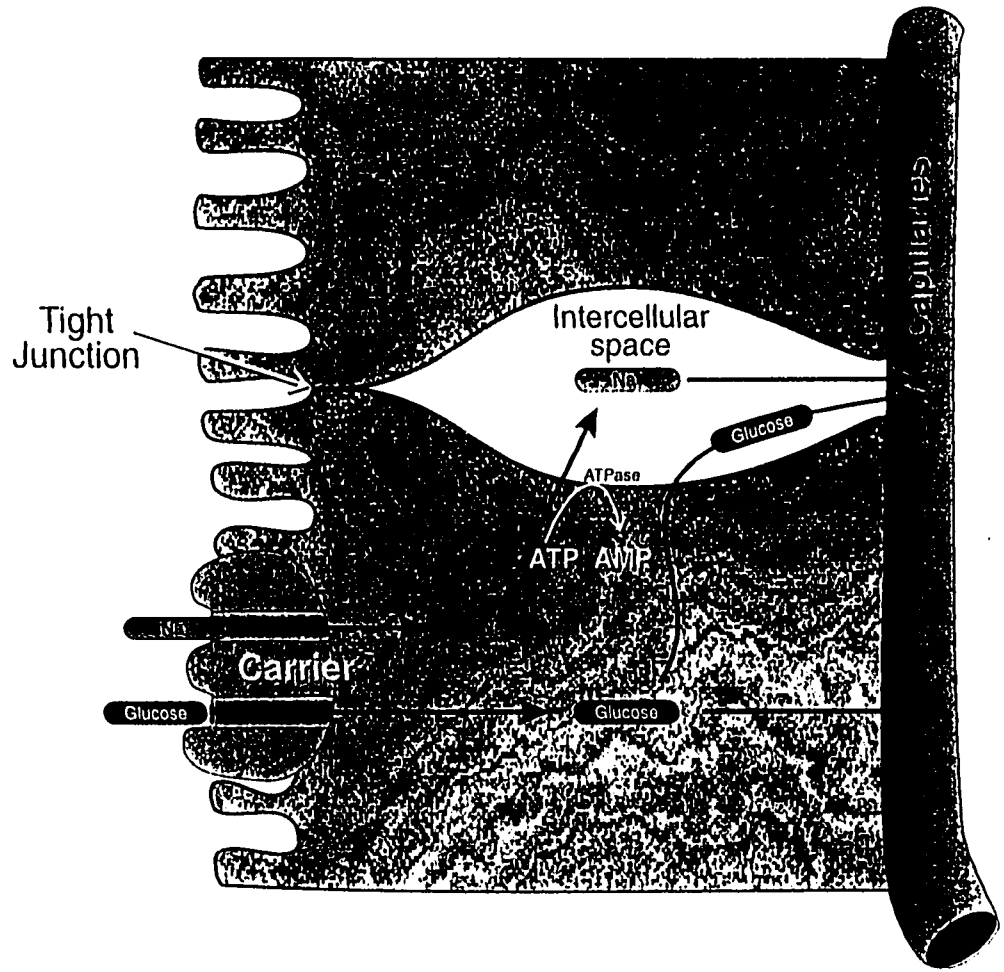


Figure 2. Nutrient Absorption



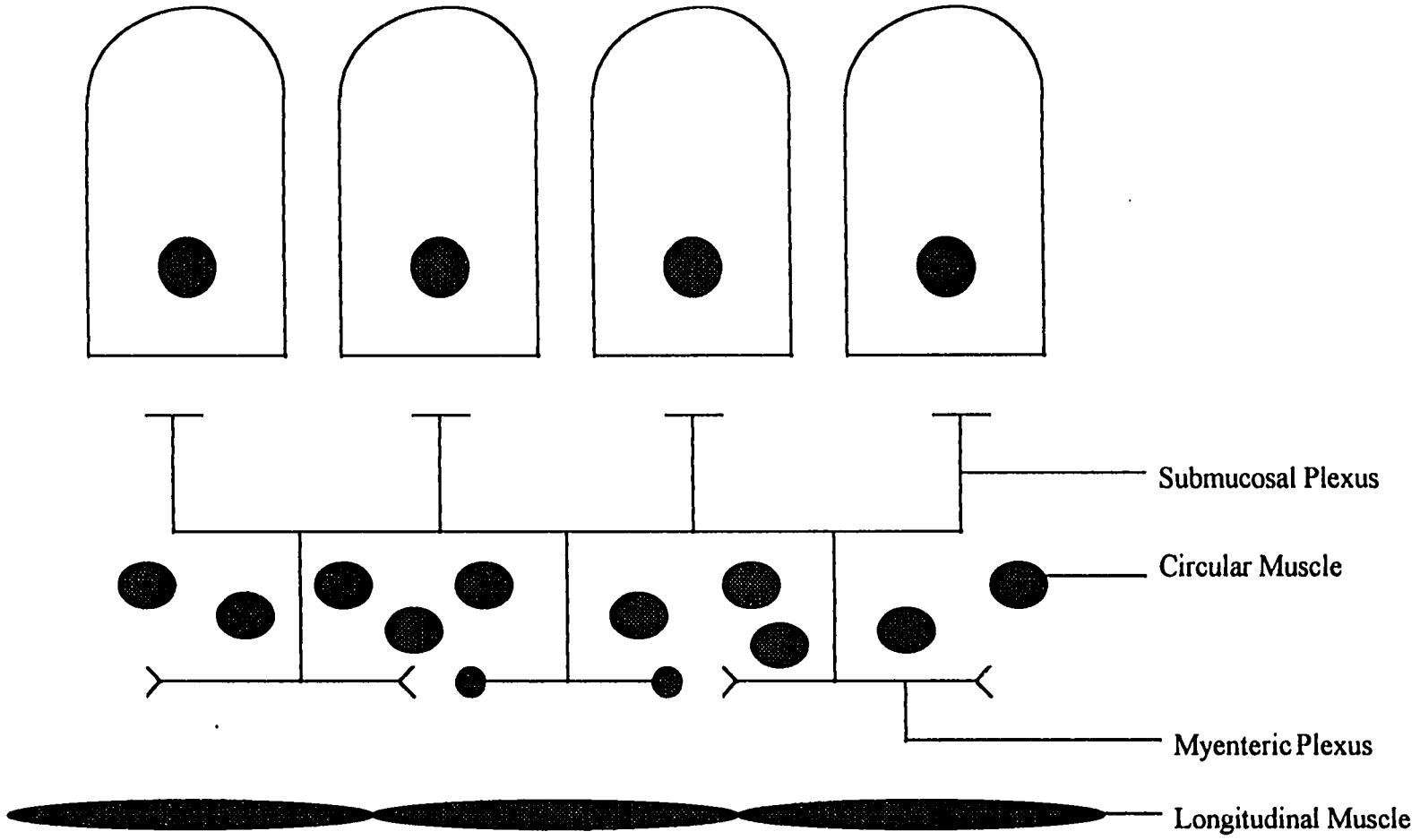


Figure 3. Intrinsic innervation of the mucosa

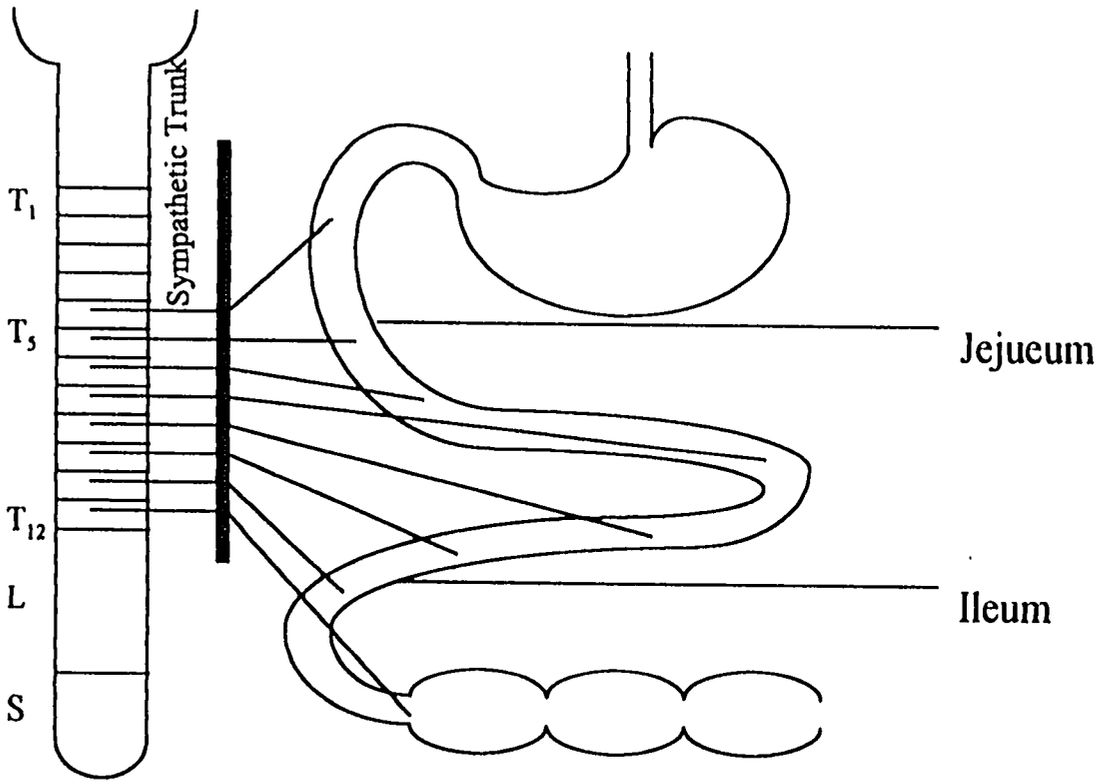


Figure 4. Extrinsic innervation of the small intestine

Figure 5. Inulin Flux 2 weeks Postoperation

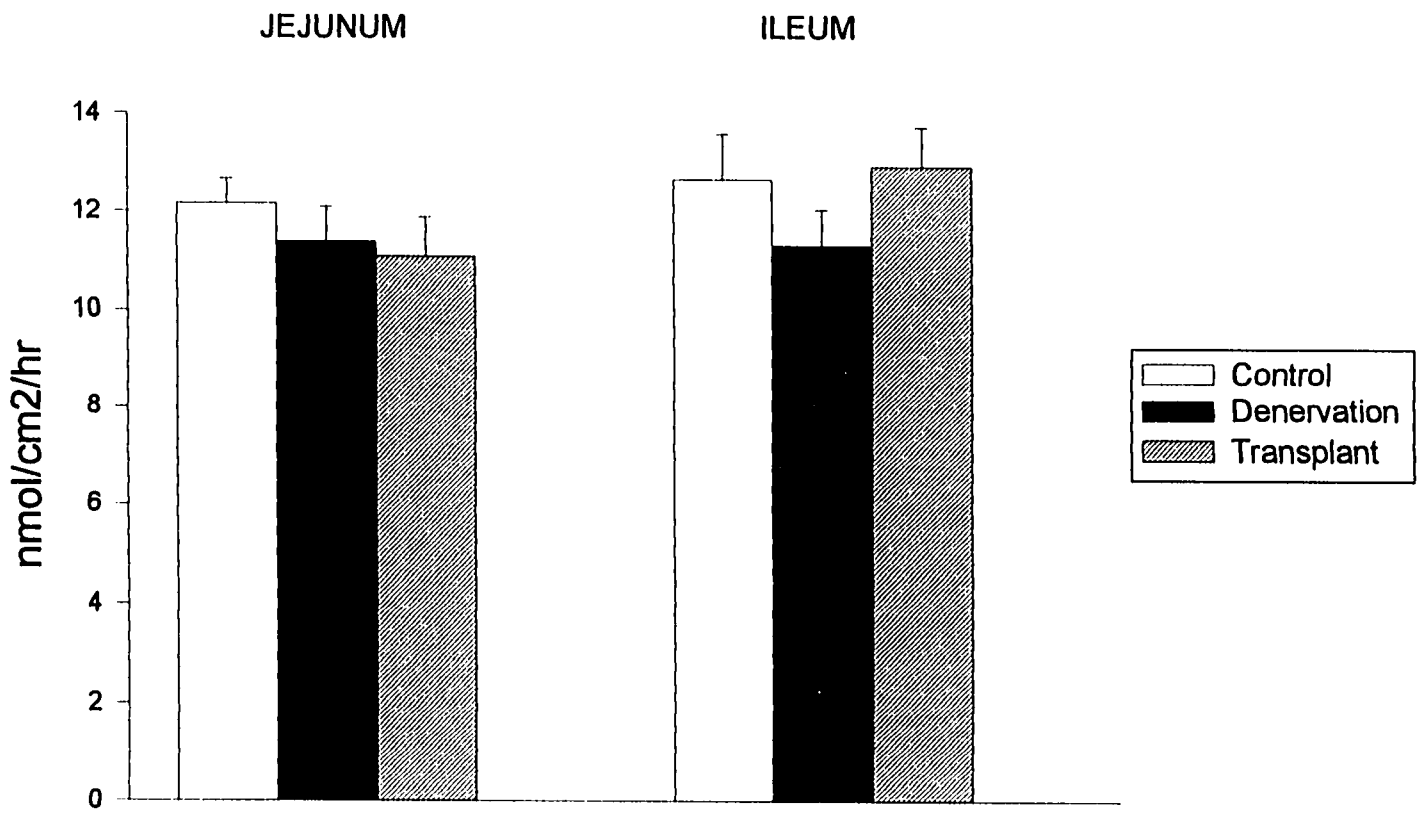


Figure 6. Inulin Flux 6 weeks Postoperation

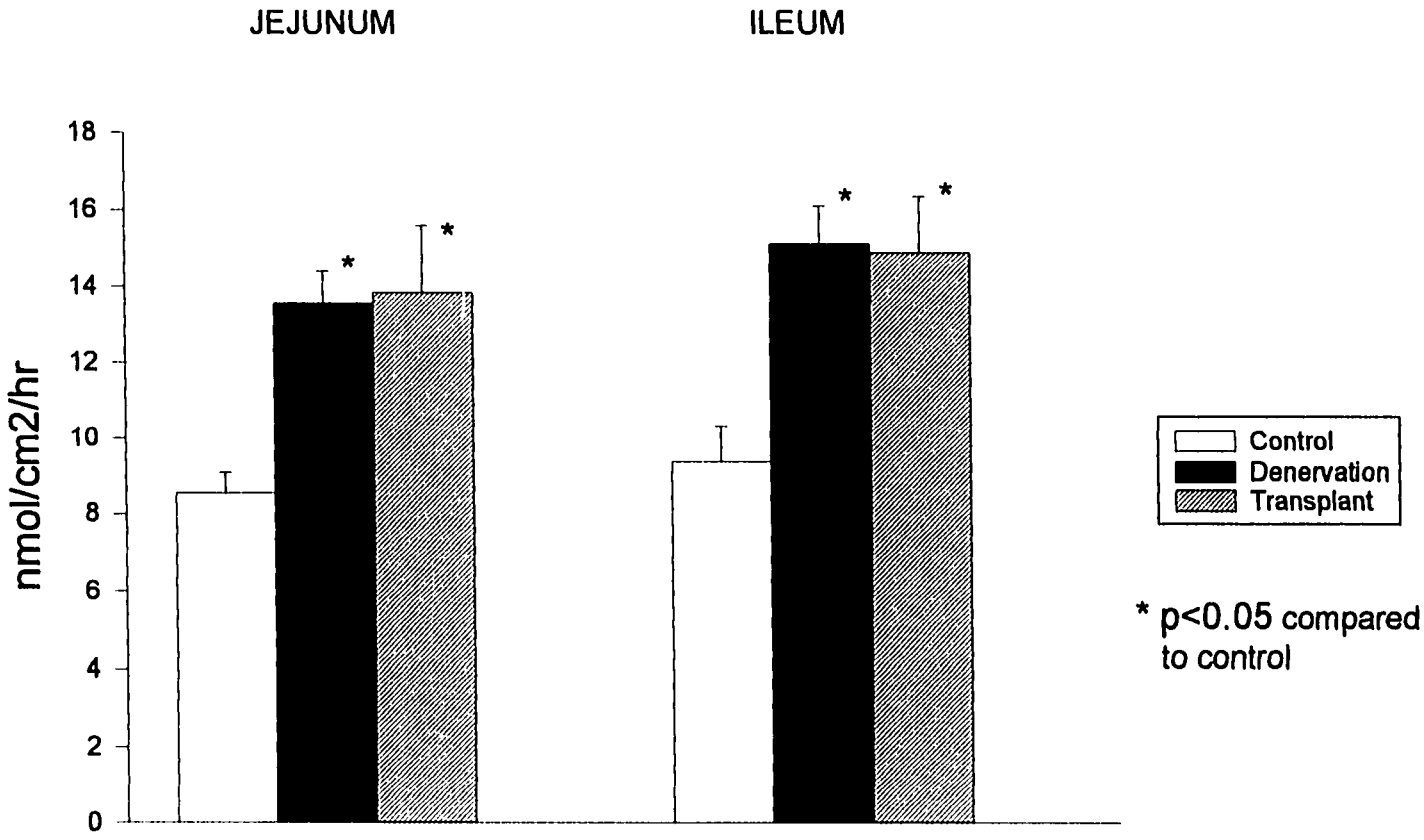


Figure 7. Inulin Flux 6 months Postoperation

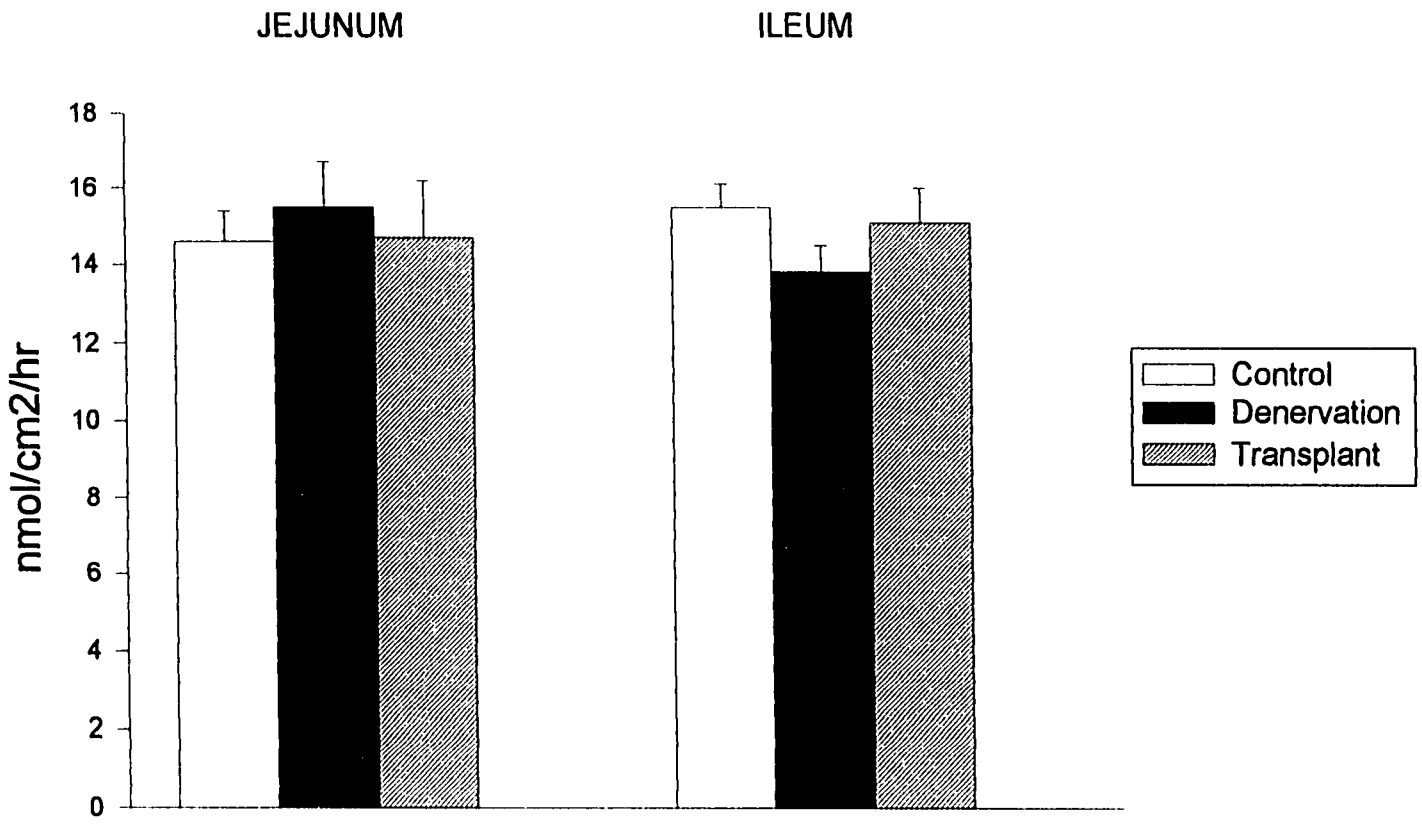
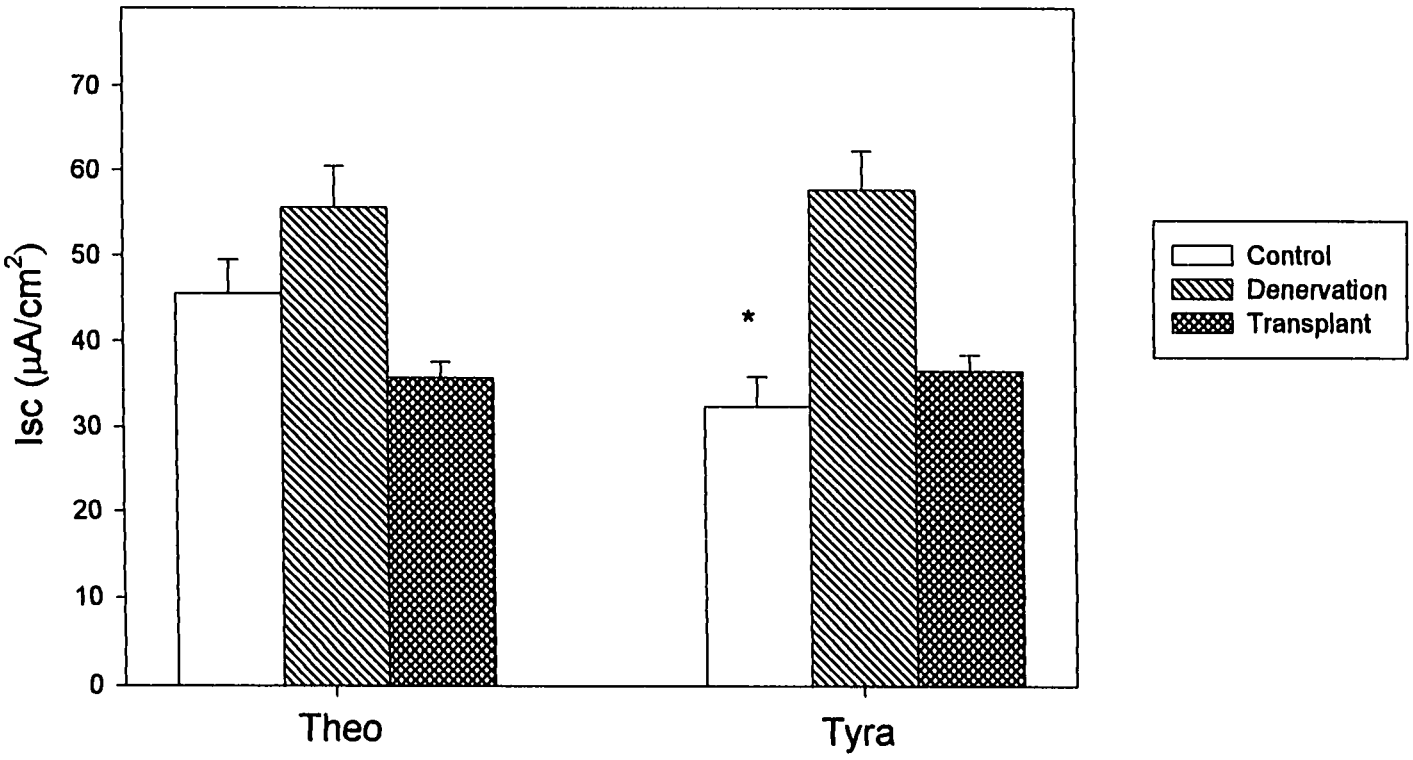
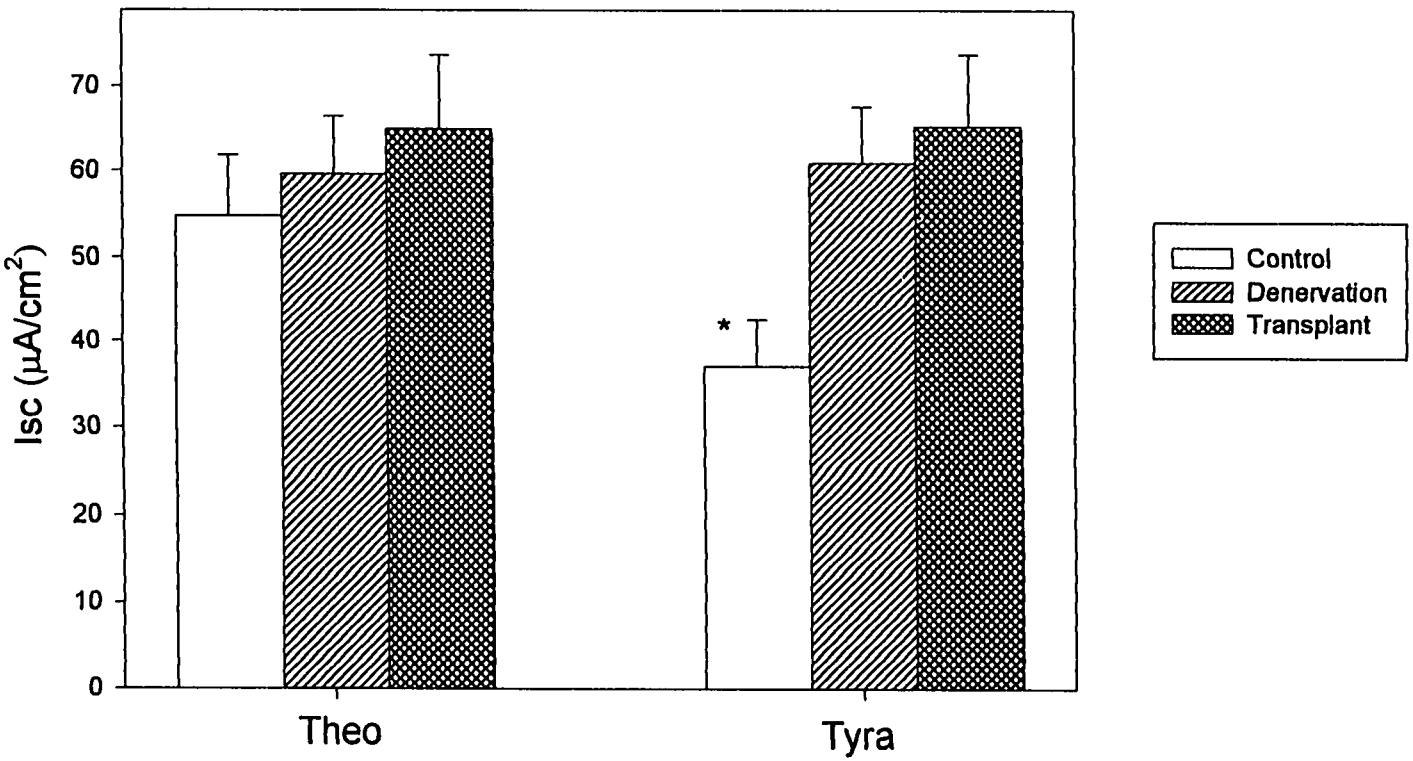


Figure 8. Isc response to Tyramine in Jejunum (2 weeks)



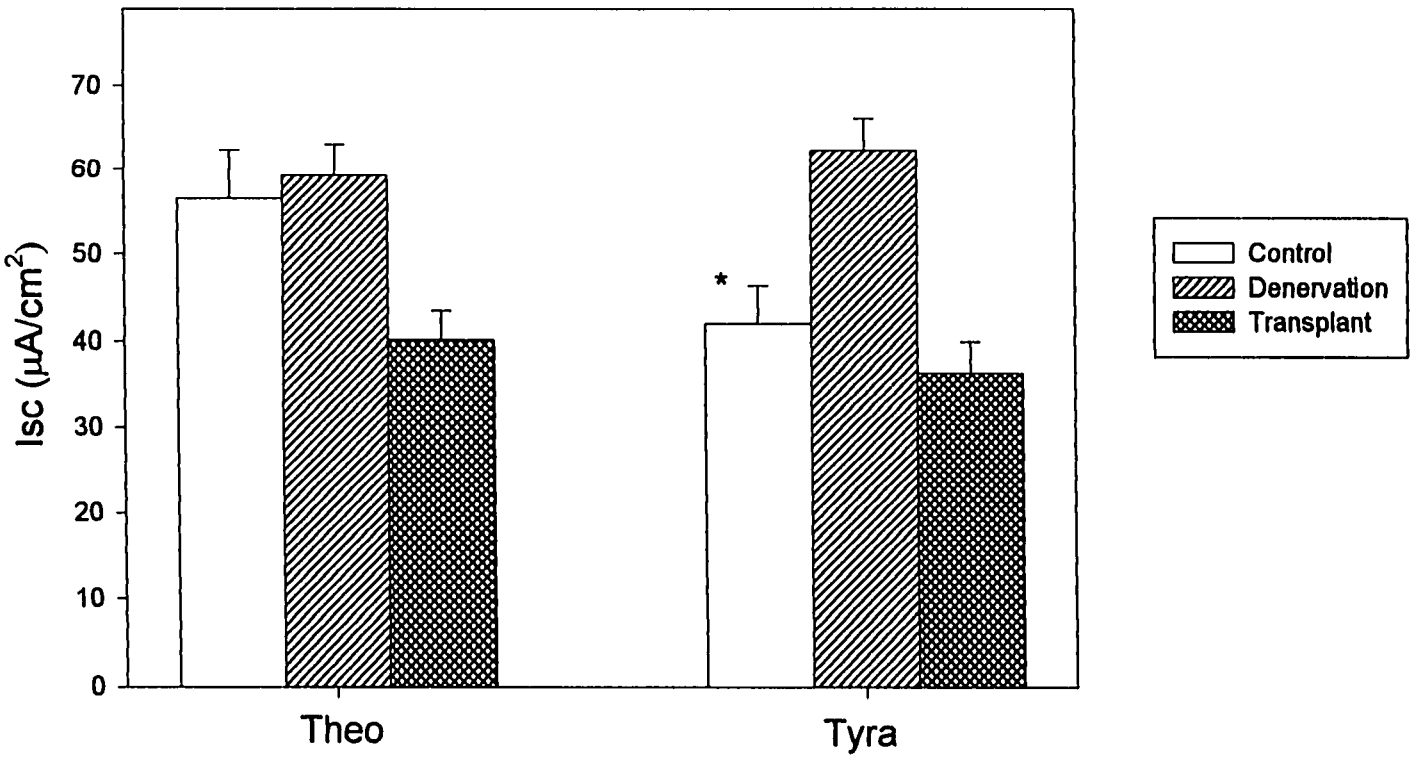
Theo - denotes treatment with theophylline
Tyra - denotes treatment with tyramine
* $p<0.05$ compared to theophylline

Figure 9. Isc response to Tyramine in Ileum (2 weeks)



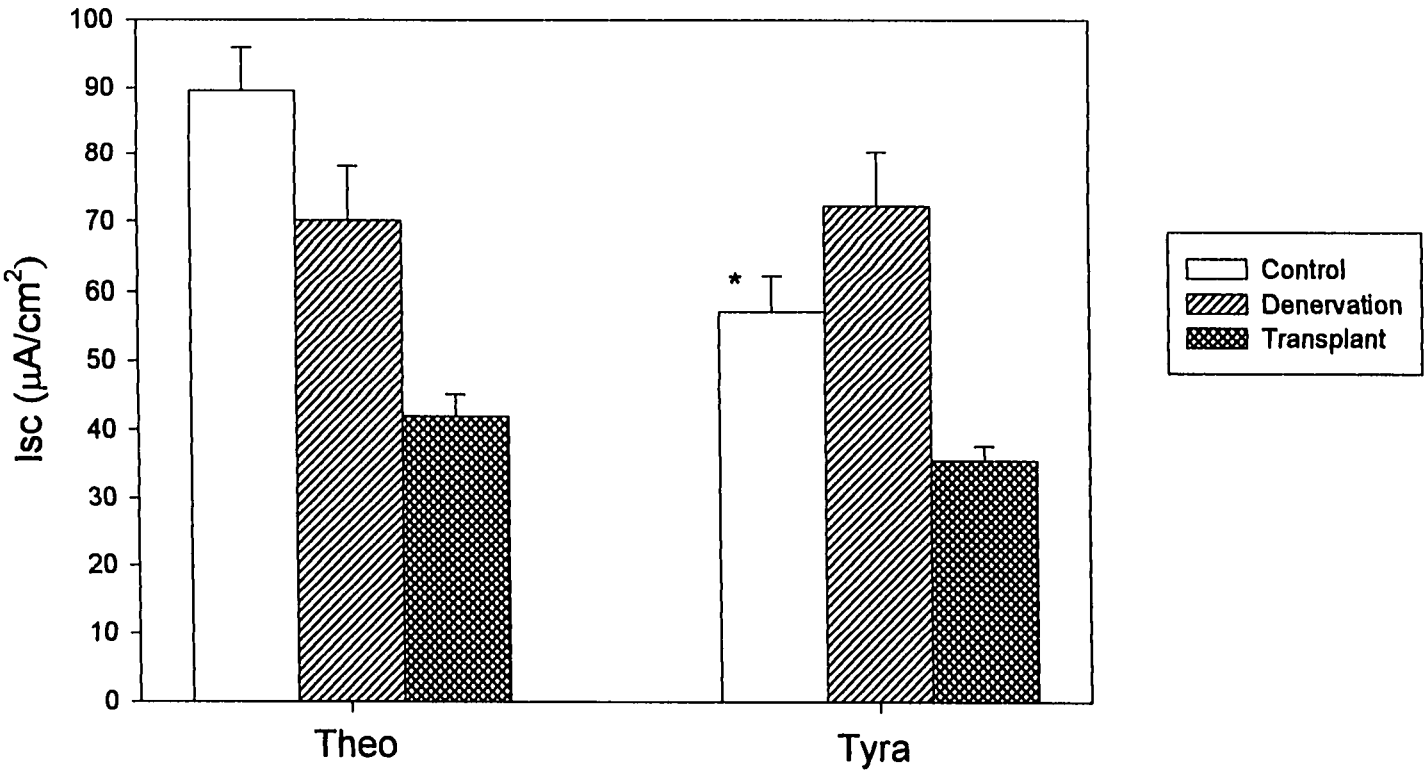
Theo - denotes treatment with theophylline
Tyra - denotes treatment with tyramine
* $p<0.05$ compared to theophylline

Figure 10. Isc response to Tyramine in Jejunum (6 weeks)



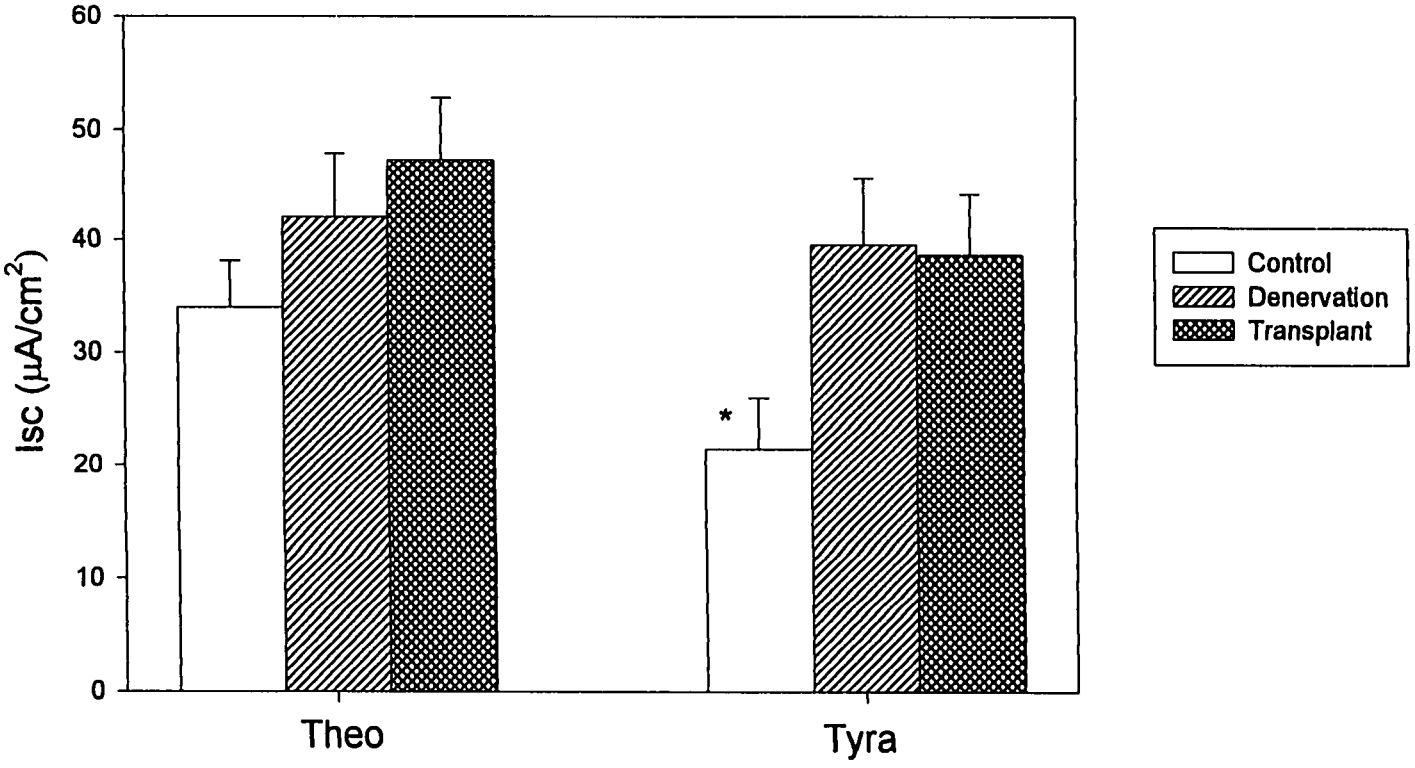
Theo - denotes treatment with theophylline
Tyra - denotes treatment with tyramine
* $p<0.05$ compared to theophylline

Figure 11. Isc response to Tyramine in Ileum (6 weeks)



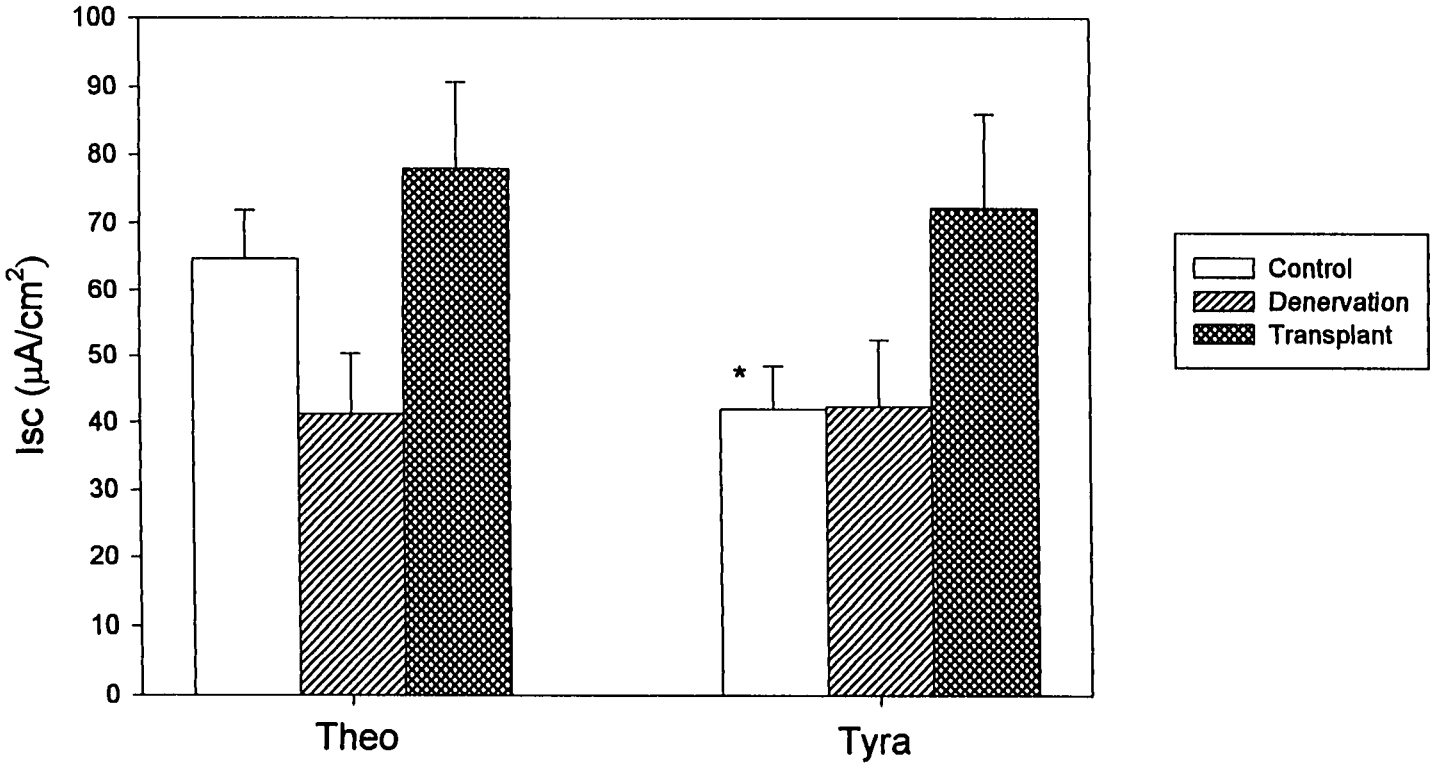
Theo - denotes treatment with theophylline
Tyra - denotes treatment with tyramine
* $p<0.05$ compared to theophylline

Figure 12. Isc response to Tyramine in Jejunum (6 months)



Theo - denotes treatment with theophylline
Tyra - denotes treatment with tyramine
* $p<0.05$ compared to theophylline

Figure 13. Isc response to Tyramine in Ileum (6 months)



Theo - denotes treatment with theophylline
Tyra - denotes treatment with tyramine
* $p<0.05$ compared to theophylline

Figure 14. Jejunum - Response to Epinephrine (two Weeks)

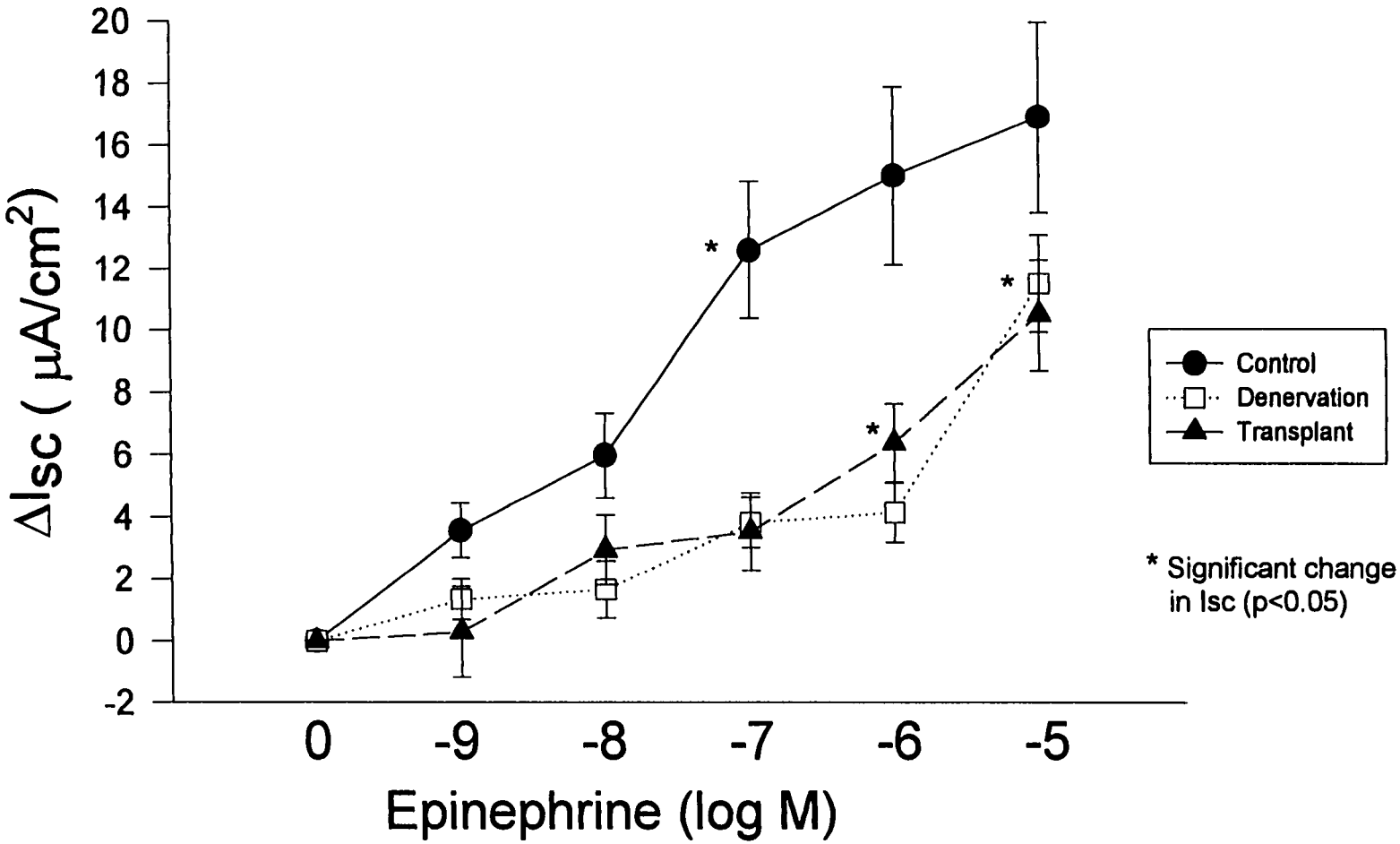


Figure 15. ILUEM - Response to Epinephrine (2 weeks)

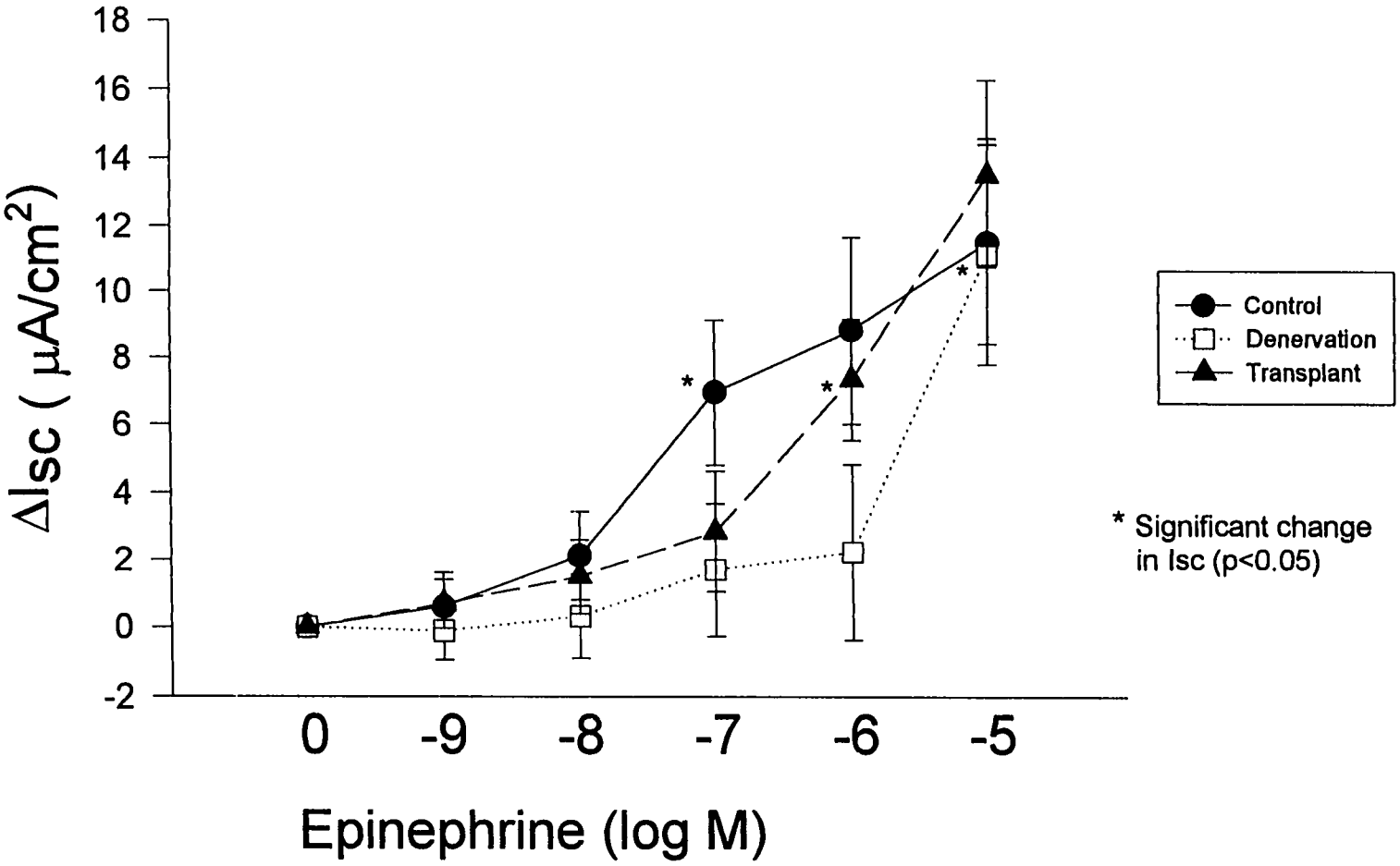


Figure 16. Jejunum - Response to Epinephrine (6 weeks)

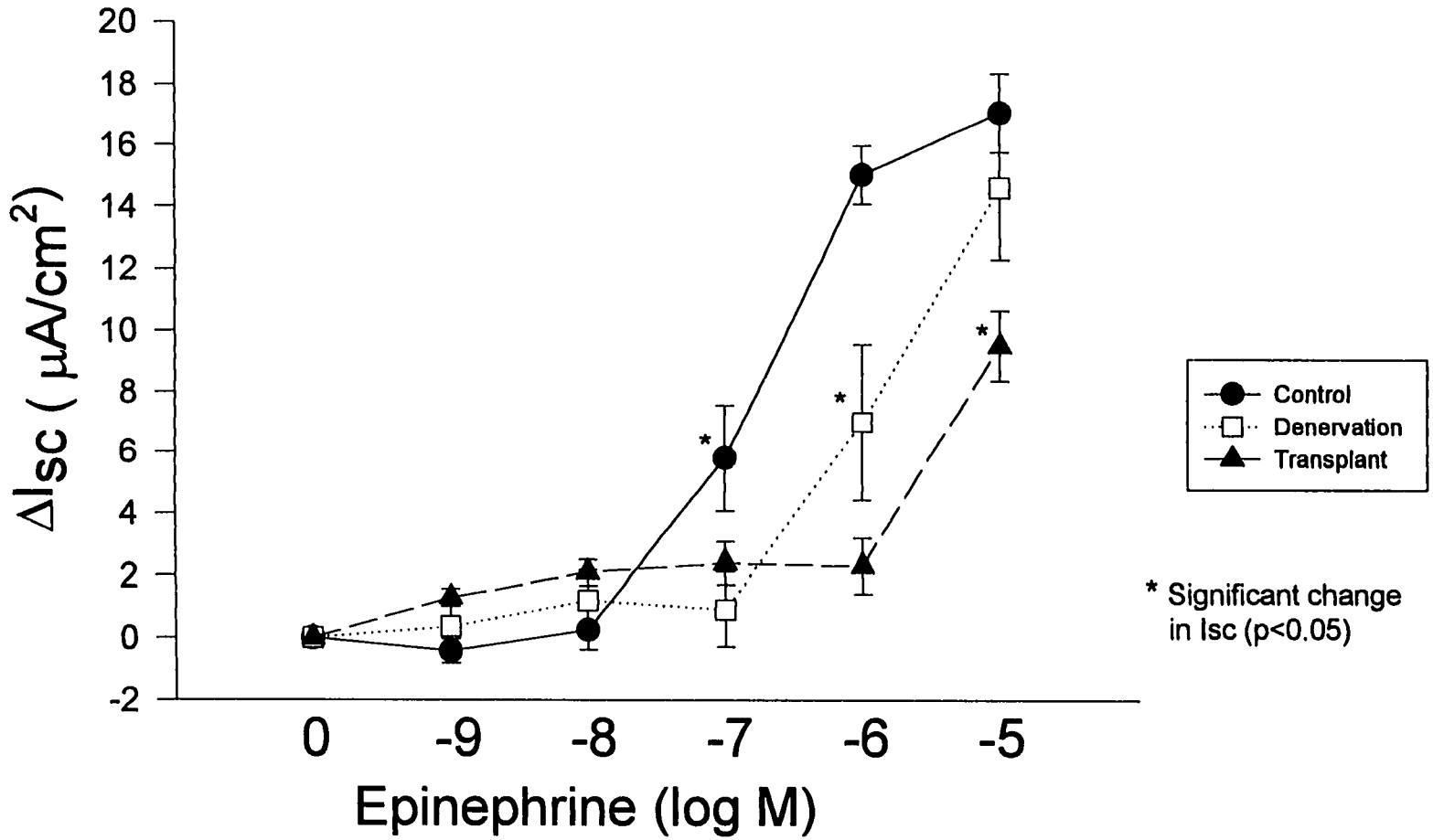


Figure 17. Ileum - Response to Epinephrine (6 weeks)

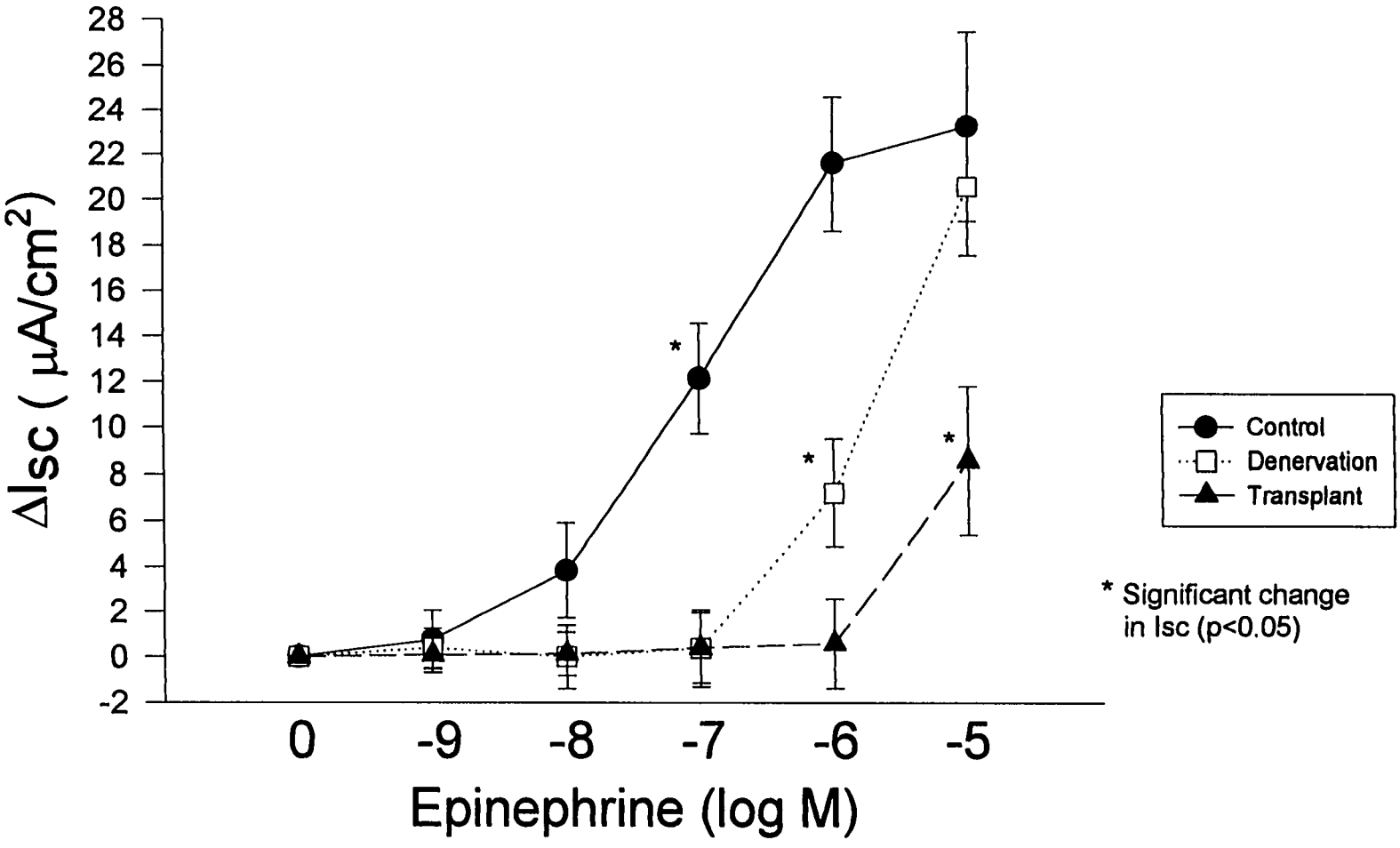


Figure 18. Jejunum - Response to Epinephrine (6 months)

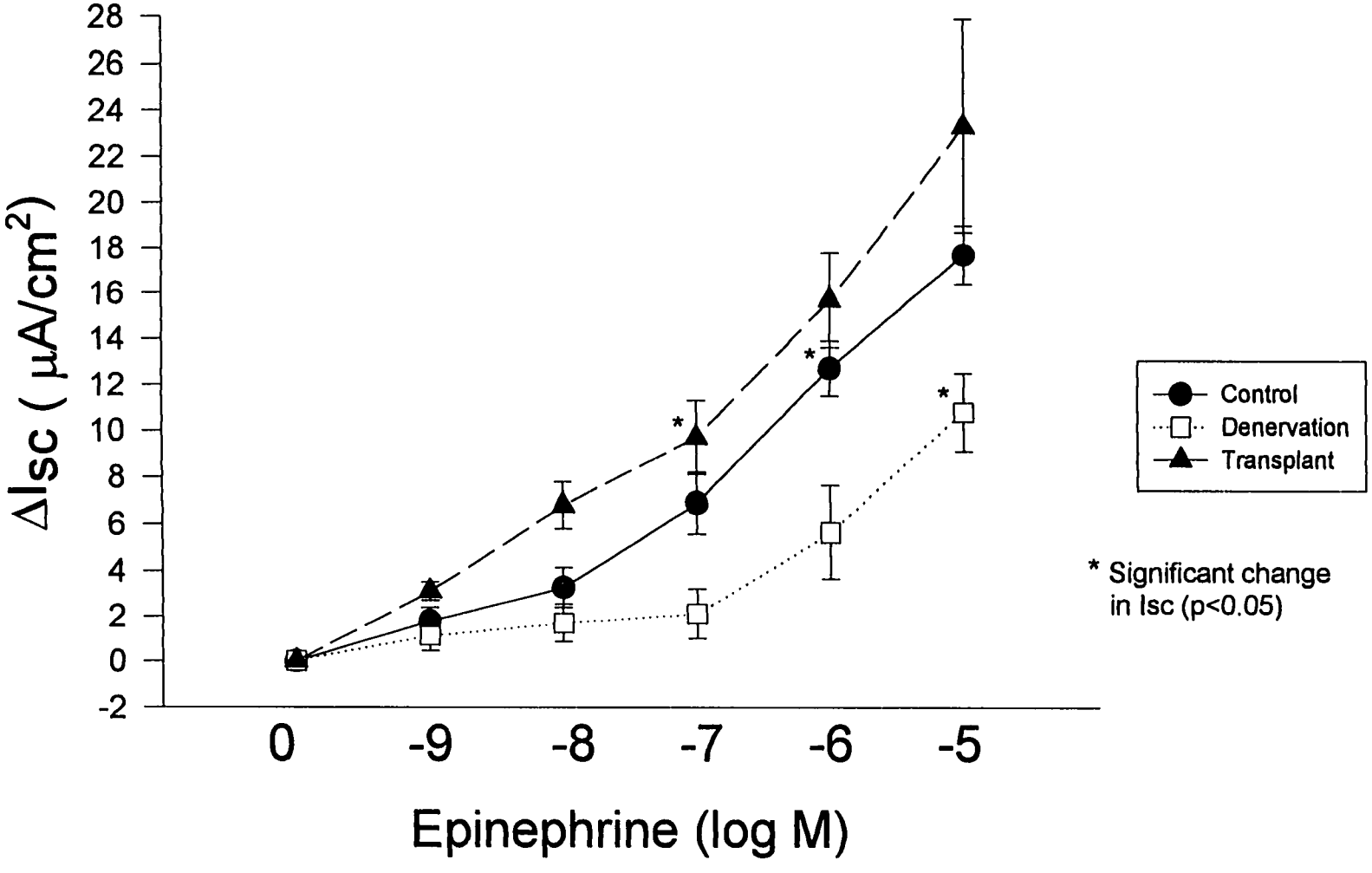


Figure 19. Ileum - Response to Epinephrine (6 months)

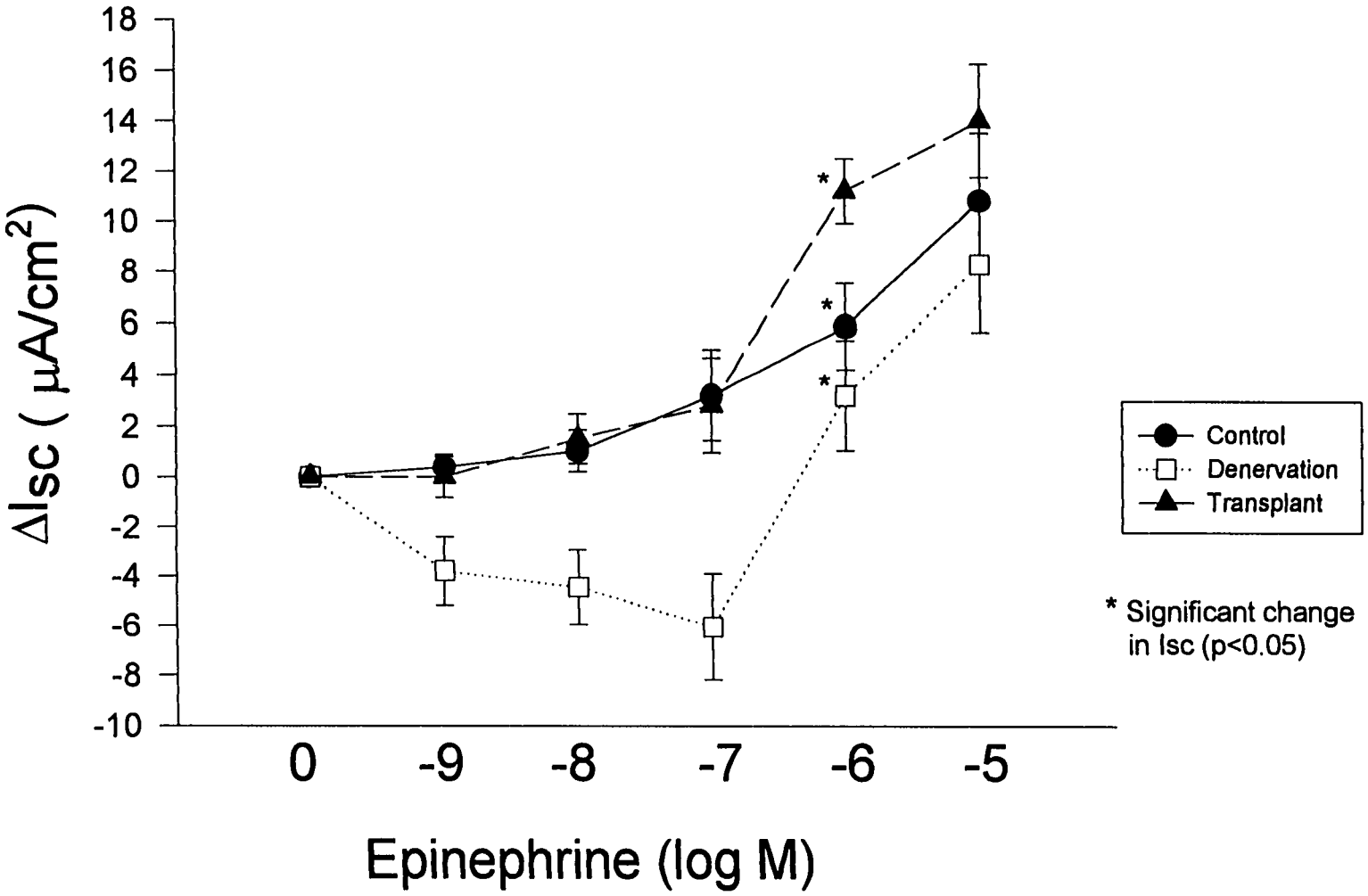


Table 1. Isc Response to Tyramine two weeks Postoperation

Jejunum	Base	Theo	Tyra	Δ Isc
Control	23.8 ± 2.8	45.5 ± 4.0	32.4 ± 3.5	-13.1 ± 1.4 *
Denervation	36.6 ± 4	55.6 ± 4.9	57.6 ± 4.7	2.0 ± 0.6
Transplant	21.0 ± 1.9	35.7 ± 1.9	36.5 ± 1.8	0.8 ± 0.7
Ileum	**Base**	**Theo**	**Tyra**	**Δ Isc**
Control	29.4 ± 6.5	54.8 ± 7.1	37.0 ± 5.5	-17.7 ± 2.4 *
Denervation	35.9 ± 6.3	59.6 ± 7	60.9 ± 6.7	1.3 ± 1.6
Transplant	32.7 ± 5.4	64.9 ± 8.7	65.2 ± 8.6	0.3 ± 2.5

Theo - denotes treatment with theophylline.
Trya - denotes treatment with tyramine.
Δ Isc - denotes the change of Isc between theophylline and tyramine.
Values are mean ± SEM and are presented as $\mu A/cm^2$
* Significant change in Isc with $p<0.05$.

Table 2. Isc Response to Tyramine six weeks Postoperation

Jejunum	Base	Theo	Tyra	Δ Isc
Control	41.6 ± 4.8	56.5 ± 5.9	41.9 ± 4.4	-14.5 ± 1.7 *
Denervation	36 ± 3.1	59.2 ± 3.8	62.3 ± 3.9	3.1 ± 1
Transplant	22.4 ± 2.3	40.1 ± 3.3	36.3 ± 3.6	-3.9 ± 1.3
Ileum	**Base**	**Theo**	**Tyra**	**Δ Isc**
Control	64.7 ± 5.8	89.6 ± 6.4	57.2 ± 5.1	-32.5 ± 3.7 *
Denervation	42.1 ± 6.8	70.1.6 ± 8	72.1 ± 8.1	2 ± 0.9
Transplant	18.3 ± 2.65	41.9 ± 3.3	35.5 ± 2.2	-6.5 ± 2.3

Theo - denotes treatment with theophylline.
Trya - denotes treatment with tyramine.
Δ Isc - denotes the change of Isc between theophylline and tyramine.
Values are mean ± SEM and are presented as $\mu A/cm^2$
* Significant change in Isc with $p<0.05$.

Table 3. Isc Response to Tyramine six months Postoperation

Jejunum	Base	Theo	Tyra	Δ Isc
Control	24 ± 4.3	34 ± 4.1	21.4 ± 4.6	-12.6 ± 1.9 *
Denervation	25.6 ± 5.1	42 ± 5.8	39.5 ± 6	-2.5 ± 2
Transplant	28.9 ± 5.1	47.2 ± 5.6	38.6 ± 5.5	-8.6 ± 1.3
Ileum	**Base**	**Theo**	**Tyra**	**Δ Isc**
Control	40.4 ± 7.7	64.6 ± 7.3	42 ± 6.5	-22.7 ± 2.7 *
Denervation	21.8 ± 6.3	41.2 ± 9.2	42.3 ± 10.1	1.1 ± 1.3
Transplant	40.5 ± 8.5	78 ± 12.7	72.2 ± 13.9	-5.8 ± 2.6

Theo - denotes treatment with theophylline.
Trya - denotes treatment with tyramine.
Δ Isc - denotes the change of Isc between theophylline and tyramine.
Values are mean ± SEM and are presented as $\mu A/cm^2$
* Significant change in Isc with $p<0.05$.